POSTER SESSION I

Iron Diseases

0001
5 YEARS OF COMBINED CHELATION THERAPY: A RADICAL CHANGE IN BETA-THALASSAEMIA MAJOR PATIENT CONDITION

K.F. Farmaki
General Hospital of Corinth, CORINTH, Greece

Background. Transfusional iron overload in Thalassaemia major is fatal in the second decade of life unless treated appropriately. The ultimate goal of iron chelation therapy is to prevent organ damage and premature death. Combined chelation with Deferoxamine (Ferriprox®) & Desferrioxamine (Desferal®) produces a synergistic iron chelating effect that is difficult to achieve with either drug alone. This approach may place all patients in negative net iron balance and lead to a significant reduction of the body iron load. Aim. To show which iron-induced complications may be reversible with the use of combined chelation in Thalassaemia major patients. Methods. 50 β-Thalassaemia major patients (TMs) aged 6-46 years, switched from Desferrioxamine monotherapy to combined chelation with oral Deferoxamine (25-30 mg/kg t.i.d) and Desferrioxamine (20-50 mg/kg, 8-12h SC or IV 2-6 days/week), in a 5 year regimen, adjusted on individual needs. The following tests were routinely performed: - mean annual Ferritin based on monthly measurements by MEIA; - annual/biannual ECG and Cardiac Echo for evaluation of cardiac function; - non-invasive heart & hepatic iron quantification, by annual Signa-MRI 1.5 Tesla, multi-echo T2 & T2* sequences; - annual endocrinology screening. Results. 1) None of the 50 TMs died since combined chelation therapy was implemented, while, with desferrioxamine monotherapy, mortality fluctuated from 13.3 to 14.3% over the last decade. 2) A trend analysis (PROC MIXED in SAS), revealed a negative trend of serum ferritin over time (p<0.0001) with a rate of decline equal to -95 ng/mL/month and a cumulative decrease in 5 years. In the 88.7% of compliant TMs the mean ferritin value at baseline (2.421 µg/mL) decreased dramatically (107 µ/L) after 5 years of treatment. 3) In 12 patients with pre-existing heart dysfunction, symptoms (arrhythmias, hypertension and edema) reversed and heart medications were stopped. Ventricular dimensions and function normalized in Echo tests. Mean LVEF increased significantly (p<0.0001) from 54% to 72% following combined therapy. No case of new onset cardiac disease or worsening of pre-existing cardiac dysfunction was evident. 4) MRI measurements (T2Heart & T2Liver) revealed significant reduction of iron overload in both organs over time leading to virtually iron free organs (Table 1).

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Mean T2H</th>
<th>Mean T*</th>
<th>Mean T1-</th>
<th>Mean T*+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal values</td>
<td>&gt;35 msec</td>
<td>&gt;28 msec</td>
<td>&gt;33 msec</td>
<td>&gt;25 msec</td>
</tr>
<tr>
<td>Desferal® monotherapy</td>
<td>28.2 msec</td>
<td>22.7 msec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 3-5ys Combined Chelation</td>
<td>38.1 msec</td>
<td>34.8 msec</td>
<td>37.2 msec</td>
<td>31.7 msec</td>
</tr>
<tr>
<td>Difference</td>
<td>9.9 msec</td>
<td>p&lt;0.0001</td>
<td>14.5 msec</td>
<td></td>
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</tbody>
</table>

5) At baseline, 7 TMs (12%, mean age 38.7 years) had Insulin-dependent Diabetes and 22 (44%, mean age 32.5 years) had Impaired Glucose Tolerance. Following addition of dferoprine, glucose metabolism improved. Insulin production increased and Insulin resistance reduced (Table 2). 6) Reversal of secondary anemia and spontaneous ovulation in individual cases was validated by LH, FSH, E2/Progesterone, ovarian and uterine ultrasound. Conclusions. Combined chelation with Desferal® & Ferriprox® seems to be the treatment of choice because of increased efficacy in a minimally intrusive way. The obvious improvement of cardiac function with reversal of cardiac complications and the removal of myocardial iron, led to zero mortality. Not only was abnormal glucose tolerance reversed, but also the cumulative glucose response improved significantly with this regimen. The reversal of secondary hypogonadism and the hope of creating a family improved the quality of life of Thalassaemia patients considerably.

Table 2.

<table>
<thead>
<tr>
<th></th>
<th>OGTT:</th>
<th>OGTT:</th>
<th>OGTT:</th>
<th>OGTT:</th>
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<tbody>
<tr>
<td>AUC Glucose</td>
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<tr>
<td>N=22</td>
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<tr>
<td>Normal values</td>
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<td>Difference</td>
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<tr>
<td>p&lt;0.0001</td>
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</table>

Iron Diseases

0002
PEARSON SYNDROME IN AN INFANT HETEROZYGOUS FOR C282Y ALLELE OF HFE GENE

T. Papajik,1 K. Kefala-Agoropoulou,1 E. Roilides,2 E. Karataz,1 A. Lazandou,1 E. Farmaki,3 P. Augoustides-Savvopoulou,1 C. Tsantali,2 J. Tsionurs1

1University Hospital, OLOMOUC, Czech Republic; 2Aristotle University, THESSALONIKI, Greece; 3Thaegeion Hospital, THESSALONIKI, Greece

Background. Pearson syndrome is a rare mitochondrial disorder characterized by sideroblastic anemia that usually presents since infancy. Liver disease, renal tubulopathy and exocrine pancreas deficiency emerge later in the course of the disease. The syndrome is due to heteroplastic mitochondrial DNA deletions and rearrangements, the lack of which, however, cannot exclude the disease. Diagnosis is made by clinical criteria and confirmed by genetic findings. Aim. To report the second case of Pearson syndrome in an infant heterozygous for C282Y allele of HFE gene. In addition, it is the first reported case successfully treated initially by deferoxamine and subsequently complicated by primary cutaneous zygomycosis. Case report. A 2-month old girl suffered from severe anemia since birth. Bone marrow examination revealed ring sideroblasts and signs indicative of dyserythropoiesis. Onset of anemia was accompanied by neutropenia that did not respond to administration of granulocyte colony-stimulating factor. M-FISH (Fluorescent in situ hybridization) canotype was normal. There was neither deletion nor metathesis of any of the chromosomes. Metabolic evaluation was initially normal. Psychomotor development was normal, but the infant grew on the 9th percentile of weight and height. Transaminemia developed when she was 8 month old, accompanied by thrombocytopenia. Transferin saturation increased to 54% and ferritin reached the level of 5000 ng/dL. Ultrasonography revealed signs of diffuse-non specific damage of the liver. Deferoxamine was initiated and liver dysfunction subsided. Genetic evaluation revealed that the patient was heterozygous for C282Y allele of HFE gene. Pyridoxine and B12 per os were initiated but the patient did not respond. Corticosteroids were also initiated and the patient initially responded to therapy. One month after the initiation of deferoxamine and corticosteroids, the infant’s course was complicated by zygomycosis. Liposomal amphotericin B was initiated while deferexamine and corticosteroids were discontinued. Ultrasongraphy revealed that diffuse liver damage had been reversed. Renal tubulopathy presented shortly before discontinuation of antifungal therapy. Enlargement of kidneys and liver was developed. Lactic acid increased (>500 mg/dL) and acidosis became severe. Acute Respiratory Distress Syndrome due to Pneumocystis carinii lung infection evolved rapidly. Neurological disturbances not due to CNS infection developed. Multiple

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organ dysfunction followed. The patient died at the age of 13 months. Conclusion. Sidringer anaemia in neonates is unusual and requires specific differential diagnosis. Metabolic disorders are among them and especially those of mitochondria. We report this case as a rare disease having uncommon complications: 1) Pearson syndrome emerged with hematological features until the age of 11 months. 2) Signs of evolution of the disease presented at a time when differentiation from amphotericin-b toxicity was difficult. The infant was subsequently shown to have mutations of the C282Y allele of HFE gene. 3) Deferoxoxamine therapy initially reversed liver damage. 4) High clinical suspicion is necessary for early recognition of rare infections (e.g. zygomycesis). Iron overload, hemochromatosis, deferoxoxamine and corticosteroids are underlying conditions for developing zygomycosis.

0003 CHELATION THERAPY WITH DEFERASIROX VERSUS DEFEROXAMINE IN TRANSFUSION-DEPENDENT SICKLE-CELL DISEASE: A COST-EFFECTIVENESS ANALYSIS FROM THE US PERSPECTIVE

E. Delea, 1 O. Sofrygin, 1 J.F. Baladi, 2 H. Berdoussi, 2 S.K. Thomas, 3 T.D. Coates 3

1 Policy Analysis Inc. (PAI), BROOKLINE, MA, USA; 2 Novartis Pharmaceuticals Corporation, FLOURHAM PARK, NJ, USA; 3 Children’s Hospital of Los Angeles, LOS ANGELES, CA, USA

Background. Patients with sickle-cell disease (SCD) receiving chronic transfusions require chelation therapy to prevent complications of iron overload. Although deferoxoxamine is an effective iron chelator, it must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance and/or quality of life. Deferasirox is an once-daily oral iron chelator that produces reductions in liver iron concentrations and serum ferritin similar to those obtained with deferoxoxamine in 5-7 days. To better characterize the cost-effectiveness of deferasirox versus deferoxoxamine in SCD patients receiving frequent transfusions (≥2 per year). As there are no long-term studies describing the complications of iron overload in patients with SCD, we focused on the short-term (i.e., one year) costs and quality-of-life effects of chelation therapy. We assumed that patients would receive dosages of deferasirox and deferoxamine that have been found to be similarly effective in patients with SCD (17.5 and 36.0 mg/kg/d, respectively). To be conservative, we assumed that all patients would be fully compliant with chelation therapy and that use of deferasirox therefore would have no effect on risk of complication of iron overload. Cost-effectiveness was measured in terms of the ratio of the difference (deferasirox vs deferoxamine) in mean quality adjusted life years (QALYs) over one year of treatment. Unit costs of deferoxoxamine and deferasirox were based on US wholesale acquisition costs. The cost of deferroxoxamine administration was based on analyses of health insurance claims data for US patients with transfusion-dependent anemias. Utilities (weights representing patient quality of life) were based on results of a study that used time-trade-off methods to estimate community-based preferences for oral versus infusional iron chelation therapy. Results. One year of treatment with deferasirox is estimated to result in a gain of 0.25 QALYs (0.82 vs 0.57 with deferoxamine). If the price of branded deferoxamine is employed, total annual costs were estimated to be $523 lower with deferasirox (0.82 vs 0.57 with deferoxamine). If the price of generic deferoxamine is employed, costs are increased by $5,527 with deferoxamine vs deferasirox. Cost-effectiveness is estimated at $29,904 vs $29,827. Deferasirox therefore dominates deferoxamine (i.e., is less costly and produces more QALYs). If the price of deferoxamine is employed, costs are increased by $5,527 with deferoxamine vs deferasirox; the cost per QALY gained with deferoxamine vs deferasirox is $15,028. Cost-effectiveness of deferoxamine vs deferasirox was sensitive to the assumed dosages of deferasirox and deferoxamine and the costs and quality of life decrements associated with infusional therapy. Conclusion. In patients with SCD receiving frequent transfusions, deferasirox is less costly and yields more QALYs than branded deferoxamine. Compared with generic deferoxamine, the cost per QALY gained with deferoxamine versus deferasirox is well within the range that is generally considered acceptable in the US. Further research is needed to assess the potential implication of deferasirox on the risk-benefit profile of transfusion therapy in patients with SCD.

0004 MANNOSE BINDING LECTIN LEVELS IN THALASSEMIC PATIENTS WITH HEPATITIS C TREATED WITH PEGINTERFERON ALPHA-2

I. Papassotiriou, 1 H. Berdoussi, 2 H. Hantzi, 2 A. Kattamis, 2 C. Kattamis, 2 V. Ladis 2

1 Aghia Sophia Children’s Hospital, ATHENS, Greece; 2 Athens University, ATHENS, Greece

Mannose-binding lectin (MBL) is a serum protein belonging to the family of collectins, which plays a critical role in the innate immune response. MBL is an acute-phase reactant of hepatic origin that can bind through its lectin domains to repeating mannose and N-acetylglucosamine sugar motifs that are characteristically displayed at high densities on bacterial and viral cells and that are lacking from mammalian cells. After binding to a pathogen, MBL initiates at least 2 protective functions that are well defined. First, through the lectin pathway, MBL can mediate the activation of the complement system without the participation of antibodies; second, MBL can promote opsonophagocytosis by lectin receptors directly. Experiments in vitro and in vivo have shown that an MBL deficiency is likely to have a major effect on innate immune activation and appears to predispose individuals to serious infection. The amounts of MBL in human plasma are genetically determined. We studied the effect of treatment with pegylated interferons–a (peginterferon alpha-2a) or peginterferon alpha-2b (PEGASYS, Roche, Basel, Switzerland). MBL levels were measured by means of a fully automated enzyme-linked immunosorbent assay on the BN-100 nephelometer (Dade Behring, Liederbach, Germany). The measurements were performed before and at the end of the treatment with peginterferon alpha-2a. MBL levels were increased significantly in 11/14 patients, independently from the therapeutic scheme, from 2.00±0.21 mg/L to 2.79±0.37 mg/L (p<0.008). In the three other patients the MBL levels remained unchanged and relatively low indicating a possible genetic influence. These findings suggest that administration of peginterferon alpha-2a in thalassemic patients with hepatitis C, additional to the reduced observed viral load, normalizes the secretion of MBL and thus restore the impaired innate immune system.

0005 EFFECTIVENESS AND SAFETY OF LONG-TERM COMBINATION IRON CHELATION THERAPY WITH DESFERRIOXAMINE & DEFERIPRONE IN MULTITRANSFUSED PATIENTS WITH THALASSEMIA

S. Fragatos-Pateraki, C. Polotis, K. Vendiadi

3rd Regional Blood Transfusion Centre, ATHENS, Greece

Background. Transfusional haemochromatosis and increased dietary iron absorption cause severe complications in thalassaemic patients and lead to death before the 3rd decade of life unless treated effectively with iron chelation therapy. In this context, accurate assessment of body iron in these patients is essential for monitoring chelation in order to avoid toxic effects of iron overload and to prevent side effects from hyper doses of the chelator. Aim. The aim of this study is to evaluate the efficacy and safety of a combination therapy with the two chelators DFO and DFP, shown to have an additive or synergistic effect when used appropriately in severely loaded thalassaemic patients presenting with cardiac or liver complications. Methods. Twelve patients (5 men and 7 women; mean age 36, range 22 to 46 years) have been treated with DFO 40 mg/Kg/day 2 days/week and DFP 75 mg/kg/day (both chelators used on the same day). Four patients had a medical history of diabetes mellitus type II. Another four had been infected with HCV and two progressed to chronic active hepatitis. Iron load was estimated with serum ferritin and 24-hour urinary iron excretion (UIE) every 3 months. Cardiac and liver function and iron load were measured annually with biochemical tests (ALT, AST and γ-GT), ECHO cardiography and magnetic resonance imaging (MRI-T2). Results. Compliance with treatment was very high throughout the study period. No side effects or adverse reactions associated with combination therapy were observed. In patients presenting with cardiac dysfunction before treatment, symptoms disappeared two years after the onset of therapy. As shown in Table 1 serum ferritin decreased significantly (p<0.01), while UIE was significantly increased. As regards myocardium and liver, T2 relaxation time was significantly increased (p=0.002 and p=0.048 respectively), while no significant changes in liver enzymes were observed after treatment. Left Ventricular Ejection Fraction (LVEF) (p=0.007) and fractional shortening were significantly increased.

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The results of the secondary endpoint revealed both therapies were associated with a significant reduction in LIC (2.4±8.2 and -1.4±4.0 for deferasirox and deferiprone respectively; p=0.001 for both). The overall reduction was due mainly to the effect of the chelators on the LIC of patients with greater baseline LIC. Conclusions. The overall success for the primary efficacy endpoint was greater for deferiprone than deferasirox. The success of the highest doses of deferasirox was similar to that of deferiprone at 75 100 mg/kg/day. Although these data were generated from distinct cohorts of patients participating in independent studies, they represent carefully conducted studies in the same types of patients and provide a means for obtaining an initial comparison. These results highlight the need for a randomized study comparing the two chelators, and one where only effective doses of deferasirox will be used.

Table 1.

<table>
<thead>
<tr>
<th>LIC at baseline</th>
<th>Exjade® (N=276)</th>
<th>Ferriprox® (N=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-&lt;7 mg/Fe/g dw</td>
<td>34/85 (40%)</td>
<td>22/27 (81%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>≥7 mg/Fe/g dw</td>
<td>112/191 (59%)</td>
<td>20/33 (61%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Overall</td>
<td>146/276 (53%)</td>
<td>42/60 (70%)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Conclusions: Our results show high acceptance of long-term combination therapy with DFO and DFP by patients who previously failed to comply with DFO monotherapy. Long-term administration of both chelators used on the same day has been shown to be safe and no deleterious effects were observed. Serum ferritin was positively correlated with cardiac ECHO and MRI. No noteworthy change was found in liver iron, possibly due to the late onset of chelation and consequent permanent liver damage.

0007
THE ROLE OF RETICULOCYTE HEMOGLOBIN CONTENT AS IRON STATUS MARKER IN HAEMODIALYSIS PATIENTS
J.M. Kim, C.H. Ihn, H.J. Kim
Eulji University Hospital, DAEJEON, South-Korea

Background. Iron deficiency leads the hyporesponsiveness to erythropoietin (rHuEPO) in hemodialysis patients and results in renal anemia. So, the earliest detection of iron deficiency is of value for the successful treatment of renal anemia. At present, serum ferritin and transferrin saturation (TS) are recommended for assessing iron deficiency. However, they have a limitation in estimating iron status because the lack of accuracy and precision in dialysis patients. The reticulocyte hemoglobin content (CHr) has been proposed as a useful tool in iron status assessment, but its cutoff value for iron deficiency varies from 26 to 32 pg in different studies. Aims. We investigate the accuracy of CHr in comparison to the conventional test and a CHr cutoff value. Also, we assess that the CHr change after administration of iron supplement related to changes in red cell count, Hb, and Hct. Methods. We selected 163 hemodialysis patients (95 females and 78 males, mean age 56.1±13.2) receiving (HuEPO and oral or intravenous iron therapy. We measured CBC, reticulocyte, CHr (using ADVIA120 autoanalyzer, Bayer Medical, USA), iron parameters (iron, TIBC, ferritin), CRP, BUN and creatinine. Iron deficiency in this study was defined as a serum ferritin <100 µ/mL or a TS <20%. In patients categorized as iron deficient, CBC, reticulocyte, and CHr were determined at 1 month after iron therapy. Results. The mean Hb in hemodialysis patient was 10.0±1.1 g/dL and 53 patients were iron deficient (19 with low ferritin and low TS, 8 with only low ferritin, 26 with only low TS). CHr were distributed with mean 55.9±14.4 pg in iron deficient group and mean CHr 29.2±1.2 pg in iron deficient group, and showed significant difference between 2 groups. CHr was positively correlated with TS (r=0.36, p=0.01), and there was no correlation with iron, ferritin, BUN and creatinine. The CHr changes were related to changes in red cell count (r=0.13, p=0.045) and Hct (r=0.21, p=0.05). Conclusions. CHr is available in measuring iron status in dialysis patients, especially in patients in iron deficiency with normal ferritin. It is considered that the CHr cut-off value 32 pg is appropriate for the assessment of iron deficiency (sensitivity 100%, specificity 80%). Also, CHr might be useful to predict the degree of erythropoietic response after iron administration.

Table 1. Success rate based on Exjade™ primary efficacy success criteria.

<table>
<thead>
<tr>
<th>LIC at baseline</th>
<th>Success rate (%) after 1 year of treatment</th>
<th>Exjade™</th>
<th>Ferriprox®</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-&lt;7 mg/Fe/g dw</td>
<td>34/85 (40%)</td>
<td>22/27 (81%)</td>
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0008
SENSITIVITY ANALYSIS ON THE COST-EFFECTIVENESS OF CHELATION THERAPY WITH DEFERASIROX OR DEFEREXOMINE IN TRANSFUSION-DEPENDENT THALASSEMAIA PATIENTS BASED ON EUROPEAN COSTS
E. Delea,1 O. Sofrygin,1 J.E. Baladi,1 S.K. Thomas,2 P.D. Phatak,1 T.D. Coates1
1 Policy Analysis Inc. (PAI), BROOKLINE, MA, USA; 2 Novartis Pharmaceuticals Corporation, FLORHAM PARK, NJ, USA; 3 Rochester General Hospital, ROCHESTER, NY, USA; 4 Children's Hospital of Los Angeles, LOS ANGELES, CA, USA

Background. Deferoxamine is an effective iron chelator but must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance, effectiveness, and/or quality of life. Deferasirox is a novel once-daily oral chelator that produces reductions in liver iron concentrations (LIC) and serum ferritin similar to deferoxamine, and has been found to have a favourable cost-effectiveness in US thalassemics. Cost-effectiveness in other settings has not been examined. Aims. To examine the sensitivity of the cost-effectiveness of deferasirox and deferoxamine among thalassemia patients to costs prevailing in various European countries. Methods. A Markov model developed previously for the
US was adapted to examine the potential cost-effectiveness of deferasirox and defereroxame in various European countries, using ranges of values for costs of chelation which may prevail across these settings. Other inputs were unchanged as they are likely to be similar across settings. Patients were assumed to have thalassemia major, be three years of age at initiation of chelation therapy, and to receive prescribed dosages of deferasirox and defereroxame that have been shown to be equivalent in patients with LIC $27 kg/\text{d}$ respectively. Compliance with defereroxame was based on analyses of health insurance claims data. Data on compliance with defereroxame versus defereroxame were unavailable, published data on compliance with the oral chelator deferiprone versus defereroxame were used. Probabilities of complications of iron overload and death by com- pliance status were estimated from published studies. Differences in quality of life with defereroxame versus defereroxame were based on a study of patient preferences for oral versus intravenous chelation therapy. The price of defereroxame was varied from $15 to $40 per 2 g vial; the price of defereroxame, from $40 to $50 per 1 g vial; and the cost of defereroxame administration, from $10 to $40 per infusion. Costs of complications of iron overload conservatively were not considered. Cost-effectiveness was defined as the incremental cost per quality-adjusted life years (QALY) gained. Future costs and QALYs were discounted at 5% annually.

Results. Compared to no chelation (which yields 7.6 QALYs), defereroxame versus defereroxame yields an additional 4.1 QALYs per patient while defereroxame yields an additional 8.1 QALYs per patient. Expected lifetime costs of chelation therapy with defereroxame range from $70,000 to $226,000 per patient; those for defereroxame range from $186,000 to $266,000 per patient. Cost-effectiveness versus no chelation ranges from $20,000 to $63,000 per QALY gained for defereroxame and from $28,000 to $35,000 per QALY gained for defereroxame. In almost all scenarios where the cost of defereroxame administration is $15 per infusion or more, the cost-effectiveness of defereroxame versus no chelation is more favorable than that of defereroxame versus no chelation. The cost-effectiveness of defereroxame versus defereroxame was less than that of $40,000 per QALY gained in all scenarios. Conclusion. Although analyses based on actual prices of defereroxame are necessary, this analysis suggests that the cost-effectiveness of defereroxame versus defereroxame or no chelation in European settings is within the range considered acceptable in these countries.

00.09
Burden of Iron Chelation Therapy Significantly Impacts Adherence to Treatment in Patients with Iron Overload
D. Rofail, M. Viala, E. Trudeau, J. F. Baladi

Background. As part of a supportive care programme, thalassemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) patients require regular blood transfusions. One consequence of this is iron overload, due to dyserythropoiesis and regular blood transfusion in β-thalassemia major patients, a major problem in Mediterranean as well as in Iran, is associated with impaired lymphocyte proliferative responses to mitogens and cell-mediated immunity. Iron mainly in its non-proliferative form, low molecular weight form, cause cellular damage by par- ticipating in the generation of the hydroxyl radical, thought to be the principal effector of oxidative DNA damage. One possibility is that telomerase activity, essential enzyme for the repair of telomeric DNA, is reduced following damage by oxygen radicals. Aims. The aim of the present study was to investigate telomerase activity in lymphocytes from patients with iron overload disease and to observe its regulation of cellular proliferation and also evaluate effect of Silymarin on this enzyme. Methods. Peripheral blood mononuclear cells (PBMC) were iso- lated from 20 patients with β-thalassemia major and 20 healthy donors. Cells were stimulated with PHA and treated with Deferoxamine and Silymarin for 72 h. Telomerase activity was measured by the telomeric repeat amplification protocol (TRAP) assay and telomerase activity was measured with TRAP assay. In addition, DNA synthesis of the cells was assayed using BrdU (bromo-2'-deoxyuridine) incorporation. Results. The results showed that telomerase activity of resting peripheral lymphocytes of healthy subjects and patients with β-thalassemia major was detectable at low level, and obviously increased after stimulation in vitro with phytohaemagglutinin (1 μg/ml) and Deferoxamine, and with treatment in vitro with Deferoxamine (DFO). The decreased telomerase activity of resting lymphocytes was found in patients with β-thalassemia major compared to that in healthy subjects. The DNA proliferation was paralleled by increase in telomerase activity. Conclusions. These results lead to important conclusions. First, the ability of T cells to upregulate telomerase activity upon activation may decrease over time (aging) and following iron overload-mediated oxidative stress. Second, Silymarin upregulates telomerase activity of T-Lymphocytes and Deferoxamine downregulates. Third There is a direct correlation between telomerase

| Table 1 |
|------------------|------------------|------------------|
| Significant predictors of thinking about stopping medication | Variance explained | p value |
| Satisfaction with burden | 30% | <0.0001 |
| Satisfaction with side effects | 26% | <0.0001 |
| Acceptability | 15% | <0.0001 |
| Age | 11% | <0.0005 |
| Perceived effectiveness | 9% | <0.0014 |
| Feelings about yourself | 4% | <0.0436 |

00.10
Effects of Silymarin on Telomerase Activity and Proliferation of Peripheral Blood T-Lymphocytes in β-Thalassemia Major Patients?
B. Bagherpour, B. Moayedi, M. Ghargozlu, M. Tahanian, H. Hourfar, L. Khodadadi, H. Mirmohammadsalehdi

School of Medicine, ISFAHAN, Iran; Omid Hospital, ISFAHAN, Iran; Royan Institute, TEHRAN, Iran; Biotechnology Research Center, ISFAHAN, Iran

Background. Iron, an essential growth trace element, is required for tissue growth. However, when iron is not handled properly and accumulates within the body, it leads to iron loading, which can lead to serious complications. Iron overload, due to dyserythropoiesis and regular blood transfusion in β-thalassemia major patients, a major problem in Mediterranean as well as in Iran, is associated with impaired lymphocyte proliferative responses to mitogens and cell-mediated immunity. Iron mainly in its non-proliferative form, low molecular weight form, cause cellular damage by participating in the generation of the hydroxyl radical, thought to be the principal effector of oxidative DNA damage. One possibility is that telomerase activity, essential enzyme for the repair of telomeric DNA, is reduced following damage by oxygen radicals. Aims. The aim of the present study was to investigate telomerase activity in lymphocytes from patients with iron overload disease and to observe its regulation of cellular proliferation and also evaluate effect of Silymarin on this enzyme. Methods. Peripheral blood mononuclear cells (PBMC) were isolated from 20 patients with β-thalassemia major and 20 healthy donors. Cells were stimulated with PHA and treated with Deferoxamine and Silymarin for 72 h. Telomerase activity was measured by the telomeric repeat amplification protocol (TRAP) assay and telomerase activity was measured with TRAP assay. In addition, DNA synthesis of the cells was assayed using BrdU (bromo-2'-deoxyuridine) incorporation. Results. The results showed that telomerase activity of resting peripheral lymphocytes of healthy subjects and patients with β-thalassemia major was detectable at low level, and obviously increased after stimulation in vitro with phytohaemagglutinin (1 μg/ml) and Deferoxamine, and with treatment in vitro with Deferoxamine (DFO). The decreased telomerase activity of resting lymphocytes was found in patients with β-thalassemia major compared to that in healthy subjects. The DNA proliferation was paralleled by increase in telomerase activity. Conclusions. These results lead to important conclusions. First, the ability of T cells to upregulate telomerase activity upon activation may decrease over time (aging) and following iron overload-mediated oxidative stress. Second, Silymarin upregulates telomerase activity of T-Lymphocytes and Deferoxamine downregulates. Third There is a direct correlation between telomerase

Significance with side effects, acceptability of ICT, age, perceived effective-
activity and cell proliferation. One possibility is that telomerase is essential for the repair of telomeric DNA following damage by oxygen radicals. Finally, because telomerase contributes to protection from telomere shortening in activated lymphocytes, it may play a critical role in immune responses and also Silymarin as a Superantioxidant may strengthen immune function through scavenging free radicals and upregulation of telomerase. However, the significance of this pathway is not yet clear.

HEPCIDIN MUTATION IN A BETA-THALASSEMIA MAJOR PATIENT WITH PERSISTENT SEVERE IRON OVERLOAD DESPITE CHELATION THERAPY

L. Duca, P. Delbini, I. Nava, A. Mee, M. La Rosa, L. Zanghì, M.D. Cappellini

University of Milan-Policlinico Hospital, MILAN, Italy; University of Messina-C. Marzio Hospital, MESSINA, Italy

Background. Hepcidin is a peptide hormone produced in the liver; it is an important negative regulator of iron absorption from the enterocytes and of iron release from macrophages. Hepcidin dysregulation is implicated in the pathogenesis of several iron disorders. Iron overload and inflammation up-regulate hepcidin synthesis decreasing dietary iron absorption, while anaemia and hypoxia suppress hepcidin expression. Thalassaemia Major (TM) is a hereditary haemolytic anaemia requiring long-life blood transfusions treatment. Iron storage in patients undergoing regular transfusions is responsible for the impaired renal function of TM patients, with high and hence reduced survival. Iron chelation therapy is required to reduce the morbidity and mortality associated with iron overload secondary to chronic transfusion therapy. Aim. Despite regular iron chelation some thalassaemia patients have persistent high ferritin levels. To get further insights in this issue, several factors have been investigated including infections, inflammatory status and coexistence of HFE and H63D mutation. No other mutations were detected in TFR2 gene, ferritin production of TM patients was measured with immunonephelometry using the Dade-Behring BN Prospec nephelometer. Results. The echocardiography showed a slight cardiac left ventricular hypertrophy and ejection fraction of 66%. Hepatomegaly and splenomegaly were found at echography. The patient had hypogonadism and hypothyroidism. Serum ferritin was 4000 ng/mL, transferrin saturation over 110% and NTBI 2,83 microM. Serum pro-hepcidin value was in normal range (167 ng/mL). Because of severe iron overload, hepcidin and other iron related genes including HFE were analysed. Hcpcidin gene was sequenced and a heterozygous 72C>T mutation previously described by Biasotto et al. (2004) was identified in the promoter region. HFE analysis revealed a homozygous genotype for H63D mutation. No other mutations were detected in TR2 gene, ferroportin and HH type 2 gene. Conclusion. The 72C>T mutation in hepcidin promoter has been previously reported in subjects with increased iron parameters and is described to aggravate the clinical phenotype and the biochemical indices of iron overload. The coexistence of the b-thalassaemia trait with hepcidin mutation and H6SD homozygosity could contribute to the development of marked iron overload poorly responsive to chelation therapy.

Anemia/Red blood cells

0012

EARLY MARKERS OF RENAL DYSFUNCTION IN PATIENTS WITH SICKLE CELL/ BETA-TALASSEMIA

E. Voksiarioud,1 E. Terpos,1 S. Michail,1 E. Hantzi,2 A. Anagnostopoulos,3 A. Margeli,1 D. Simirgoglou,1 D. Loukopoulos,1 I. Papassotiriou4

1Thalassemia Center, Laikon Gen. Hospital, ATHENS, Greece; 251 General Airforce Hospital, ATHENS, Greece; 1Laikon General Hospital, ATHENS, Greece; 3Agia Sophia Childrens Hospital, ATHENS, Greece; University of Athens Medical School, ATHENS, Greece, 4Academy of Athens, ATHENS, Greece

Background. Progressive renal failure is one of the main complications in sickle cell/b-thalassemia (HbS/b-thal). Detection of the progressive renal damage using conventional parameters, such as serum creatinine levels (Cr) or clearance of creatinine (Ccr) is often misleading. The early development of glomerular hypertrophy enhances creatinine excretion and gives false normal results of both Cr and Ccr. Therefore, the renal dysfunction becomes evident rather late. For that reason, the identification of markers that indicate early renal dysfunction as well as further progression to end-stage renal disease is highly desirable. Cystatin C (Cys-C) is a cysteine protease inhibitor, which serves as an endogenous parameter of GFR, while b2-microglobulin (b2-M) is a sensitive marker of the glomerular filtration capacity of the kidney. Finally, N-acetyl-β-D-glucosaminidase (NAG), a widely distributed lysosomal enzyme found predominantly within the renal proximal tubule is also a sensitive indicator of renal injury. Aim. The aim of this study was to evaluate whether Cys-C, b2-M and NAG excretion may serve as early indicators of renal dysfunction in a large cohort of Hbs/b-thal patients. To our knowledge, such studies are not available in the literature. Patients and Methods. We studied 87 compound Hbs/b-thal patients (64M/51F; median age 39 years) and 80 healthy controls. All patients were Caucaussians, of Greek origin, had stable disease at the time of evaluation, without sickle-cell crises or infections, and had not been transfused for at least three months before. Serum Cys-C and b2-M were determined by particle enhance immunonephelometry using the Dade-Behring BN Prospect nephelometer (Dade Behring, Liederbach, Germany). Urine NAG activity was measured photometrically at 580 nm using a colorimetric assay (Roche Diagnostics, Mannheim, Germany) and expressed as daily output in U/day. Results. Cys-C, NAG and serum b2-M levels were higher in patients than controls (p<0.01, p<0.0001, and p<0.0001, respectively). The incidence of patients with high levels of Cys-C, NAG and b2-M was 32.1%, 74.7% and 70.1% respectively, while only 6.8% of patients had increased serum creatinine levels. Cys-C and serum b2-M showed a strong correlation with Ccr (r=0.48, p<0.0001; and r=0.38, p<0.001, respectively), while NAG positively correlated with proteinuria (r=0.546, p<0.0001). An inverse strong correlation was also shown between hemoglobin and b2-M (r=-0.53, p=0.004). NAG and Cys-C levels (r=0.515, p=0.002). Seven patients with proteinuria received therapy with ACE-inhibitors. Changes of proteinuria positively correlated with NAG levels (r=0.691, p<0.001). Conclusions. These results indicate that Cys-C is an accurate marker of renal dysfunction, and urinary NAG excretion can be considered as a reliable index of the tubular toxicity, and possible predictor of proteinuria and eventual renal impairment in Hbs/b-thal patients. Furthermore, NAG measurement may be used for monitoring ACE-inhibitors therapy in Hbs/b-thal patients with proteinuria.
males. Methods: In this randomized, single blind, and placebo (PBO)-controlled study, 44 patients were enrolled in 5 dose cohorts. In Stage 1, 35 subjects received a single IV administration of 0.03, 0.09, 0.3, 0.9 mg/kg CNTO 528 or PBO. In Stage 2, 9 subjects received fractionated IV administrations of CNTO 528 or PBO on Days 1, 3 and 5 (3 infusions of 0.09 mg/kg or PBO). Results. Pharmacodynamics: In subjects treated with IV CNTO 528, a dose dependent increase in reticulocyte counts was observed from baseline on POD 6 and occurred back to baseline between days 22 through 29. Hemoglobin (Hgb) concentration increased in a dose dependent manner with a maximum effect occurring at day 22. Mean Hgb concentration remained 0.4 g/dL above baseline values at the last measurement, approximately 2.5 months after a single dose administration. A dose dependent increase in RBC count was observed with all RBC indices (MCV, MCH, MCHC) within normal range, indicating an increase in normocytic, normochromic RBCs. In all CNTO 528 treated subjects, a dose-dependent increase in soluble transferrin receptor concentration was observed. A dose-dependent increase in endogenous EPO concentration was observed, followed by a dose dependent decrease in endogenous EPO concentration. Pharmacokinetics: In the single dose part of the study, Cmax and AUC increased in an approximately dose proportional manner. The mean terminal half-life ranged between 6-7 days in the higher dose cohorts. Safety: Treatment with CNTO 528 was generally well tolerated. There were no serious adverse events (AEs) and few CNTO 528-related AEs. Two subjects in the 0.9 mg/kg cohort met the protocol pre-specified indication rule of Hgb > 17.5 g/dL and underwent phlebotomy. In these subjects, high Hgb concentrations were not associated with AEs or clinical symptoms. All AEs were determined by the investigator to be mild to moderate in intensity. The most common AE across all groups was headache, occurring in both CNTO 528- and PBO-treated subjects. There was no dose-related trend across groups, and most subjects who experienced headaches were in the lowest 2 dose groups. There was no indication that any patterns of AEs or significant safety laboratory, vital signs, or ECG abnormalities were associated with the administration of CNTO 528. Immunogenicity: None of the 24 subjects who received single IV administration of CNTO 528 were positive for antibodies to CNTO 528. Conclusions. Single and fractionated IV administrations of CNTO 528 were well tolerated and resulted in prolonged, dose-dependent erythropoietic responses with notably low inter-subject variability. PK of IV CNTO 528 was linear and approximately dose proportional. This data provides the first proof of concept in humans for erythropoietic responses and an increase of endogenous EPO levels by an erythropoietic mimetic antibody fusion protein.

0014
CORRECTION OF ANEMIA OF THE POST-OPERATIVE PERIOD AFTER ORTHOPEDIC SURGERY BY ORAL VERSUS INTRAVENOUS IRON VERSUS INTRAVENOUS IRON + EPO: A PROSPECTIVE RANDOMIZED TRIAL

P. Berri, F. Verholen, M. Sadowski, M. Noger, P. Hoffmeyer
Geneva University Hospitals, GENEVA, Switzerland

Background. Approximately 20% of patients after orthopedic surgery (hip or knee replacement) present moderate to severe anemia (Hb between 75 and 105 g/L). They are often treated by oral iron for up to three months. Aims. We performed a prospective randomized pilot study to investigate the potential of iv iron or iv iron + EPO to treat this kind of anemia as compared to oral iron therapy. Methods. Of the 57 patients included in the study, 47 completed the trial and received either 80 mg of oral iron/day (Tardyferon®) for three months (19 patients), or 200 mg iv iron sucrose (Venoferr®) at post-operative-days (POD) 1, 3, 5 and 8 (18 patients), or 200 mg iv iron sucrose (Venoferr®) on the same PODs plus 150 IU/kg of EPO (epoetin alpha, EPREX®) on PODs 1, 3 and 5 (18 patients). Results. The hemoglobin values in the three groups were similar for all these parameters. At baseline the three groups were similar for all these parameters. In particular, Hb values were 141.3, 135.0, and 134.1 g/L, respectively. Nadir post-operative mean Hb values were 98 (POD 10), 91 (POD 8), and 94.5 (POD 8) g/L for the three groups respectively. At POD 20, Hb (compared to nadir) was 12.5 g/L in the oral iron group, 15.5 g/L in the iv iron group, and 25.5 g/L in the iv iron + EPO group (p=0.016 group 3 versus groups 1 & 2). At POD 30, Hb was 27.5, 29, and 32.5 g/L respectively, and 45.3, 48, and 42.5 g/L at POD 90. At day +30, 37%, 46%, and 60% of patients had normalized their Hb value (p=0.0266 group 3 versus groups 1 & 2). The venous iron + EPO group's iron stores were 51, 48, and 51 g/L, respectively. All patients developed an acute inflammatory state with CRP mean value at POD 1, 8, and 10 of 117, 56, and 34 mg/L respectively. Finally, ferritin levels at POD 90 were -29, +75, and +84 ug/L respectively. Conclusions. This pilot study clearly shows that in moderate to severe post orthopedic surgery anemia, the highest and most rapid increase in Hb was seen in the group of patients treated by iv iron + EPO. This difference is, in our opinion, due to the acute inflammatory state which developed secondary to surgery and lasted for almost 15 days. The study also shows that therapy with iv iron is well tolerated and, unlike oral iron therapy, allows a complete restoration of iron stores. The impact of this accelerated Hb recovery on quality of life, hospital stay duration and incidence of post-operative complications should be studied in a future trial with a larger patient population.
M.D. Cappellini, C. Vermyle, L. Gathmann, J.M. Ford

Background. In chronically transfused patients it is important to understand how much iron is removed by chelation therapy for a given rate of iron intake, to allow tailoring of treatment regimens to achieve the desired iron balance, ie maintenance in a well-controlled patient or reduction in an iron-overloaded patient. Deferasirox (Exjade®; ICL670) is a novel, once-daily oral iron chelator that was recently approved for the treatment of chronic transfusional iron overload in adult and paediatric patients aged ≥2 years. The efficacy and safety of deferasirox have been established in patients with a range of transfusion-dependent anemias. Importantly, the efficacy of deferasirox is similar across a wide dose range (~28% over 5-30 mg/kg/day), indicating that the higher the dose, the more iron will be removed from the body. Aims. The aim of this post-hoc analysis, which pooled data from four pivotal deferasirox clinical trials, was to evaluate deferasirox in relation to change in net body iron (excretion) and the impact of transfusional requirements (intake) and to determine the iron excretion:intake ratio. Methods. A total of 1,005 patients (deferasirox n=652, deferoxamine [DFO, Desferal®] n=353) were stratified according to their transfusional requirements while on study, measured in mL/kg/month of packed red blood cells: <7 (low), 7-14 (intermediate) or >14 (high). In practical terms, 7 and 14 mL/kg/month correspond to approximately 2 and 4 adult units of blood, respectively. In each treatment arm, iron balance was calculated (g/year) and the doses necessary to achieve the desired iron balance were assessed. Results. Among the pooled population of deferasirox-treated patients, most (n=419, 64.5%) had intermediate transfusional requirements. When evaluating mean net iron balance and iron excretion:intake ratio in completing patients with baseline and end-of-study liver iron concentration (LIC) assessments and recorded positive iron intake (approximately 90% of overall population), a transfusion- and dose-related response pattern was observed with both deferasirox (n=556) and DFO (n=525). Mean net iron balance results for 10, 20 and 30 mg/kg/day doses are presented in Figure 1.

Figure 1. Mean net iron balance (g/year) by treatment, dose and transfusional requirements.

The mean iron excretion:intake ratio was less than 1 (intake exceeded excretion) in all patients receiving deferasirox 5 mg/kg/day, irrespective of transfusional requirements (0.52, 0.67 and 0.34 in the low, intermediate and high cohorts, respectively). Conclusions. Based on this analysis, deferasirox 10 mg/kg/day maintained iron balance in patients with low transfusional requirements. 20 mg/kg/day maintained or reduced iron balance in patients with low and intermediate requirements, while 30 mg/kg/day decreased iron balance in most patients, irrespective of transfusional requirements. Since deferasirox efficiency does not vary across doses, it is now known that 5 mg/kg/day is insufficient to maintain or reduce iron balance relative to patients' transfusional requirements. Comparable effects were observed between DFO and deferasirox doses in a 2:1 ratio, indicating that an effective deferasirox dose will be around half that of an effective DFO dose. Deferasirox dosing should therefore be guided by transfusional requirements, severity of iron overload and treatment goal. In addition, as regular transfusions lead to iron accumulation, it is important to monitor transfusion rates, serum ferritin levels and/or LIC.
0018

PATIENT CONTROLLED ANALGESIA VERSUS CONTINUOUS INFUSION OF MORPHINE DURING VASO-OCCUSSIVE CRISIS IN SICKLE CELL DISEASE: A RANDOMIZED CONTROLLED TRIAL

Academic Medical Centre, AMSTERDAM, Netherlands

Background. Pain during vaso-occlusive crisis (VOC) in sickle cell disease (SCD) is commonly treated with continuous intravenous infusion (CI) of morphine. During CI the treating physician titrates the dose of morphine until adequate relief of pain has been established. Patient controlled analgesia (PCA) allows the patient to self-administer doses of morphine for the relief of pain and has shown to be equianalgesic in post surgical patients with lower morphine consumption than with the CI of morphine. Morphine has many dose-related side-effects and high plasma levels of morphine are associated with serious complications. Aim: To compare the administration of morphine with PCA versus CI in sickle cell patients with VOC we conducted the first randomized controlled trial in this setting. Methods. Patients were randomized between PCA and CI of morphine within 24 hours after hospital admission. Endpoints of the study were: the mean and cumulative morphine dose, pain intensity and quality of life (Gol). Pain intensity was measured daily using a ten-point-scale verbal pain score. Reduction of pain intensity was measured by subtracting a pain score on a ten point visual analogue scale (VAS) from randomization from the same measurement two days after randomization. Gol was measured using the Medical Outcomes Study 36-Item Short Form Healthy Survey (SF36). Results. Twenty-five consecutive episodes of VOC in 19 patients with SCD were included. Patients with PCA demonstrated to have significantly lower morphine consumption as compared to patients randomized to CI. The mean and total cumulative morphine dose was 0.5 mg/h and 33 mg in the PCA-group versus 2.1 mg/h and 275 mg in the CI-group, respectively (p<0.001 and p<0.001). In addition, a non-significant reduction in median duration of hospitalisation was found (6 versus 10). Despite the markedly reduced cumulative dose of morphine in the patients treated with PCA, no difference in pain intensity was found between the groups. The mean daily ten-point-scale verbal pain score was 4.9 in the PCA group versus 5.3, in the CI-group (NS). Also no difference in Gol was found. Conclusion. We conclude that the use of PCA in sickle cell patients with VOC results in adequate pain relief at a significant lower morphine dose as compared to morphine administration by continuous infusion.

Figure 1. Mean Morphine dosage per patient.

0019

EX VIVO ANALYSIS OF PKLR MUTATIONS THAT AFFECT CORRECT PROCESSING OF PKLR MRNA CAUSING PYRUVATE KINASE DEFICIENCY

R. van Wijk, A. van Wesel, G. Rijksen, W.W. van Solinge
University Medical Center Utrecht, UTRECHT, Netherlands

Background. Red blood cell pyruvate kinase (PK) deficiency is the most common cause of nonspherocytic hemolytic anemia due to defective glycolysis. The clinical picture varies from severe hemolysis causing neonatal death to a well compensated hemolytic anemia. PK deficiency is inherited in an autosomal recessive manner and caused by mutations in the PKLR gene. Most of these are missense mutations affecting conserved residues in structurally and functionally important domains of the protein. More rarely, PK deficiency is caused by mutations that lead to aberrant processing of PKLR pre-mRNA. Aims. We aimed to study the effect of mutations associated with PK-deficiency and postulated to affect PKLR pre-mRNA processing. Methods. Pro-erythroblasts were cultured ex vivo from patient-derived CD34+ cells and used as a source of erythroid-specific RNA. We used RT-PCR with fluorescent-dye-labeled primers, fragment analysis, cloning, and DNA sequence analysis of the clones to identify and characterize PKLR transcripts. Results. Five different mutations were studied. Two were located at the 5’ splice site of exon 3 (c.283G>A) and intron 11 (c.1618+1delC). Two mutations were located at the 5’ splice site of intron 4 (IVS4-2A>C) and intron 11 (IVS11-3C>G). The fifth mutation was located in exon 8 (c.990C>T). The missense mutation c.283G>A in exon 3 encodes a substitution of glycine by arginine at residue 95. More importantly, this mutation altered the 5’ splice site of IVS3. As a result most transcripts did not contain exon 3, coding for a PK monomer that, if translated, lacks amino acids 34 to 94. Similarly, the one-bp deletion at the exon/intron boundary of IVS11, c.1618+1delG, altered the 5’ splice site of IVS11. This caused skipping of exon 11 in the majority of transcripts, encoding a shortened PK monomer due to a premature end of translation at residue 154. At the 5’ splice site, the two main effects of the novel IVS4-2A>C base change were retention of IVS4 and the simultaneous skipping of both exons 5 and 6. Retention of IVS4 predicts the in-frame insertion of 32 additional amino acids between residues 125 and 126. Skipping of both exons 5 and 6 renders a transcript with a premature stop codon in exon 7. The main effect of the IVS11-3C>G mutation was a strongly reduced amount of transcripts. The remaining transcripts were processed normally or at an alternative donor site 5 nt upstream in exon 12. The novel c.990C>T base substitution in exon 8 does not change the codon for serine at residue 300. Interestingly, however, this mutation was associated with an increased amount of transcripts processed at an alternative donor site at nt 965. Consequently, this in-frame deletion would remove residues 329 to 372 from the PK monomer. Conclusions. The results of our studies provide insight into the molecular mechanisms by which the herein described mutations lead to PK deficiency. It shows in particular that any type of mutation may affect pre-mRNA processing. This will contribute to the better understanding of the pathophysiology of PK deficiency and, in general, the complex regulation of pre-mRNA processing.

0020

ASSESSMENT OF COGNITIVE EFFECTS OF ONCE-WEEKLY EPOETIN ALFA IN ANEMIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING CHEMOTHERAPY: RESULTS OF THE EPOLYM TRIAL

H. Tesch,1 A.M. Liberati,1 N. Ifrah,1 Epolym Investigators
1Onkologische Gemeinschaftspraxis, FRANKFURT, Germany; 2Clinica Medica Generale, PERUGIA, Italy; 3CHU d’Angers, ANGERS, France; 4Universit y Hospitals and Clinics, EUROPE, Switzerland

Background. Increasing evidence suggests that chemotherapy can produce cognitive dysfunction in cancer patients, and while the cognitive deficits tend to be subtle, they can have a negative impact on the patients’ social, educational, and professional activities, and overall quality of life (QOL). Clinical evidence suggests an association between chemotherapy-related decreases in hemoglobin (Hb) level and an increased risk of cognitive dysfunction. Aims. To assess changes in cognitive function in patients undergoing chemotherapy and receiving once-weekly (QW) epoetin alfa to maintain Hb levels and prevent subsequent fatigue, symptoms of anemia, and deficits in QOL. Methods. EPOLYM was a 24-week, prospective, international, multicenter, open-label, Phase IIIb trial in anemic (Hb ≤12.0 g/dL) patients receiving chemotherapy (N=1034) for Hodgkin’s disease (HD), non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). Epo-
etin alfa therapy was initiated at a dose of 40,000 IU SQ administered subcutaneously, with dosage adjustments to be made based on clinical response (target Hb, 11.5-13.0 g/dL). Cognitive function was evaluated at baseline, Weeks 1, 6, 12 and at 24 weeks or study completion. Summary statistics were calculated for each measure at each assessment. The Cognitive Drug Research (CDR) Computerised Cognitive Assessment System (CCAS) battery of tests (tasks) performed by subjects, was used to assess changes in parameters of cognitive function, including tasks of attention (Simple Reaction Time, Choice Reaction Time, Digit Vigilance), working memory (Numerical Working Memory), and secondary memory (Immediate and Delayed Word Recognition, Picture Recognition). Because of the relationship between affect and cognition, depression and anxiety were assessed at the time of cognitive assessment using the Hospital Anxiety and Depression Scale (HADS). Changes from baseline in Hb levels, transfusion requirements, and QOL measures were also evaluated (transfusion and QOL results not reported here). Results. Analyses were performed on the cognitive data (904/1054 patients) and HADS scores (978/1054 patients). Performance on attention tasks was slightly impaired over the duration of the study, reaching significant decrease from baseline at weeks 12 and 24 (p<0.05). Continuity of attention, the ability to sustain attention and avoid error, had a pattern of improvement from baseline over time with a significant improvement at week 12 (p=0.027). Speed of memory improved from baseline, achieving significance (p<0.005) at each evaluation point. HADS scores were near the high normal range at baseline and improved slightly from baseline during the study, reaching significance (p<0.001) from week 6 onward. The baseline up to week 24 significant improvement in HADS scores was associated with increase in Hb level, with those experiencing an Hb increase >1 g/dL had the most improved HADS score. Similarly, the indications of clinical improvement in cognitive function were related to a significant (p<0.0001) increase in Hb from 10.4±1.3 g/dL at baseline to 12.0±1.7 g/dL at 24 weeks. Conclusion. Overall, the assessment of data indicated a positive change in cognitive function parameters and HADS scores over the 24 week study. These improvements were associated with an increase in Hb level achieved with QW epoetin alfa.

0022
DEFERASIROX (EXJADE, ICL670), THE NOVEL, ONCE-DAILY ORAL IRON CHELATOR, IS WELL TOLERATED AND EFFECTIVE IN TREATING TRANSFUSIONAL IRON OVERLOAD IN PATIENTS WITH A RANGE OF RARE ANAEMIAS
C. Rose,1 N. Gattermann,2 E. Glimm,3 B. Rabault4
1Hôpital Saint Vincent de Paul, LILLE, France; 2Heinrich-Heine-University, DÜSSELDORF, Germany; 3Novartis Pharma AG, BASEL, Switzerland

Background. Deferasirox (Exjade®, ICL670), the novel, once-daily oral iron chelator, is currently approved for use in eight countries for the treatment of transfusional iron overload in patients aged 22 years. Deferasirox has been shown to be effective and well tolerated in patients with various transfusion-dependent anaemias, including β-thalassaemia. There are, however, a number of rare anaemias that may also require transfusion therapy, meaning that patients are at risk for iron overload. To date, little has been published regarding iron overload and chelation therapy in these rare anaemias. Aims. To evaluate the severity of iron overload, as well as the efficacy and safety/tolerability of deferasirox in transfusion-dependent patients with a range of rare anaemias (a subpopulation of a Phase II study). Methods. The overall study was an open-label, multicentre, 1-year trial that enrolled 22 patients with a range of rare anaemias, including: aplastic anaemia (n=5), α-thalassaemia (n=3), sideroblastic anaemia (n=3), myelofibrosis (n=2), pure red cell aplasia (n=2), pyruvate kinase deficiency (n=2), autoimmune haemolytic anaemia (n=1), Fanconi’s anaemia (n=1), hereditary sideroblastic anaemia (n=1), erythropenia (n=1), and unspecified anaemia (n=1). Patients were assigned deferasirox doses according to baseline liver iron concentration (LIC). Results. Enrolled patients (median age 52 years; range 4-83) received deferasirox 50 mg/kg (15 patients), 20 or 30 mg/kg (4 patients), and 10 mg/kg (3 patients). The median duration of exposure to deferasirox was 52.1 weeks (15.7-66.9). The median number of transfusions during study was 13.5 (25-75% percentiles; 6.0-18.0), while the median blood transfused was 0.31 mL red blood cells/kg/day (25-75% percentiles; 0.12-0.43). Mean baseline LIC in this sub-population was high (15.1 mg Fe/g dw; SD±6.2), but decreased by 5.7 mg Fe/g dw (SD±6.6) after 1 year of deferasirox treatment. Mean serum ferritin level at baseline was 3144 µg/L (SD±1850), and fell by 750 µg/L (SD±1517) during study. The mean rate of iron excretion (0.41±0.19 mg/kg/day) exceeded iron intake (0.31±0.19 mg/kg/day). Seventeen patients (77.3%) completed the study; three subjects discontinued as they no longer required study drug and two withdrew due to adverse events (AEs). There were no deaths in this patient subgroup. All 22 patients reported at least one AE, the majority of which were transient and mild to moderate in severity. The most common drug-related AEs were mild, transient gastrointestinal disturbances such as diarrhoea (n=8, 36.4%), nausea, vomiting (n=4, 18.2% for each) and abdominal pain (n=2, 9.2%). Mild, non-progressive serum creatinine increases >33% of baseline were observed in 12 patients receiving deferasirox 20 and 30 mg/kg/day (within the normal range in seven patients, >ULN in five). There were no incidences of drug-induced neutropenia or arthralgia. Conclusions. In these patients with diverse rare anaemias, the LIC was severe and above the published clinically acceptable thresholds. This suggests that these patients are at increased risk for developing co-morbidities with a resultant negative impact on survival. Once-daily, oral deferasirox was effective and generally well tolerated, resulting in a clinically relevant reduction in overall body iron burden.

0023
GLYCOCALYX PERTURBATION IN PATIENTS WITH SICKLE CELL DISEASE: IMPLICATIONS FOR VASCULAR VULNERABILITY
E.J. van Beers, M. Nieuwdorp, H. Vink, L.M. Evers, B.J. Biemond
Academic Medical Centre, AMSTERDAM, Netherlands

Background. Activated endothelium plays a pivotal role in the pathogenesis of sickle cell disease (SCD). The activation of the endothelium is caused by hypoxia, repuffusion damage, high shear rates and pro-inflammatory mediators, like thrombin and TNF-α. The central role of the glyocalyx (a layer of hyaluronan and proteoglycans covering the endothelium) has been established as an antiadhesive and anti-inflammatory barrier. Recently, we have validated a technique to determine the glyocalyx thickness in vivo. In this study, we evaluated the impact of activated endothelium on the glyocalyx in humans and demonstrated that the glyocalyx volume is strongly diminished in diabetic patients with microangiopathy. Aim. Sickle cell patients are known to have a strongly activated endothelium and may also develop microangiopathy. Therefore, we assessed the glyocalyx volume in patients with SCD in comparison with carriers of SCD. Methods. The glyocalyx...
cocalyx was measured in 20 patients with SCD (HbSS, HbSC and HbSB) and 10 sex- and age-matched carriers of SCD. We determined the total systemic glycocalyx volume by comparing the intravascular distribution volume of a glycocalyx permeable tracer (dextran 40) to that of a glycocalyx impermeable tracer (autologous labelled erythrocytes).

**Results.** Patients with SCD demonstrated to have a significant reduced glycocalyx volume of 0.48±0.14 litres as compared to 1.26±0.27 litres in the carriers of sickle cell disease (*p*=0.009, expressed as mean±SEM). However, no correlation between glycocalyx volume and microangiopathy, disease severity or genotype was found. **Conclusions.** The strongly diminished glycocalyx volume in sickle cell patients resembles the chronic state of activation of the endothelium in SCD that may also may be responsible for the enhanced adhesion of leukocytes and erythrocytes as well as the prothrombotic state of these patients. Since the glycocalyx layer serves as an important barrier between the endothelium and the circulating blood cells to prevent the adhesion of leukocytes, therapies that may restore or preserve glycocalyx function are warranted in SCD.

![Figure 1. Glycocalyx volumes in litre (mean and SEM).](image)

**Table 1. BMD values of treatment and control group.**

<table>
<thead>
<tr>
<th>BMD</th>
<th>Treatment Mean±SD (z-score)</th>
<th>p-value</th>
<th>Control Mean±SD (z-score)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar Spine</td>
<td>2.84±0.57 &lt;0.01</td>
<td>2.77±0.78 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>2.39±0.55 0.04</td>
<td>2.74±0.55 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>2.20±0.76 0.04</td>
<td>2.92±0.76 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>-1.37±0.74 0.04</td>
<td>-1.75±0.35 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>-1.29±0.66 0.04</td>
<td>-1.76±0.21 0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>-1.18±0.65 0.04</td>
<td>-1.86±0.28 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Hip</td>
<td>-1.62±0.59 &lt;0.01</td>
<td>-1.60±0.14 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>-1.40±0.68 0.04</td>
<td>-1.56±0.57 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>-1.34±0.68 0.04</td>
<td>-1.70±0.35 0.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results.** Both groups had no significant difference with respect to age, gender and baseline BMD. Patients taking zoledronic acid had a significant increase in their lumbar spine, femoral neck, and total hip BMD.
 measurements over the 12-month period. Patients in the control group did not have any significant change in BMD measurements. Table 1 shows the BMD values of the treatment and control groups.

There was a significant change in the levels of OC and BAP over the 12-month follow-up. There was a significant decrease in the number of painful sites experienced by the patients over the whole treatment period (p=0.01). Pain and analgesic scores significantly decreased over the whole treatment period (p=0.00 and 0.01 respectively). Pain interference with general activity and ECOG score, on the other hand, did not show any significant change. Reported adverse events included joint pain in 9 patients (50%) after the 1st dose and in 2 (11.1%) after the 2nd dose and responding very well to oral analgesics. Two patients (11.1%) had per- oral numbness and 3 (16.7%) had low grade fever after the 1st dose. No treatment-related adverse events were reported after the 3rd and 4th doses. None of the patients experienced elevated serum creatinine lev- els and none discontinued the study. Conclusions. Treatment of tha- lassemic osteoporotic patients with zoledronic acid, administered at a dose of 4 mg intravenously every 4 months over a period of 12 months, is safe and very effective in increasing BMD at the lumbar spine and hip and in reducing pain and is well-tolerated.

0026

THE DISTINCTION BETWEEN HAEMOLYSIS DUE TO HEREDITARY SPHEROCYTOSIS AND THAT DUE TO A CATION PERMEABILITY DISORDER OF THE RED CELL MEMBRANE IN A REGULAR HAEMATOLOGY LABORATORY

City Hospital Triemli, ZURICH, Switzerland; University Hospital, ZURICH, Switzerland; University College London, LONDON, United Kingdom; University Laboratory of Physiology, OXFORD, United Kingdom; Institute of Biochemistry, ZURICH, Switzerland

Background. An increased fraction of hyperchromic RBCs, reticulocy- tosis, splenomegaly and reduced osmotic resistance of RBCs leads in routine haematology to the diagnosis of spherocytosis (HS). If this condition shows cation permeability disorders (CPD), as found in various forms of disorders with regard to clinical severity, gene defects and mode of inheri- tance. Splenectomy is not indicated in cases of haemolysis that show cation permeability disorders (CPD), as found in various forms of hereditary stomatocytosis (Stewart et al., Br. J. Haematol 93, 1996, 403- 410). We have previously reported a patient, initially diagnosed as hav- ing HS, with persistent post-splenectomy haemolysis, in whom further analysis confirmed cryohydrocytosis, a rare disorder with abnormal permeability of the red cell membrane to sodium and potassium (Hematol J., 5, Suppl. 2, 2004, Abstract 250). Based on a detailed study of this patient we designed a simple test to recognize time and temperature- dependent CPD. All blood samples with a significant as determined elevated percentage of hyperchromic RBC were stored on ice for two hours and re- analysed by routine counting in order to detect swelling due to a CPD of the red cell membrane. Methods. Routine haematology testing, includ- ing reticulocyte counts and erythrocyte histogram with percentage- assessment of hypochromic, hyperchromic, microcytic and macrocytic RBC were evaluated using a flow cytometer (Bayer Advia 120). To assess CPD of the red cell membrane we routinely measured mean cellular vol- ume (MCV), mean cellular haemoglobin concentration (MCHC) and percentage of hyperchromic erythrocytes in whole heparinized blood stored for two hours on ice. Results. Approximately one year after the beginning of our study at the University Hospital Zurich, we found a fur- ther patient with a mild haemolytic condition who had been splenec- tomised at another clinic due to the estimated diagnosis of HS. The find- ings were: Haemoglobin 15.4g/dL (normal range 13.5-17.2), reticulo- cytes 52% (6-17), MCV 95 fl (80-100), MCHC 39.2 g/dL (31-36), hyper- chromic erythrocytes 32.1% (0-1.5), microcytic erythrocytes 1.8% (0- 1.5). Analysis after storage on ice for two hours showed the following values: Haemoglobin 15.6g/dL (normal range 15.5-17.2), MCV 105.5fl (80-100), MCHC 34.7g/dL (31-36), hyperchromic erythrocytes 0.1% (0- 1.5), microcytic erythrocytes 10.9% (0-1.5). Further studies on the patient’s RBC revealed that after 2h incubation at 0°C there was a 5.22-fold increase in plasma [K]. These data strongly suggested the diagno- sis of cryohydrocytosis. The isotopic flow studies at 37°C confirmed an increased ouabain-humetanide-resistant influx of potassium compared to control RBC. The temperature dependence of this leak showed a U- shaped profile with a minimum at about 23°C and a maximum at 0°C. Conclusion. Cryohydrocytosis seems to be an underdiagnosed disorder mimicking in daily practice typical hereditary spherocytosis with mild haemolysis. Hence, in cases with suspected HS the regular blood tests should be repeated routinely after a cold storage of whole blood, to exclude or eventually verify cryohydrocytosis.

0027

RITUXIMAB AND FLUDARABINE COMBINATION THERAPY FOR CHRONIC COLD AGglUTININ DISEASE

S. Berentsen, G.E. Tjønnfjord
Haugesund Hospital, HAUGESUND, Norway; Rikshospitalet University Hospital, OSLO, Norway

Background. Chronic cold agglutinin disease (CAD) is an autoimmune haemolytic anaemia characterized by the production of monoclonal antibodies, most often IgM kappa, against erythrocyte sur- face antigens. A clonal lymphoproliferative bone marrow disorder can be demonstrated in most cases. Rituximab single agent therapy has been shown in prospective studies to induce remission in more than 50% of patients. Aim. We wanted to improve on response rates achieved by ther- apy directed against the underlying B cell proliferation. Methods. In a prospective phase II trial, eligible CAD patients received rituximab 375 mg/sqm intravenously d 1, 29, 57 and 85, and fludarabine tablets 40 mg/sqm d 1-5, 29-33, 57-61 and 85-89. Clinical, haematological, immunological and histological data were recorded, and responses were classified according to previously published criteria as complete (CR), partial (PR), or no (NR) response. Results. By Feb 2006 we had treated six patients with a median age of 70. All patients had monoclonal IgM kappa and considerable or severe cold-induced circulatory symptoms. All had been previously treated with rituximab single agent therapy, result- ing in one CR, one PR and four NR. After the combination therapy, cir- culatory symptoms resolved completely in four patients and improved in one additional patient. Haemoglobin levels increased by > 3 g/dL in two of four anemic patients. Overall, two patients achieved CR, two achieved PR and two were non-responders. Haematological toxicity was recorded in three patients (grade 2, 3 and 4, respectively), infection grade 2 in one and nausea in one. Conclusions. Rituximab and fludarabine combination therapy is feasible even in elderly patients with CAD. Response rates are promising, but superiority over rituximab single agent therapy remains to be proven until more patients have been treated.

Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Indication for therapy</th>
<th>Bone marrow histology</th>
<th>Hb (g/dL)</th>
<th>Increase in Hb (g/dL)</th>
<th>Change in circulatory symptoms</th>
<th>Overall response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>F</td>
<td>HA,CS</td>
<td>LPL</td>
<td>9.9</td>
<td>3.8</td>
<td>Resolution</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>F</td>
<td>CS, HA</td>
<td>UCL</td>
<td>10.3</td>
<td>0.3</td>
<td>Improvement</td>
<td>NR</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>M</td>
<td>CS</td>
<td>LPL</td>
<td>12.2</td>
<td>1.0</td>
<td>Resolution</td>
<td>PR</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>M</td>
<td>CS, HA</td>
<td>LPL</td>
<td>16.0</td>
<td>0.3</td>
<td>Resolution</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>F</td>
<td>CS, HA</td>
<td>LPL</td>
<td>10.4</td>
<td>3.1</td>
<td>Resolution</td>
<td>PR</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>M</td>
<td>HA,CS</td>
<td>LPL</td>
<td>7.8</td>
<td>1.18</td>
<td>No change</td>
<td>NR</td>
</tr>
</tbody>
</table>

0028

IN VIVO OXIDATIVEERYTHROCYMEMEMBRANE PROTEIN DAMAGE IN HEREDITARY SPHEROCYTOSIS

I. Margetis, M. Antonelou, F. Karababa, A. Loutradi, L. Margaritis, I. Papassideri
University of Athens, ATHENS, Greece; Center of Thalassemias, ATHENS, Greece

Background. Hereditary spherocytosis (HS) is a heterogeneous group of disorders with regard to clinical severity, gene defects and mode of inheritance. Most patients are presented with mild or moderate hemolysis. The abnormal red cell morphology (resulting in shortened cell sur- vival) is due to a deficiency of, or a dysfunction in, spectrin, ankyrin, band 3 or pallidin. Previous in vitro studies suggested that the spherocytes are sensitive to the action of oxidative agents. Furthermore, higher Hb autoxidation rate and abnormal oxidant sensitivity of spectrin, have also been reported in HS. Aims. To determine the possible oxidant-related protein alterations and the oxidative index of the membrane ghosts and cytoskeletons in clinically diagnosed cases of HS. Methods. Twelve patients with clinical and laboratory diagnosis of mild to mod- erate HS [α-thalassemia N=4, 6; betathalassemia N=3, B3(-)Hb N=5, spleenectomized N=2, concomitant carriers of α- or β-thalassemia N=4] and twelve healthy subjects used as controls were examined. Total ghosts and cytoskeletons were analyzed by SDS-PAGE densitometry and probed for

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hemoglobin, human immunoglobulins (IgG's) and various membrane proteins using erythroid specific antibodies. Carboxylated protein content was determined following 2,4-dinitrophenylhydrazine derivatization and SDS-PAGE coupled with western blotting with anti-DNP moiety antibody. Results. Protein degradation, formation of high molecular weight aggregates and increased Hb and IgG’s binding to the membrane were found by means of SDS-PAGE and immunoblotting analysis in the majority of the HS patients examined. The protein band (22 kDa) was also increased in 8/12 patients, half of which had concomitantly increase in Hb. Probing of the HS ghost membranes for Hb clarified that the membrane-associated globin was in the form of probably oxidized/denatured Hb or hemichromes. Subsequent analysis of the Triton-extracted membrane skeletons revealed pathologically increased amounts of membrane-associated Hb, monomers and higher order aggregates, representing globin oligomers and complexes with membrane protein components, in 30% of the samples. Immunoblotting with dinitrophenol-specific antibody showed increased RBC membrane and cytoskeleton protein carbonyls in the majority of the HS patients. In comparison to control membranes, there was an evident increase in the number and the intensity of the carboxylated protein bands appearing in the immunostained gels, ranging from MW 240 kDa to 15 kDa. In approximately 70% of the HS samples that were examined.

Summary/Conclusions. The red cells in HS in vivo are characterized by oxidative alterations in Hb and various membrane proteins and increased post-translational modifications defects in this. Similar to the RBCs that had embolic/thrombotic events in vivo stored and senescent RBCs are dictated by increased oxidative stress and are positively correlated with perturbations in membrane properties. These data corroborate the evidence for the occurrence of oxidative damage in membrane proteins in HS and add some new insight in the field of HS pathophysiology.

0029 DARBEPOETIN ALFA ADMINISTERED EVERY 3 WEEKS WITH OR WITHOUT PARENTERAL IRON IN ANAEMIC PATIENTS WITH NONMYELOID MALIGNANCIES RECEIVING CHEMOTHERAPY: INTERIM RESULTS FROM A RANDOMISED OPEN-LABEL STUDY
A. Vandenbroeck,1 S. Altintas,2 B. Gaede,3 K. Smith,4 B. Yao,5 M. Schupp6 1Ziekenhuisnetwerk Antwerpen, ANTWERPEN, Belgium; 2Universitair Ziekenhuis Antwerpen, EDEGEM, Belgium; 3Schweizisches Praxis Hämatologie/Onkologie, HANNOVER, Germany; 4Amgen Ltd, CAMBRIDGE, United Kingdom; 5Amgen (Europe) GmbH, ZUG, Switzerland

Background. Patients with cancer receiving chemotherapy often have chemotherapy-induced anaemia (CIA) and reduced quality of life. Darbepoetin alfa is an erythropoiesis-stimulating agent (ESA) that can effectively treat CIA when administered once every 3 weeks (Q3W). In patients with CIA, limited data in the literature suggest that administration of intravenous (IV) iron with ESA therapy may increase clinical response. Aims. This randomised, multicentre, open-label, 16-week study evaluated the safety and efficacy of 500 mcg darbepoetin alfa administered Q3W with oral iron or no iron in patients with CIA (haemoglobin < 11 g/dL). Methods. Patients were randomly assigned to receive either IV iron or standard practice for iron administration. The dose of IV iron was 200 mcg administered either Q3W with darbepoetin alfa Q3W or, if required, as 2 doses (200 mcg total) within a 3-week period. Patients who received 1 dose of darbepoetin alfa and who had completed the 16-week study period by October 19, 2005 are included in this interim analysis. The planned sample size is 400 patients and accrual will be complete by the time of the conference. Randomisation was stratified by tumour type and baseline haemoglobin (<10 or ≥10 g/dL). The incidence of patient-reported adverse events and serious adverse events, in particular embolic/thrombotic events, was summarised. Efficacy endpoints included the crude% (95% CI) of patients achieving the target haemoglobin (>10 g/dL) from week 5 to the end of treatment period (EOTP) and the crude% (95% CI) of patients receiving red blood cell transfusions from week 5 to EOTP. Haemoglobin values within 28 days of a transfusion were not included in any efficacy analysis. Results. Of the 114 pts included in this interim analysis, 65% were women, 99% were Caucasian, the mean (SD) age was 60 years (12), and 26% had lung or gynaecological tumours. Adverse events were reported by 79% of patients in the IV-iron group and 7% of patients in the standard-practice group. Treatment-related SAEs occurred in 3% of the IV-iron group and 4% of the standard-practice group; 9% of patients in the IV-iron group and 7% of patients in the standard-practice group had embolic/thrombotic events. Haemoglobin and transfusion endpoints stratified by baseline-1haemoglobin category are shown in the table. Summary/Conclusions. Based on the interim results, the safety profile for patients receiving 500-mcg darbepoetin alfa Q3W with IV iron appears to be comparable to patients receiving 500-mcg darbepoetin alfa Q3W with oral iron or no iron. The percentage of patients who achieved the target haemoglobin (≥11 g/dL) appeared higher, and the percentage of patients who required transfusions appeared lower, in the group receiving IV iron. This trend was consistent in patients in both baseline-anaemia groups.

Table 1. Study endpoints.

<table>
<thead>
<tr>
<th></th>
<th>IV iron</th>
<th>Standard practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) Bl Hb, g/dL</td>
<td>9.3 (0.54)</td>
<td>10.6 (0.39)</td>
</tr>
<tr>
<td>Crude % (95% CI) pts achieving target Hb (&gt;11 g/dL) from week 5 to EOTP</td>
<td>81 (58 to 95)</td>
<td>91 (76 to 98)</td>
</tr>
<tr>
<td>Crude % (95% CI) pts receiving RBC transfusions from week 5 to EOTP</td>
<td>19 (5 to 42)</td>
<td>12 (3 to 28)</td>
</tr>
</tbody>
</table>

BL: baseline; Hb: haemoglobin; EOTP: end of treatment period; pts: patients; RBC: red blood cell.

LS: 114 pts were randomised, but 1 pt in the IV-iron group was not treated.

*Based on the number of pts (for IV iron, n=21 for BL Hb < 10 g/dL and n=34 for BL Hb ≥10 g/dL; for standard practice, n=24 for BL Hb<10 g/dL and n=30 for BL Hb>10 g/dL) who were in the study until at least Day 29.

0030 CHARACTERISATION OF INDIVIDUAL NADH-CYTOCHROME B5 REDUCTASE VARIANTS USING A HETEROLOGOUS EXPRESSION SYSTEM
M.J. Percy,1 L.J. Crowley,1 C.A. Davis,3 J. Boudreaux,2 D.M. Layton,1 M.E. McMullin,2 T.R.J. Lappin,3 M.J. Barber1 1Belfast City Hospital, BELFAST, United Kingdom; 2University of S. Florida College of Med, TAMPA, FLORIDA, USA; 3Children’s Healthcare of Atlanta, ATLANTA, GEORGIA, USA; 4Imperial College. LONDON, United Kingdom; 5Queen’s University. BELFAST, United Kingdom

Background. Recessive congenital methaemoglobinaemia (RCM) arises from deficiency of NADH-cytochrome b5 reductase (cb5r) and manifests as cyanosis from birth. It exhibits two clinical phenotypes, benign type I and more severe type II, where the cyanosis is associated with neurological impairment. The physiological basis for the phenotypic variation between type I and type II RCM is poorly understood. Several mutations, Arg199del and Val252Met, have been found associated with both types suggesting that it is the combination of both alleles and thus the residual activity of cb5r variants that influences the development of type II as opposed to type I RCM. To date more than 40 mutations of cb5r have been described with a cluster in exon 9 of the DIA1 gene. To characterise individual cb5r variants a heterologous expression system has been developed based upon the X-ray crystallographic structure of the rat cb5r protein. Expressed proteins can then be purified to homogeneity and investigated for protein stability, catalytic efficiency, EAD cofactor properties and NADH+NAD+ substrate affinities. Aims. To characterise five different cb5r variants, Gly75Ser, Arg199Ter, Asp293Gly, Val252Met, Pro275Leu and Gly291Asp, recently described as causing type I RCM in four patients, who all showed markedly reduced red cell cb5r enzyme activity. Methods. The different RCM variants were generated using a bacterial expression system for the soluble, diaphorase domain (residues 133-F300) of rat hepatic cb5r. Four mutants and the wild-type domain were purified to homogeneity and characterised using absorption and CD spectroscopies, initial-rate kinetics of NADH:cytochrome and NADH:cytochrome b5 reductase activities, thermostability measurements and dye-mediated redox titrations of the EAD prothetic group. Results. Four of the expressed variants, Gly75Ser, Asp293Gly, Val252Met, Pro275Leu and Gly291Asp, were found to
exhibit decreased enzyme activity when compared to the recombinant wild type cb5r protein. The Arg159del variant protein was unstable and could not be purified thus preventing further characterisation. Although four variants, Gly75Ser, Val252Met, Pro275Leu and Gly291Asp, exhibited impaired protein stability the Asp239Gly had near wild type protein stability. A reduction of 40-45 and 48-57-fold respectively in the affinity of cb5r towards NADH co-factor was found in the Gly75Ser and Pro275Leu variants. Using predictions from the rat model, residue Asp239 is essential for the selection of NADH over NADPH and reduces cb5r affinity for NAD+. Summary. The heterologous expression system has been a useful tool for providing insights into the impact of type 1 RCM mutations on the structure and function of cb5r. It may allow the relationship between the clinical phenotype and cb5r activity to be examined and may lead to a better understanding of the pathophysiology of the two types of RCM.

0031

DEFERASIROX (EXJADE, ICL670) PROVIDES 24-HOUR PROTECTION FROM LABEL PLASMA IRON (LPI), IN IRON OVERLOADED β-TALASSEMIAS PATIENTS PREVIOUSLY CHELATED WITH MONO- OR COMBINATION THERAPY

S. Daar,1 A. Taher,1 A. Pathare,1 U. Krahn,1 C. Ressayre-Djaffer,1 H. Nick,1 D. Hadler1

1Sultan Qaboos University, MUSCAT, Oman; 2American Univ Beirat-Chron-ic Care Center, BEIRUT, Lebanon; 3Novartis Pharma AG, BASEL, Switzerland

Background. Chelation therapy aims to reduce iron burden, as patients are at increased risk for developing co-morbidities with a resultant negative impact on survival unless excess iron is removed. This can be achieved by reducing free or non-transferin bound iron (NTBI). LPI, one form of NTBI, is redox-active and can be taken up by cells, resulting in an increase in the cellular iron pool and an increased propensity for radical formation with ensuing oxidative stress. Direct capture of LPI has been suggested to avoid accumulation of cellular iron and to prevent its adverse consequences. Aims. To evaluate baseline data from the ongoing ESCALATOR trial and to measure LPI change in a patient subgroup. Methods. The overall aims of the ESCALATOR study are to investigate the efficacy and safety of the novel, once-daily oral iron chelator deferasirox (Exjade®, ICL670; 20 mg/kg/day) in 232 iron overloaded β-thalassemia patients previously chelated with mono- or combination therapy. Methods. Patient characteristics (159 aged 2-15 years; 73 aged ≥16 years) were analyzed to determine baseline iron burden. Pre-administration and 2-hour post-administration LPI levels were measured in a subgroup of 14 patients, at baseline and following repeat administration (weeks 4 and 16). Results. Despite previous chelation, baseline iron burden in the overall population was very high, indicating severe iron overload; mean baseline liver iron concentration (LIC) was 18.0 mg Fe/g dw (SD±9.1) and serum ferritin was 4148 µg/L (SD±3019), with both measures greater in all population was very high, indicating severe iron overload; mean baseline liver iron concentration (LIC) was 18.0 mg Fe/g dw (SD±9.1) and serum ferritin was 4148 µg/L (SD±3019), with both measures greater in

<table>
<thead>
<tr>
<th>LPI, mmol/L</th>
<th>Baseline (n=13)</th>
<th>Week 4 (n=13)</th>
<th>Week 16 (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre vs post</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0119</td>
<td>p&lt;0.1948</td>
</tr>
<tr>
<td>Baseline</td>
<td>p&lt;0.0187</td>
<td>p=0.0007</td>
<td></td>
</tr>
</tbody>
</table>

Although baseline LPI levels were high, Table 1 demonstrates a significant reduction in post- versus pre-administration levels at baseline and week 4. Pre-administration LPI levels were within normal parameters (0-0.4 µmol/L) by week 4, and were further reduced by week 16; post- ver-
**0033**

**GENETIC AND LEUKAEMIA-SPECIFIC FACTORS ASSOCIATED WITH P-GLYCOPROTEIN EXPRESSION AND FUNCTION IN AML BLASTS**

C. Seedhouse,1 M. Grundy,1 P. White,2 Y. Li,3 J. Fisher,4 D. Yakunina,2 N. Russell,1 A. Burnett,2 M. Pallis1

1 Nottingham City Hospital, NOTTINGHAM, United Kingdom; 2 Cardiff University, CARDIFF, United Kingdom

Background. P-glycoprotein (PgP), expressed on acute myeloid leukaemia (AML) blasts, is associated with failure to respond to chemotherapy in AML. This study aimed to determine whether expression and function of PgP may be linked to polymorphisms of the encoding gene (MDR1, also known as ABCB1) and whether leukaemia-specific changes in cell biology may override genetic factors in predicting PgP expression.

Methods. Genotyping of C3435T and G2677T polymorphisms in MDR1 (RFLP analysis) was performed in AML samples. Protein expression was determined by flow cytometry; drug sensitivity was evaluated using mitoxantrone (MTX). Statistical analyses were performed using MedCalc.

Results. In univariate analysis, white blood cell count, cytogenetic risk group, age at diagnosis, secondary AML/MDS and MDR1 haplotype were all strongly associated with PgP protein expression. In multivariate analysis white blood cell count, cytogenetic risk group, age and MDR1 haplotype remained significant. Cell cycle analysis of 39 consecutive fresh trial samples showed that PgP was associated with a lower proportion of cycling cells; median percentage of cycling cells in pgP-negative/low samples 4.5%, and in pgP intermediate/high samples 1.0% (p=0.01). Conclusions. We conclude that there is an extended MDR phenotype of indolent, pgP positive cells in AML, particularly in the elderly which is affected both by genetic factors and acquired leukaemia-specific factors.

Table 1. Factors associated with pgP protein expression.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Low pgP protein</th>
<th>High pgP protein</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median WBC (&gt;10x10⁹/L)</td>
<td>32</td>
<td>12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median age</td>
<td>57</td>
<td>63</td>
<td>0.006</td>
</tr>
<tr>
<td>Good risk cytogenetics</td>
<td>68%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>Intermediate risk cytogenetics</td>
<td>63%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td>Poor risk cytogenetics</td>
<td>36%</td>
<td>65%</td>
<td>0.008</td>
</tr>
<tr>
<td>De novo AML</td>
<td>62%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Secondary AML</td>
<td>36%</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>25%</td>
<td>75%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2617T/3435T haplotype ≥ 2 WT allele</td>
<td>56%</td>
<td>44%</td>
<td></td>
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<tr>
<td>2617T/3435T haplotype T/T VAR</td>
<td>78%</td>
<td>22%</td>
<td>0.002</td>
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</tbody>
</table>

**0034**

**FCGRIIA 158 V/V GENOTYPE IS ASSOCIATED WITH INFERIOR RESPONSE TO RITUXIMAB AND CHOP IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA**

Z.M. Mitrovic,1 I. Auer,1 I. Radman,1 R. Ajdukovic,2 J. Sertic,3 B. Labar1

1 University Hospital Center Zagreb, Zagreb, Croatia; 2 Clinical Hospital Dubrava, Zagreb, Croatia; 3 University Hospital Center Zagreb, Zagreb, Croatia

Background. Patients with follicular lymphoma or Waldenstrom’s macroglobulinemia and a homozygous valine/valine (V/V) at position 158 of the FcγRIIIa (CD16) receptor have a superior response to rituximab monotherapy. This could be related to higher affinity binding of rituximab to FcγRIIIa and NK cells with FcγRIIIa 158 V/V genotype to FcγRIIIa 158 F/F. The contribution of IgG1, suggesting antibody-dependent cellular cytotoxicity (ADCC) as an important mechanism of rituximab action in indolent lymphomas. Similarly, a histidine(3)/arginine(9) dimorphism in position 131 of the FcγRIIIa (CD163) may also be related to the treatment response. There is no data whether these dimorphisms affect response to combination of rituximab and chemotherapy in aggressive lymphoma. Aims. We examined the correlation of FcγRIIIa and FcγRIIIa gene dimorphisms with response in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab and CHOP (R-CHOP). Methods. FcγRII and FcγRIIIa gene dimorphisms were determined in 46 previously untreated patients with DLBCL presenting within the first 3 months of diagnosis. Results. Complete or partial remission (CR or PR) at 12 months after the R-CHOP therapy was achieved in 74% patients (34/46). Three patients achieved partial remission and nine patients had no response to the treatment. The frequency of FcγRIIa 158 V/V, V/F and F/F was 22%, 63%, 15%, respectively. The frequency of FcγRIIa 158 H/H, H/R, R/R was 39%, 48%, 15%. There was no difference in age, sex or IPI between the groups. Surprisingly, patients with FcγRIIIa 158 V/V genotype had significantly lower CR rate comparing to FcγRIIa 158 F carriers (40% vs. 83%, p=0.011). There were no significant differences in CR rate between the patients with different FcγRIIa 131 genotypes. Summary/conclusions. Contrary to the previous reports of response to rituximab in follicular lymphoma, FcγRIIa 158 V/V genotype in DLBCL was associated with significantly lower response rate to R-CHOP therapy. These results support the hypothesis that antibody dependent cytotoxicity (ADCC) does not mediate rituximab activity in DLBCL. Some other mechanisms, such as chemosensitization or direct apoptosis may be involved in synergistic effects of rituximab and CHOP in DLBCL. Considering the wide inter-individual variability in pharmacokinetics of rituximab, it is possible that FcγRIIa 158 V/V genotype provides more effective elimination of rituximab over the hypothetical benefit of ADCC in patients with DLBCL treated with R-CHOP.

**0035**

**DEFINED BONE MARROW NICHES COMPONENTS MEDIATE THE IN VITRO RESISTANCE OF AML SAMPLES TO THE TYROSINE KINASE INHIBITOR AG1299 AS WELL AS TO CYTOSINE ARABINOSIDE**

M. Pallis,1 U. Mony,2 D. Cardosa,3 C. Seedhouse,2 N. Russell1

1 Nottingham City Hospital, NOTTINGHAM, United Kingdom; 2 University of Nottingham, NOTTINGHAM, United Kingdom

Background. Patients with AML tend to respond well to induction chemotherapy, but relapse is frequent, suggesting protection of minimal residual disease cells in the bone marrow niche. Aims. We sought to determine the effect of defined bone marrow niche components’ fibronectin and cytokines - on the survival and chemoresistance of AML cells. Methods. We examined the effects of the cell adhesion substrate fibronectin and/or the cytokines IL-3, IL-6, SDF-1, angiopoietin 1, stem cell factor (SCF) and several combinations to the vitro adhesion, survival and response to the tyrosine kinase inhibitor AG1299 and to the nucleoside analogue cytosine arabinoside (ara-C) in 48 hr suspension culture of presentation samples from AML patients. 12 of the 16 samples selected for study had internal tandem duplication of the FLT3 gene, since relapse rates are high in these patients and tyrosine kinase inhibitors are an attractive therapeutic strategy. Results. In vitro adhesion to fibronectin in primary AML samples at 2 hours was enhanced by coculture with the cytokines IL-3 (69% increase, p=0.04) and stem cell factor (89% increase, p=0.04). In vitro survival at 48 hours in serum-free sus-
pension culture was enhanced 25% by adhesion to fibronectin (p=0.02, n=10) and further enhanced 32% by IL-3 (p=0.03), and 44% by a four cytokine cocktail (IL-3, IL-6, SCF and angiopoietin 1, p=0.02), but not by SCF, IL-6 or angiopoietin 1 individually. In vitro resistance to 15 µM AG1296 was enhanced 18% by adhesion to fibronectin (n=10, p=0.007) and further enhanced 45% by IL-3 (n=10, p=0.005), 57% by IL-6 (n=6, p=0.049) and 88% by the four cytokine cocktail (n=5, p=0.012). Similar-ly, in vitro chemoresistance to 500 ng/ml ara-C was enhanced 25% by adhesion to fibronectin (p=0.008, n=10) and further enhanced 31% by IL-3 (n=10, p=0.05) and 93% by the four cytokine cocktail (n=8, p=0.017). Conclusion. Adhesion to fibronectin increases survival and chemoresistance in AML samples and these effects are enhanced by cytokines, particularly IL-3. The tyrosine kinase inhibitor AG1296 and the cytotoxic drug cytosine arabinoside evoked similar patterns of resist-ance. It may be necessary to target cell-adhesion-mediated mechanisms of drug resistance in order to prevent relapse in AML.

0038

OVERCOMING CHEMoresistance IN HUMAN CHRONIC MYELOID Leukemia K562 Cells by SIRNA INHIBITION OF SHINGosine KInase-1

Y.B. Baran, 1 J.B. Bielawski, 2 C.E.S. Senkal, 3 B.O. Ogretmen, 2 U.G. Gunduz 2

1Middle east Technical University, ANKARA, Turkey; 2Medical University of South Carolina, CHARLESTON, USA

Background. Sphingosine-1- phosphate (S1P), the product of sphingosine kinase-1, is essential for cell survival and proliferation. To test if S1P antagonism could sensitize leukemia cells, we hypothesized that SK1 inhibition would sensitize the imatinib resistant Ph(+) human K562 cells to GC.

Materials and Methods. We used RT-PCR and Western blot analysis to confirm the inhibition of SK1. The dose-response curve for SK1 inhibition by siRNA was determined. The resistant human cell line K562/IMA-0.2, that was selected over increasing concentrations of imatinib (IC50 =11.6 ± 0.5 µM), was selected to determine the ability of SK1 inhibition to overcome the GC resistance. The determination was performed using a caspase-3 colorimetric assay and the measurement of DNA fragmentation and caspase-3 activity. The IC50 for dexamethasone was 6.8 ± 0.3 µM. The GC-sensitizing effect of SK1 inhibition in SK1 silenced K562/IMA-0.2 cells was additionally confirmed by an NFkB activity assay. mRNA levels of all tested genes remained unaltered, suggesting an inactive state of NFkB, which was confirmed by an NFkB activity assay. In summary, our data show that dasatinib as a single agent or in combination with 2Ca counteracts growth of neoplastic mast cells and may thus represent a promising new candidate drug for the treatment of SM.

0039

SENSITIZING LEUKEMIC CELLS TO GLUCOCORTICOSTEROIDS BY INHIBITING NFkB ACTIVATION


VU University Medical Center, AMSTERDAM, Netherlands

Glucocorticoids (GC) are commonly used in childhood leukemia, and induce apoptosis in GC-sensitive leukemic cells. Resistance to GC is a major adverse prognostic factor, occurring in ±20% of newly diagnosed childhood acute lymphoblastic leukemia (ALL) and in >50% of relapsed ALL, while acute myeloid leukemia (AML) is largely unresponsive to GC. Nuclear factor -kappaB (NFkB) is a transcription factor regulating the expression of cell survival genes, counteracting GC-induced cytotoxic effects both directly, via binding to the glucocorticoid receptor (GR) and indirectly. Blocking NFkB might prove a successful strategy to increase GC-sensitivity of leukemic cells. Chronic exposure of CEM-C7 T cell leukemia cells to dexamethasone (SSZ) downregulated NFkB activity, sensitized these primary sensitive cells even 10-20 fold further for GC (Van der Heijden et al., 2004). Two myeloid leukemia cell lines, THP1 and U937, with inherent resistance to GC (IC50 for dexamethasone >10 µM) were also markedly sensitized for GC upon long-term exposure to SSZ (IC50 <0.1 µM). In SSZ-exposed cells GR, NFkB p50 and IkB protein expression was markedly increased. Expression of NFkB p50 remained unaltered, suggesting an inactive state of NFkB, which was confirmed by an NFkB activity assay. mRNA levels of all tested genes remained unchanged, suggesting that the GC-sensitizing effect is due to a non-genomic mechanism of protein degradation. Consistently, co-incubation with a proteasome inhibitor further enhanced (5-fold) GC sensitivity in SSZ-exposed cells. To determine whether GC sensitization could also be achieved in cells that became GC-resistant due to previous GC exposure, we tested the GC sensitive CEM cell-line C7H2 and six CEM-C7H2 sublines with acquired GC resistance after GC exposure. All cells were exposed to SSZ, after which dexamethasone (dex) sensitivity was measured. Three cell-lines (IC50 dex >11.6 µM) gained increased GC sensitivity (IC50 dex 1.7-3.0 µM). One cell-line (IC50 dex >6 µM) remained resistant (IC50>6 µM) although some degree of sensi-tization could be seen (IC20 from 2.48 µM to 0.46 µM). Two cell-lines only showed a transient increase in GC sensitivity. We are currently measuring the potential GC sensitizing effects of the proteasome inhibitor Bortezomib since it has been shown that this drug inhibits IKK by inhibiting the degradation of IkBa. In addition, we have found earlier that co-incubation with Bortezomib further enhanced (5-fold) GC sensitivity in SSZ-exposed cells. These experiments will be per-
formed both in cell lines and primary patient samples. In conclusion, several promising new agents with mechanisms of action different from standard chemotherapy have been developed during the past few years. These agents may be able to improve the outcome of multiple myeloma, especially if they are able to enhance the response towards conventional therapeutics when given in combination. Aims. In order to provide rationale for combination therapies, we performed in vitro studies on the effect of combinations of conventional and novel drugs on human myeloma cell lines. Methods. Two cell lines, JK-6L and L363, were incubated with various concentrations of drugs, alone or in combination, in the presence or absence of human bone marrow stromal cells (BMSC). Cell growth was measured in an MTS assay and results were evaluated for synergism. Results. BMSC, dexamethasone (Dex) and rapamycin (Rapa) inhibited the growth of JK-6L cells in a synergistic fashion (e.g. 0.4 µM Dex reduced cell growth to 89% of that observed in untreated controls, 0.25 nM Rapa reduced growth to 56% of untreated controls, combining both agents reduced growth to 27%), whereas no synergism was observed for the L363 cell line. Interestingly, in the presence of BMSC, growth of both JK-6L and L363 cells was blocked in a synergistic fashion. Closer examination of the results from the cell growth assays revealed that JK-6L cells are resistant to Dex, regardless of the presence or absence of BMSC. Rapa was able to overcome this resistance (i.e. in the presence of Rapa, a strong dose-dependent reduction of cell growth by Dex was observed), hence explaining the observed synergy between both compounds. In contrast, L363 cells were already Dex sensitive in the absence of BMSC, and sensitivity was not enhanced by the addition of Rapa. However, in the presence of BMSC, L363 cells became Dex resistant and treatment with Rapa was able to overcome this protective effect and, as in JK-6L cells, restored Dex sensitivity. Furthermore, we determined the rate of apoptosis, upon treatment of JK-6L cells with either agent alone or in combination, by Annexin V (AxsV) staining. Addition of Rapa (which by itself did not alter the apoptotic rate) was able to enhance the cytotoxic effect of Dex, confirming the data obtained from the cell growth assays. Summary/Conclusions. Rapa synergizes with Dex in overcoming inherent (JK-6L) and BMSC dependent (L363) Dex resistance of malignant plasma cell lines. This in vitro study provides rationale to explore the use of combinations of these agents in patients with multiple myeloma.

MULTIDRUG RESISTANCE MECHANISMS IN HUMAN CHRONIC MYELOID LEUKEMIA CELLS

Y.B. Baran, 1 A.U. Ural, 1 B.O. Ogretmen, 1 U.G. Gunduz 1

1Middle east Technical University, ANKARA, Turkey; 1Gulhane Military Medical School, ANKARA, Turkey; 1Medical University of South Carolina, CHARLESTON, USA

Background. Chronic myeloid leukemia (CML) is diagnosed by finding a specific translocation between chromosomes 9 and 22. The resulting Bcr-Abl codes for a fusion protein with tyrosine kinase activity leading to uncontrolled cell growth. Imatinib, a Bcr-Abl inhibitor, induces apoptosis in CML cells by stabilizing the non-ATP-binding form of Bcr-Abl, and in turn, phosphorylation of its substrates. Aims. Despite the excellent clinical results with imatinib in CML, most patients have minimal residual disease and others will develop resistance which may eventually progress. In this study, the mechanisms responsible for imatinib resistance have been investigated. Methods. The Pb human K562 and Meg-01 cells were exposed to step-wise increasing concentrations of imatinib. Subpopulations of cells that were able to grow in the presence of 0.2 and 1 µM imatinib, were then selected, and referred to as K562/ or Meg-01/IMA-0.2 and Meg-01/IMA-1, respectively. The IC50 values were determined from cell survival plots obtained by MTT. The expression patterns of Bcr-Abl, MDR1 and apoptotic proteins were detected by RT-PCR and western blotting. Caspase-3 activity was determined using the caspase-3 colorimetric assay. Mitochondrial membrane potential (MMP) was measured using a JC-1 MMP detection kit. Cell cycle profiles of cells were analyzed by flow cytometry. Results. K562/IMA-0.2, K562/IMA-1, Meg-01/IMA-0.2 and Meg-01/IMA-1 expressed about 2.3, 19, 2- and 5-fold resistance to imatinib, as compared to their parental counterparts. There were an increased expression of Bcr-Abl, MDR1, Bcl-2, and Bcl-XL and decreased expression of Bax protein in resistant cells as compared to their parental counterparts. A decrease in caspase-3 activity and an increase in MMP was detected in resistant cells compared to parental cells. Exposure to 500 nM IMA for 48 hr resulted in apoptosis in about 75% and 60% of the population in K562 and Meg-01 sensitive cells, while there were no apoptosis in K562/IMA-0.2 and only 20% of apoptosis in Meg-01/IMA-0.2 cells. Summary/Conclusions. Various diverse mechanisms have been reported for their involvement in the multidrug resistance. In this study, it has been well documented that the degree of BCR/ABL expression appears to be directly proportional to the levels of imatinib resistance. In addition, there have been BCR/ABL-independent mechanisms reported for deriving resistance against imatinib. Our results revealed that besides Bcr-Abl overexpression, imatinib resistance also depends on the inhibition of apoptosis as a result of up-regulation of anti-apoptotic Bcl-2 and Bcl-XL proteins, down-regulation of pro-apoptotic Bax protein, decreased caspase-3 activity, and increased MMP K562/ or Meg-01/IMA-0.2 and Meg-01/IMA-1 cells.

IDENTIFICATION AND CHARACTERIZATION OF A HOMO-DIMER OF ABCG2 IN MATURE HUMAN ERYTHROCYTES

M.L. Leimane, E.G. Georges

McGill University, MONTREAL, Canada

Background. Human ATP-binding cassette G2 (ABCG2, also known as breast cancer resistance protein, mitoxantrone resistant protein, and ABC placenta) is a member of the ABC family of transporters. Similar to other well characterized ABC transporters that are expressed in humans, namely P-glycoprotein (P-gp) and Multi-Drug Resistance Protein 1 (MRP1), ABCG2 has been shown to transport xenobiotics and other normal cell metabolites and anti-cancer drugs. ABCG2 is termed a homodimer in mature erythrocytes. Methods. Erythrocyte plasma membranes were isolated from whole blood drawn from consenting blood donors. ABCG2 protein was visualized using western blotting technique. Activity was determined using FACS analysis whereby the active transport of Pheophorbide a (a chlorophyll catabolite, similar in structure to Protoporphyrin IX) was inhibited using an ABCG2 specific inhibitor Fumitremorgin C. The results show the first evidence of an ABCG2 homo-dimer expression in erythrocytes. It appears that the levels of ABCG2 oligomerization varies in human erythrocytes, however, no correlation was found between expression levels from genders, different blood types, as well as samples from different racial groups. The protein was found to be active as demonstrated using FACS analysis to transport Pheophorbide a (a chlorophyll catabolite, similar in structure to Protoporphyrin IX) and the subsequent inhibition of transport of Pheophorbide a was achieved using an ABCG2 specific inhibitor Fumitremorgin C. The results show that ABCG2 in erythrocytes have more of the homo-dimer as compared to MCF7/Mitoxantrone resistant cell line. This is likely due to the formation of disulfide bonds resulting from a highly oxidative environment in erythrocytes. The biological significance of this finding is not clear at the present time but may contribute to the physiologic function(s) of ABCG2 in erythrocytes and its possible role in maintaining heme homeostasis. Further studies to characterize the possible functions of ABCG2 in mature erythrocytes are currently in progress.
FUNCTIONAL AND GENOME-WIDE ANALYSIS OF ACQUIRED RESISTANCE TO TRAIL/APPOL2 MEDIATED APOPTOSIS OF HL60 LEUKEMIA CELLS

P. Klenér,1 R. Královics,2 P. Procházka,2 A. Vicha,2 T. Eckschlager,2 E. Necas,3 L. Andera,2 J. Živny2

1Charles University, First Medical Faculty, PRAHA, Czech Republic; 2Austrian Academy of Sciences, VIENNA, Austria; 3Charles University, PRAHA, Czech Republic

Background. Acute leukemia comprises malignant diseases of clonal character, to which specific treatment remains limited. Apoptosis induced by death receptor activation (i.e. by tumor necrosis factor-related apoptosis inducing ligand, TRAIL/APPOL2) is a potential anti-tumor therapeutic mechanism. TRAIL, a member of the TNF family of death ligands, appears to specifically and efficiently kill tumor cells of diverse origin while sparing normal tissues. The TRAIL receptor family consists of five receptors: two death receptors (DR4/TRAIL-R1, DR5/TRAIL-R2), two decoy receptors (DcR1/TRAIL-R3, DcR2/TRAIL-R4), and osteoprotegerin (OPG). Aims: Functional analysis of individual TRAIL receptors in HL60 myeloid leukemia cells and analysis of the molecular basis of TRAIL resistance. MATERIALS AND Methods. TRAIL-resistant cells were selected from the original HL60 population using pressure of recombinant His-tagged TRAIL (200-2000 ng/mL). The expression of TRAIL receptors and CD14 were analyzed by flow cytometry using fluorescent labeled antibodies and/or by real-time RT-PCR. Percentage of apoptotic cells was measured by flow cytometry using Annexin-V-FITC/Propidium iodide apoptosis detection kit. The contribution of individual TRAIL receptors on the transmission of apoptotic signal was measured using blocking antibodies to TRAIL receptors. The TRAIL resistance related genome aberrations were analyzed by genome-wide loss of heterozygosity (LOH) screening with marker density of 10cM and comparative genomic hybridization (CGH) assay. Results. The blockage of DR4 receptor significantly reduced the number of apoptotic HL60 cells compared to untreated controls. The blockage of DR5 receptor also inhibited TRAIL-induced cell death but the results did not reach statistical significance. Combination of anti-DR4 and anti-DR5 antibodies almost completely abrogated TRAIL-induced HL60 cell death and significantly reduced apoptosis compared to control or anti-DR4 antibody alone (p<0.01). Blocking of decoy receptors (DcR1, DcR2) or OPG of HL60 TRAIL-sensitive and TRAIL-resistant cell lines did not significantly affect the apoptotic signaling. Two distinct HL60 TRAIL-resistant phenotypes were identified based on the expression of TRAIL-receptors and CD14. Phenotype-1 (n=4) was characterized by the decreased expression of TRAIL receptors DR4, DR5, DcR1, and DcR2, CD14 and unchanged expression of OPG as compared to control TRAIL-sensitive HL60 cells. Phenotype-2 (n=5) was characterized by the decreased expression of DR5 receptor, increased expression of CD14, and undetectable expression of OPG compared to control TRAIL-sensitive HL60 cells. Using LOH assay we identified two genotypes. The first exhibiting deletions/uniparental disomy on the short arm of chromosomes 2, 3, 6, and 14. The second genotype corresponded to TRAIL-resistant phenotype-1 and phenotype-2, respectively. CGH assay confirmed the loss of genomic material of whole chromosome 18. Further, the CGH detected a gain of genomic material at 1q21-23 of TRAIL-resistant phenotype-1 while the phenotype-2 cells did not show genomic defects of chromosome 1. Summary/Conclusions. In HL60 cells TRAIL-specific apoptotic signal is transduced predominantly through TRAIL receptor DR4. Decoy receptors, including OPG, did not play a role in TRAIL resistance. The identified TRAIL-resistant phenotypes are associated with distinct genomic conditions. Supported by: IG A MZ NR83174-7 and GAUK 50/2004/4.

OPTIMIZATION OF THERAPY FOR THIOPURINE S-METHYLTRANSFERASE DEFICIENT CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

B. Petrucci,1 L. Dokmanovic,2 N. Tosic,3 D. Canic,3 N. Jovanovic,3 S. Pavlovic2

1IMGC, BELGRADE, Serbia and Montenegro; 2University Children Hospital, BELGRADE, Serbia and Montenegro

Background. Thiopurine S-methyltransferase (TPMT) is an enzyme that catalyzes the conversion of thiopurine drugs, such as 6-mercaptopurine (6-MP) and thioguanine, commonly used in therapy for childhood acute lymphoblastic leukemia (ALL). In BFM protocol for childhood ALL, 6-MP is administered during maintenance therapy. Patients with low TPMT activity experience severe hematological toxicity when standard 6-MP doses are used. It is now well established that lower TPMT activity can be due to TPMT gene mutations. Three alleles account for more than 95% of the clinically relevant TPMT variants: TPMT*2, TPMT*3A and TPMT*3C. Wild type has been designed as TPMT*T1. TPMT*T2 allele contains single G238C mutation, TPMT*T3C-A719G mutation, TPMT*T3B-G460A mutation and TPMT*T3A allele has two mutations (G460A and A719G). Aim. The purpose of this study was to determine the relevance of TPMT gene mutations in the management of childhood acute lymphoblastic leukemia (ALL). METHODS. Blood samples from 100 children with ALL were analyzed for TPMT mutations, using polymerase chain reaction-based assays (PCR-RFLP and ARMS). For 50 patients TPMT variant alleles were determined retrospectively, after completing the therapy protocol modification in a way that if leucopenia was noticed, only the dose of 6-MP was reduced but there were no reduction of thioguanine, commonly used in therapy for childhood acute lymphoblastic leukemia. In the remaining 50 patients the pharmacokinetics of cyclophosphamide in plasma and to measure the levels of mRNA (by real time PCR), protein (by western blot) and enzyme activity (by microsomal incubation with cyclophosphamide) of CYPs, respectively. Results. mRNAs of CYP2B1 and 2B2 were significantly induced with repeated dosing. Protein levels were also induced and autoinduction of CPA metabolism to 4-hydroxylation was found. Conclusion. Repeated dosing of CPA leads to autoinduction of CPA metabolism and induction of CYP2B2 mRNA in rat. This knowledge may help in optimizing the dosing regime of cyclophosphamide in patients to keep plasma levels within the therapeutic range. It may also help in minimizing drug-drug interactions and hence increase the therapeutic efficacy and reduce side effects of cyclophosphamide in cancer patients.

THE ADMINISTRATION OF REPEATED DOSES OF CYCLOPHOSPHAMIDE, INDUCES CYTOCHROME P450 IN RAT

P. Afsarian,1 Y. Terelius,2 Z. Hassan,4 S. Lundgren,4 M. Hassan1

1Karolinska Institute, STOCKHOLM, Sweden; 2Department of Research DMPK, AstraZeneca, SDERTJ, STOCKHOLM, Sweden; 3CAST, Karolinska Univ. Hospital, Huddinge, STOCKHOLM, Sweden; 4Clinical Pharmacology, Karolinska Institut, STOCKHOLM, Sweden; 5Laboratory of Hematology, Karolinska Univ. Hospital, HUDDINGE, STOCKHOLM, Sweden

Background. Cyclophosphamide is used in high doses as a part of the conditioning regimen prior to stem cell transplantation. It is usually given for two or four consecutive days, primarily to facilitate engraftment of donor cells. Cyclophosphamide is activated in the liver by a 4-hydroxylation reaction catalyzed by cytochrome P450 (CYP) enzymes. Several studies have shown that cyclophosphamide induces its own metabolism, which affects its pharmacokinetics and pharmacodynamics after repeated doses. Aim. In the present study, we aimed to investigate the effect of repeated doses of cyclophosphamide on the CYPs in rat. The levels of mRNA, protein, and enzyme activity were investigated. Methods. Male Wistar rats were given 4 consecutive doses of CPA (2 dose levels). Plasma and livers were collected to study the pharmacokinetics of cyclophosphamide in plasma and to measure the levels of mRNA (by real time PCR), protein (by western blot) and enzyme activity (by microsomal incubation with cyclophosphamide) of CYPs, respectively. Results. mRNAs of CYP2B1 and 2B2 were significantly induced with repeated dosing. Protein levels were also induced and autoinduction of CPA metabolism to 4-hydroxylation was found. Conclusion. Repeated dosing of CPA leads to autoinduction of CPA metabolism and induction of CYP2B2 mRNA in rat. This knowledge may help in optimizing the dosing regime of cyclophosphamide in patients to keep plasma levels within the therapeutic range. It may also help in minimizing drug-drug interactions and hence increase the therapeutic efficacy and reduce side effects of cyclophosphamide in cancer patients.
(M/M). Among 50 patients retrospectively analyzed for TPMT variants 6 were found to be W/M. Mean duration of full dose therapy was signif-
ificantly longer (p<0.01) in W/W patients (54 weeks) than in W/M (87.5
weeks). Mean duration of period with no therapy was significantly
longer (p<0.01) in W/M patients (11.3 weeks) than in W/W (3.4 weeks).
Neutropenic fevers occurred in all of the patients (1-4 times). For four
respectively detected W/M patients, therapy protocol was modified
dosage reduction of 6-MP by 25-50%). In contrast to W/M patients re-
spectively analyzed, these W/M patients neither missed the therapy
nor developed febrile neutropenia. Conclusion. The ability to tolerate 6-
MF based maintenance therapy was used as a surrogate marker of hema-
tological toxicity in childhood ALL. We found that even patients hett
erogeneous for TPMT variant alleles are at greater risk of thiopurine drug-
related leucopenia. Lowering doses of 6-MP in heterozygous TPMT
deficient patients while allowing administration of full dose of MTX,
might be an optimal way of treatment for this group of patients. These
results justify performing TPMT genotyping before initiating thiopurine
therapy in all children diagnosed with acute leukemia to minimize con-
sequent toxicity.

0045

IMATINIB AND HUMAN ORGANIC CATION TRANSPORTER 1 (hOCT1):
CHARACTERISATION OF TRANSPORT IN STABLY TRANSFECTED MYELOID CELLS
A. Giannoudis, L. Wang, M. Pirmohamed, R.E. Clark
University of Liverpool, LIVERPOOL, United Kingdom

Imatinib is an important drug for treating chronic myeloid leukaemia
(CML). However, not all patients achieve a major cytogenetic response
(MCR) to imatinib. We investigated if induction therapy with the cellu-
lar uptake and efflux of imatinib, and particularly whether this may
influence clinical outcome. A previous study in our lab showed that the
influx of imatinib is mediated by hOCT1 while efflux is through MDR1
(Thomas et al., Blood, 2004; 104: 3739-3745). We also analysed the
expression levels of hOCT1 and MDR1 in 67 imatinib-treated CML
patients. Forty patients achieved a MCR (of which 33 were complete)
and 27 had no cytogenetic response (NCR) after 6 months of imatinib.
Prior to commencement of imatinib, hOCT1 expression levels were
greater in patients destined to achieve MCR than in NCR patients.
MDR1 expression was high in initial MCR patients who subsequently
lost their cytogenetic response, and most of these had developed a BCR-
ABL kinase domain mutation at progression. These data are compatible
with the view that hOCT1 expression prior to imatinib is an important
determinant of the outcome of imatinib treatment. However, our non-
clinical studies of imatinib transport used a panel of pharmacological
inhibitors of several drug transporters, and many of these are not truly
specific for individual transporters. Here we use cells stably transfected
with hOCT1 to further characterise imatinib uptake. The BCR-ABL pos-
itive cell line KCL22 and the BCR-ABL negative human embryonic kid-
ney line HEK293 were transfected with pcDNA3-hOCT1 (provided by
Gründemann D, Cologne, Germany) with the empty vector pcDNA3.1
used as a negative control. KCL22 was selected as it has low constitut-
ive expression of hOCT1 in comparison to other BCR-ABL positive
cells such as LAMA4, KY01 and K562. KCL22 and HEK293 cells were
transfected using Nucleofector technology and Fugene 6 respect-
ively. Positive clones were selected using the G418 antibiotic
(Combi) Several stable clones for each line were obtained, express-
ing hOCT1 at different levels. These clones were then used for transport
experiments, using cold and 14C radio labelled pure imatinib (gift of
Novartis). The influx of imatinib was greater in the stably transfected
cells compared with the untransfected or cells containing the empty
vector. Current data suggest that uptake is greater in clones with high
hOCT1 expression than in those with lower expression. A time course
assay also showed that uptake was very quick, but reached a plateau
after 30 minutes. These findings support the view that hOCT1 is an
important determinant of imatinib influx into BCR-ABL positive cells.

0046

PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE MONITORING ON
RELAPSE-FREE SURVIVAL IN CBFB-MYH11 ACUTE MYELOID LEUKEMIA
A. Corbacioglu,1 C. Scholl,2 K. Eiwen,1 L. Bullinger,3 S. Frohling,4
H. Döhner,3 R.F. Schlenk,1 K. Döhner1
1University Hospital of Ulm, ULM, Germany; 2Harvard Medical School, BOSTON, USA

Background. Detection of minimal residual disease (MRD) in acute
myeloid leukemia (AML) associated with specific gene fusions is an
important tool for the assessment of response to treatment and the indi-
vidual risk of relapse. The real-time quantitative RT-PCR (RQ-PCR)
method allows the quantification of fusion transcript levels at distinct
time points during treatment. While in acute promyelocytic leukemia
(APL) MRD monitoring has been clearly shown to be predictive for clin-
ocutaneous toxicity.

results justify performing TPMT genotyping before initiating thiopurine
therapy in all children diagnosed with acute leukemia to minimize con-
sequent toxicity.

Molecular diagnostics

0041

CHARACTERIZATION OF THREE CASES OF ANEMIA/MENTAL RETARDATION SYM-
DROMES (ATR-16) IN THE NETHERLANDS, USING MULTIPLEX LIGATION-DEPEN-
DENT PROBE AMPLIFICATION (MLPA)
C.L. Hartveld,1 M. Kriek,2 E.K. Bijlsma,1 Z. Erjavec,3 A. Voskamp,1
D. Balak,1 P.C. Giordano2
1Leiden University Medical Center, LEIDEN, Netherlands; 2Delftich Zieken-
hus, DELFZIJL, Netherlands

Background. Two distinct and rare syndromes of a-thalassemia asso-
ciated with mental retardation are known to date. One is characterized
by the occurrence of large deletions involving the a-globin gene cluster
on chromosome 16p (ATR-16 syndrome) and is most likely a continu-
guage X linked XNP gene, coding for helicase-2, a putative global transcription-

"
The value of allo-SCT needs to be revisited in the various genotype subsets.

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<th>N</th>
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Methods. The Cepheid Xpert® BCR-ABL Monitor assay is designed to co-amplify the BCR-ABL transcript and the ABL transcript (the endogenous control). The assay only requires a few simple manual pipetting
steps, followed by fully automated nucleic acid purification, nested RT-PCR, and data analysis. A 200 ul aliquot of whole blood is mixed with proteinase K and lysis reagent to inactivate cells and release the nucleic acid from the cells. After addition of 1 mL of ethanol to the lysed sample, the mixture is added to the test cartridge using a transfer pipette. Wash, rinse, and elution reagents are also added to designated ports in the test cartridge, the lid is closed, and the cartridge is loaded into the GeneXpert, Dx System. By moving the sample and reagents into different chambers in the cartridge during the test process, the GeneXpert (1) isolates the total RNA from lysed whole blood by binding the RNA to the solid phase purification material, (2) washes and rinses away inhibitors, (3) elutes the RNA, (4) hydrates the reagent beads and combines the mixture with the eluted RNA, (5) moves the sample and reagent mixture into the reaction tube, (6) performs quality checks to ensure that reagent preparation was successful, (7) performs a one step RT-PCR followed by nested real-time PCR (8) reviews the signal from both the ABL endogenous control and the BCR-ABL transcript for acceptability, and (9) calculates the delta Ct between the two signals. The test process for BCR-ABL takes approximately 2 hours and 20 minutes.

Results and Conclusions. The specificity of the assay was tested using 42 Congress of the European Hematology Association R. Hehlmann, | haematologica/the hematology journal | 2006; 91(s1) We wished to assess Harmonization of BCR-ABL mRNA quantification is feasible In order to standardize results, b3a2 BCR-ABL and In a series of consensus meetings G. Saglio, T. Ernst, The JAK2V617F mutation was detected in 31 1. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-1061.
0052
IDENTIFICATION OF A COMMONLY USED CD3 REGION OF LGHS TCR ALPHABETA+/CD4+ TCR Vβ 13.1 PATIENTS

P. Garrido,1 A. Orfao,1 J. Canton,1 J. Almeida1 M. Lima,3 P. Barcena,1 M.A. Lopez-Nevo1, F. Garrido,1 F. Ruiz-Cabello1
1Virgen de las Nieves Hospital, GRANADA, Spain; 2Cancer Investigation Center, SALAMANCA, Spain; 3Santo Antonio General Hospital, PORTO, Portugal

Background. Monoclonal TCRβ+/-CD4+/CD8-/+dim T represent a subgroup of monoclonal LGL lymphoproliferative disorders different from both CD8+ T-LGL and NK-cell type LGL leukemias. The recently described TCRβ+/CD4+/CD8- T-LGL leukemia/lymphocytosis has been shown to be associated in around one third of cases with a neoplastic clone other than the T-LGL, which prompted us to hypothesize that the TCRβ+/CD4+/CD8- T-LGL may proliferate and expand as an effort of the immune system to control tumor growth, supporting in some way the antigen-driven selection model. We typed for HLA class I and II genes in patients with different TCR-Vβ expansions. TCR clonotypes and VDJ rearrangement structure were analyzed in a cohort of patients with CD4+LGL expansions.

Aims. Analyse the possible association between the TCR Vβ family expanded with HLA and CD83 hypervariable region expressed in patients with Vβ expansions, LGL TCRαβ+/CD4+ /CD8−/dim. Methods. Total of 26 individuals (19 males and 7 females; mean age 64±11 years, ranging from 40 to 81 years) having a TCRβ+/-CD4+/CD8-/+dim monoclonal T-LGL lymphoproliferative disorder were studied. For the immunophenotypic studies a panel of 24 monoclonal antibodies (MAb) directed against an identical number of members of 21 different TCR-Vβ families was used. A genotype for HLA-ABC and -B8 was determined by SSPO-PCR. DNA was amplified and clonal products from the VH gene PCR were sequenced directly using the BigDye Terminator Cycle Sequencing Reaction Kit. Results. In all cases studied, expanded CD4+ LGL T-cells showed relatively high SSC features as compared to normal PB CD4+ T-lymphocytes and common phenotypic characteristics, consisting of TCRβ+/-CD4+/CD8-/+dim cells with a typical cytotoxic (granzyme B+, CD56+). Flow cytometric analysis of the TCR-Vβ repertoire of CD4+/CD8-/+dim LGL T-cells was consistent with a (mono)clonal expansion in all cases studied, which accounted for 75%±26% of all PB CD4+ T-cells. In 27 cases the expanded TCR-Vβ family was identified with the panel of TCR-Vβ reagents used, corresponding to TCR-Vβ13.1 in 15 cases (42%), TCR-Vβ 21 in 2 (5.6%), TCR β V8.1 in 2 (5.6%), TCR-Vβ 8.2 in 2 (5.6%), TCR-Vβ17.1 in 2 (5.6%), TCR-Vβ 22 in 2 (5.6%) and TCR-Vβ11 or TCR-Vβ14 in one case each (2.8%). In the remaining 9 patients the expanded TCR-Vβ family was not identified (25%) with the panel of MAB used. All 15 patients who showed expansions of TCR-Vβ 13.1 + CD4+ T-cells were HLA-DRB1*0701+. Comparison of CD83 size distribution in clonal CD4+/CD8-/+dim T-cells from the same patients showed a highly restricted usage of VHDJH segments and shared CDR3 configuration and sequences. These findings suggest the expansions were selected for this unique TCR structure. This results strongly suggest that Vβ13.1 CD4+ T cells with the described CD83 motif may recognize a specific antigen presented by DR7 molecules, indicating the existence of a common associated antigen.

0054
REAL-TIME RT-PCR ASSAY USING THE TAQMAN PROBES WITH LNA (LOCKED NUCLEIC ACID) MODIFICATION TO DETERMINE JAK2 GENE V617F MUTATIONS IN MYELOPROLIFERATIVE DISEASES (MPD)

J. Marková,¹ J. Schwarz²
¹Institute of Hematology and Blood Transf, PRAGUE, Czech Republic; ²Institute of Hematology & Blood Transfusion, PRAGUE, Czech Republic

Background. JAK2 V617F mutations are frequently found in MPDs. JAK2 mutations confirm clonality in MPD, and according to some authors, they may be relevant even prognostically. In the near future, therapy by JAK2 inhibitors may be foreseen. The classical method of detection of JAK2 V617F mutations published by Baxter et al.1 takes advantage of mutation-specific primers in PCR and requires sequencing to distinguish between homo- and heterozygous mutations. Aims. We have developed a more straightforward, real-time RT-PCR method, to demonstrate JAK2 mutations, allowing zyosityc discrimination.

0055
MDR1, MRP AND LRP EXPRESSION IN PATIENTS WITH UNTREATED ACUTE LEUKEMIA: CORRELATION WITH TC-99m MIBI BONE MARROW SCINTIGRAPHY

I. Ak, G. Demirel, Z. Gulbas
Eskisehir Osmangazi University, ESKISEHIR, Turkey

Background. Multidrug-resistance (MDR) phenotype concerns altered membrane transport that results in lower cell concentrations of cytotoxic drug in many cancer types, including leukemia and is related to the overexpression of a variety of proteins that act as ATP dependent extrusion pumps. TC-99m Sestamibi (MIBI) is a transport substrate for Pgp pump. Aim. We assessed the bone marrow uptake of TC-99m MIBI and its correlation with messenger RNA (mRNA) levels of MDR1, Multidrug-Resistance Associated protein (MRP) and Lung Resistance Protein (LRP) in acute leukemia. A total of 26 patients with newly diagnosed acute leukemia (8 ALL and 18 ANLL) were included in the study. The expression of MDR1, MRP, and LRP on mRNA levels were assessed by semi quantitative RT-PCR (Roche Light Cycler System, Metis Biotechnology primers and probes for MDR1, MRP and LRP) in the blast cells from the bone marrow samples. Planar images of the pelvis and thorax were acquired 20 min after injection of 740 MBq TC-99m MIBI. The MIBI uptake in the bone marrow was evaluated using a quantitative scoring system with determination of the tumour-to-background ratios for the bone marrow in areas that included the proximal femur, anterior iliac crest and sternum. The correlation between the RT-PCR results and MIBI uptakes was analysed by using Spearman’s rank correlation coefficients with two-tailed test of significance. Results. There was an inverse relationship between TC-99m MIBI uptake of bone marrow and both mRNA levels of MDR1 and MRP (p=0.000, r=- 738 and p=0.001, r=-610, respectively). No correlation was found between MIBI uptake and mRNA levels of LRP. Conclusion: Increased expression of MDR1 and MRP correlates with a low accumulation of TC-99m MIBI in bone marrow areas in patients with acute leukemia. As a functional imaging, TC-99m MIBI bone marrow scintigraphy can identify the MDR1 and MRP phenotype, but not LRP, in patients with acute leukemia.
Methods. Peripheral blood granulocytes were separated from altogether 151 patients with already diagnosed or suspected Ph- MPD. Patients with polycythaemia vera (PV), secondary polycythaemia (SP), essential thrombocytocytosis (ET), idiopathic myelofibrosis (IMF) and unmutilated MPD (MPD-U) were included in the study. The cells were lysed and RNA extracted using the Trizol reagent. Following reverse transcription, two methods were employed to detect JAK2 mutations. 1) The method described by Baxter et al. (2005), using them hybridizing to the mutated allele and a common reverse primer recognizing both the mutated and unmutated JAK2 alleles. Homozygosity or heterozygosity of the mutated gene was discriminated by sequencing analysis. 2) The allelic discrimination real-time KT-PCR assay that uses one pair of primers and two dual labeled TaqMan probes with LNA modified nucleotides. The probes differ at the polymorphic site, one of them is complementary to the wild-type JAK2 allele and the other to the mutated one. The result is given by the curves arising from measured fluorescence of two different reporter dyes during the real-time PCR (FigURE 1).

Results. Altogether 151 samples of patients with suspected Ph-MPD were analyzed using both of the above mentioned methods for JAK2 detection. In both of the assays, the result was obtained: JAK2 mutation being found in the same 71 out of 151 patients (47.0%). Ten of the 71 JAK2 mutations (14.1%) were homozygous, half of which were found in PV patients. In ET, JAK2 mutations were demonstrated in 22/57 (38.6%) patients, none of them was homozygous. Of 45 patients with PV, 35 had mutations (77.8%), whereas only 1/10 patients with SP had the mutated allele of JAK2 gene. Six of 20 (50.0%) individuals with IMF had JAK2 mutations (3 were homozygous). In the remaining 21 MPD-U patients, 9 mutations (42.9%) were detected. Conclusions. The TaqMan allelic discrimination assay yields the same results as the method of Baxter et al. (2005). In contrast to the latter, it is very simple and does not require sequencing to distinguish between homo- and heterozygotes. Thus it is less laborious and time-consuming and therefore also suitable for routine clinical laboratory testing.

References

Supported by the Research project of the Czech Ministry of Health 00235/60001.

0056

NPM1 AND FLT3 MUTATIONS, DUPLICATIONS OF MLL AND EXPRESSION OF GENES WT1, EVI AND BAALC AS PROGNOSTIC FACTORS IN PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA

S. Ballestre,1 E. Barargán,1 J. Cervera,1 P. Bolufer,1 E. Afan de Ribera,1
P. Fernández,1 R. Andreu,1 R. Collado,1 G. Martín,1 P. Montesinos,1 J.C. Pajuelo,1 M. Collado,1 M. Maiques,1 C. Martin,1 M.A. Sanz2
1Hospital La Fe, VALENCIA, Spain; 2Hospital General Universitario, ALICANTE, Spain; 3Hospital Dr. Peset, VALENCIA, Spain

The cytogenetic analysis allows to classify the AML in different risk groups, at least in about 50% of AML patients carry normal karyotype by conventional cytogeneticists and they lack of prognostic markers. Recently, several molecular alterations have been related to AML. This study analyze the prognostic impact of FLT3 mutations (ITD and DSS5 mutations), NPM1 mutations, partial tandem duplications (PTD) of MLL and as well as WT1, EVI1 and BAALC gene expression in a group of 100 adult patients (48 female and 52 male; median age 62 years, range 22-94) with AML de novo. Screening for NPM1 was performed using a melting curve assay based Lightycler (Schmittgen et al. Blood 2005) and confirmed by direct sequencing in ABI 310. The presence of FLT3 ITD was detected according to the method of Nakao M et al. (Leukemia 1997) and DSS5 using a melting curve based Lightycler assay designed by Tib Molbiol (Berlin, Germany). MLL PTD was analyzed according to the method of Caligiuri MA et al. (Cancer research 1996). Gene expression quantification for WT1, EVI1 and BAALC was performed by real-time PCR ABI Prism using β-glucuronidase (GUS) as control gene and TaqManTM probes technology. Frequency of mutations: NPM1 mutations were found in 25/82 patients (30.5%) and four different mutations were detected: type A (72%), B (8%), D (12%), K (8%). FLT3 mutations were present in 16/97 patients (16.5%) (12 ITD and 4 DSS5) and the incidence of mutation for MLL PTD was of 4/74 patients (5.4%). Gene Expression: WT1, EVI1 and BAALC showed a median gene expression ratio: 0.28 (range 0-7.03), 0.013 (range 0-3.35) and 0.01 (range 0-18.90) respectively. Overexpression criteria was defined according to the median expression ratio Clinical characteristics: FLT3 and NPM1 mutations were significantly associated with a high white blood cell count (WBC) (p=0.003 and p=0.002 respectively). In addition, NPM1 mutated cases were significantly associated with FLT3 mutations (p<0.0001), normal karyotype (p=0.024), and the monocytic lineage (FAB M4/M5, p=0.056). Prognostic impact: The response to induction showed no relation with any of the molecular markers. The disease-free survival (DFS) was significantly influenced by overexpression of WT1 (p=0.045), FLT3 mutations (p=0.007), a high WBC (p=0.047) and the cytogenetic risk group (p=0.0034). In conclusion, our data show that the analysis of WT1 expression accompanying the FLT3 mutations may be useful to predict prognosis beside cytogenetic findings. This study partially has been supported by grant FIS03/0400.
ACCUVATE V617F JANUS KINASE 2 MUTATION GENOTYPING COMBINING ARMS PCR AND CAPILLARY ELECTROPHORESIS

F. C. Lambert,1 A. Schioppa,2 S. Castermans,3 L.Y. Wang,4 D816H,5 patients were 54±14.1% and 81±8.8%, respectively (p=0.191). The 5-year EFS were 56±13.7% for c-KIT(-) patients (p=0.323). The 5-year OS for those carrying any one of RTK/Ras mutations was 60±8.0%. The 5-year OS of c-KIT- and c-KIT+ patients, respectively (p=0.191). The 5-year EFS were 56±13.7% for c-KIT+ and 76±9.5% for c-KIT(-) patients (p=0.323). The 5-year OS for those carrying any one of RTK/Ras mutations was 60±11.9% compared with 81±10.0% for those without mutation (p=0.263); and the 5-year EFS was 61±11.7% for mutation+ compared with 75±10.9% for mutation- patients (p=0.444). Conclusions. Our study showed that 53% of childhood CBF AML had RTK/Ras mutations, with c-KIT being the most common (41%). Patients carrying c-KIT mutations relapsed with the identical patterns indicating that c-KIT mutations play a crucial role in the leukemogenesis in a subset of CBF AML.

Background. During the last decade CML has paved the way for tyrosine kinase-targeted therapy. Recently, several groups have demonstrated the pivotal role of the acquired V617F Janus kinase 2 (JAK2) mutation in Ph– Chronic Myeloproliferative Disorders, indicating a novel potential therapeutic target in CMPDs. Although this mutation has been described in the major CMPDs subtypes, controversy still remains about its exact prevalence in each subcategory. This could partially indicate the difficulties of achieving a precise diagnosis relying on the current WHO and FVSG diagnosis criteria. On the other hand, different technologies characterized by various sensitivity and specificity have been used in the phase I studies, highlighting the need for standardized, accurate and sensitive assays. Aims. We describe here a new V617F JAK2 mutation screening approach. The analytical properties of this assay are described and compared with the traditional PCR sequencing strategy, site-specific restriction analysis and ARMS PCR followed by slab-gel electrophoresis. Finally, we report our experience in genotyping 20 controls and 222 patients sent to our lab for typical and atypical MPD diagnosis. Clinical parameters, as well as previously described clonality assay, i.e. granulocytes PRV-1 expression, were evaluated in regard of the JAK2 V617F genotype. Methods. We developed a new V617F JAK2 mutation screening assay which combined the previously described amplification refractory mutation system (ARMS) and capillary electrophoresis performed with the Agilent 2100 Bioanalyzer apparatus (ARMS-cap). Serial dilutions of 100% V617F-homozygous HEL cell line into non mutated Jurkat cell line were assessed by ARMS PCR, ARMS-cap, PCR sequencing and BsaXI site-specific restriction analysis. The sensitivities of these tools were compared.

Results. ARMS PCR followed by slab-gel electrophoresis presented a JAK2 V617F detection sensitivity ranging from 1/32 (DNA) to 1/256 (RNA), which is far better than the PCR sequencing resolution (1/8 to 1/16). BsaXI cleavage improved the sensitivity when starting from DNA (1/64-1/128) whereas its best sensitivity level was the same as ARMS RNA PCR (1/256). ARMS-cap, offering a resolution of 1/64 (DNA) and 1/512 (RNA), improved the sequencing approach as well as the ARMS PCR followed by slab-gel electrophoresis. These results were in the same range than the BsaXI cleavage assay (1/128-1/256). Using this tool to assess our cohort of patients, we found the following incidence of JAK2 V617F mutation: PV, 90% (18/20); TE, 44% (12/27); IMF, 80% (4/5) and aCMPD, 24% (16/66). None of the 10 ALL; 10 de novo AML, 14 primary AML, 10 NHL, 10 HES/CML, 25 MDS, 11 CMMI were found to be mutated whereas we found 1 CNL and 4% MDS (1/25) harboured the mutation. Conclusions. JAK2 ARMS PCR assay combined with capillary electrophoresis represents a new and sensitive assay for an accurate V617F JAK2 mutation screening. The analytical properties of this assay overcome the classical PCR-sequencing or ARMS PCR followed by slab-gel electrophoresis approaches. Whereas this approach only slightly improve the raw sensitivity level achieved by BsaXI site-specific restriction analysis, electrophoresis followed by automated data analysis and recording offers an objective and reproducible tool for V617FJAK2 mutation screening.
**0059**

**SENSITIVE DETECTION OF C-KIT POINT MUTATIONS IN PATIENTS WITH MASTOCYTOSIS BY D-HPLC**


Background. The majority of patients with systemic mastocytosis (SM) are associated with an activating mutation in codon 816 of c-kit (CD117), a tyrosine kinase receptor on the surface of mast cells. This abnormality is regarded as being causative for the pathogenesis of the disease and is a potential target for therapeutic intervention. The sensitivity of screening procedures for mutations by direct sequencing might be compromised by a small proportion of malignant cells in the bone marrow (BM) sample. Therefore sensitive methods are required for diagnosis and surveillance of pts during therapy. Imatinib inhibits the c-kit tyrosine kinase at pharmacological doses with an IC50 of 0.1 µM, but it does not affect D816V mutations. Recent in vitro data suggest that both dasatinib, nilotinib (AMN107) and midostaurin (PKC412) have inhibitory effects on c-Kit D816V mutant cells. Aim. We sought to set up a sensitive strategy to detect c-kit mutations in BM and peripheral blood (PB) samples using D-HPLC (denaturing high-performance liquid chromatography) and conventional direct sequencing. Methods. D-HPLC has been established using serial dilutions of D816V c-kit positive HMC-1 cells in a background of NB4 cells harboring wildtype c-kit. The technique was then applied to 79 pts fulfilling the WHO criteria for SM. In case of a positive D-HPLC signal, c-kit exon 17 was sequenced to confirm the mutation. Results. D-HPLC was optimized to detect down to 0.1-0.5% HMC-1 cells. In comparison, the detection limit for D816V point mutations by conventional sequencing was 10%. BM (n=79) and/or PB (n=7) samples from 77 pts (42 m, 35 f) have been investigated. Median age was 51 yrs (range 25-85). At diagnosis, D-HPLC was positive in BM samples from 70/79 cases (89%), conventional sequencing revealed the D816V mutation in 56 pts, one pt was positive for the D816H mutation. In addition to D816V, an I798I polymorphism was observed in one pt. The analysis of PB only revealed D-HPLC positivity in 5/7 pts with a consecutive detection of a D816V mutation in three pts. Conclusions. (i) D-HPLC combined with conventional sequencing is a reliable and sensitive method to detect c-kit mutations in the majority of pts with SM. (ii) The method is eligible for the surveillance of pts during therapy with novel tyrosine kinase inhibitors.

**0060**

**MUTATIONAL SCREENING IN A POPULATION OF HAEMOPHILIA A SUBJECTS FOLLOWED AT THE HAEMOPHILIA CENTRE IN CENTRO HOSPITALAR DE COIMBRA**


**Centre Hospitalar de Coimbra, COIMBRA, Portugal**

Introduction. Haemophilia A (HA) is an X-linked hemorrhagic disorder associated with blood coagulation factor VIII (FVIII) deficiency. FVIII gene mutations type are closely correlated with the FVIII activity levels, however, the same mutation can generate different phenotypes and not all the haemophilia patients with FVIII levels <1% bleed with the same intensity and frequency. Aim. In order to provide the HA carriers identification and eventual prenatal diagnosis, we performed the FVIII gene molecular studies in the HA patients followed at our Haemophilia Centre. In this study we present the mutations found, their correlation with the severity of the disease and the development of FVIII inhibitors. In the severe haemophiliacs group we screened the Factor V Leiden (FVL) and prothrombin G20210A variant (FRT G20210A) to evaluate their role as phenotype modulating factors. Material and Methods. S3 proposita with HA, and 11 affected relatives, classified as severe (S3), moderate (S2) and mild (16) according to ISTH criteria. FVIII gene molecular studies were performed in 24 and intron 1 rearrangements were studied by PCR techniques; other mutations were screened by direct sequencing of PCR fragments spanning the promoter region, all the exons and the intron exon boundaries. The NHL and FRT G20210A were screened by Multiplex Allele-specific PCR. Results. Twenty nine different mutations were identified in the HA patients, 15 of which have not been previously reported in HAMSteRS. In the group of severe HA patients (n = 32) seventeen (53%) have the IVS22 inversion, two (6%) have the IVS1 inversion, I has a missense mutation, 2 have splicing mutations, 4 have small deletions, 2 have large deletions and 3 have insertions. In one patient we are still looking for the mutation. Missense mutations were the most common (81%) among the moderate and mild HA patients (n=21). In this group we found a frameshift mutation, due to the small deletion c.3637delA p.I1194fsX4 in exon 14, in one haemophilia with c.FVIII-199. Five out of 35 severe HA patients developed FVIII inhibitors: two, high responders, carry the IVS22 inversion (2/14), 2 have the IVS1 inversion (2/3) and 1 has a large deletion (1/2). One mild haemophilic with a missense mutation developed inhibitors and is a lower responder. Four severe HA carried prothrombotic risk factors (4/35): 2 have FVL (one is homozygous) and 2 have the FRT G20210A. Conclusions. In our HA patients, the FVIII gene mutation is mostly associated with gene rearrangements (IVS22 and IVS1) (19/32). Six out of 7 frameshifts identify are responsible for premature stop codons. The exception is the deletion c.3637delA p.I1194fsX4, associated with a mild phenotype, in which a reading frame correction probably occurs at the mRNA level. In the moderate and mild phenotypes the majority of mutations identified are missense transitional mutations. The mild haemophilic who developed inhibitors after treatment for surgery, has a mutation near an antigenic determinate region of FVIII. Amelioration of the phenotype is evident in the patient homozygous for FVL, as he only needs 2-3 treatments per year and his first hemorrhagic episode was traumatic, at the age of 5.

Table 1. New mutations identified at the Haemophilia Centre in CHC.

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<td>Promotor-3</td>
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<tr>
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<td>nt (5962-5964 del)</td>
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<td>Mild</td>
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<tr>
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<td>-</td>
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<tr>
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<td>1T at codon 24</td>
<td>Frameshift</td>
<td>A1</td>
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<td></td>
<td>14</td>
<td>Duplic 15 bp (nt 836-840)</td>
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rt: nucleotide; del: deletion; duplic.: duplication; bp: base pair; cd: codons.

**0061**

**MOLECULAR CLINICAL CORRELATION OF G6PD DEFICIENCY IN WESTERN SAUDI ARABIA**

S.K. Al Jaouni

King Abdulaziz University, JEDDAH, Saudi Arabia

**Background.** High frequencies of Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency have been reported in most countries in the region. In Saudi Arabia, G6PD deficiency exists at variable frequency in different provinces of the country. G6PD deficiency may cause neonatal jaundice, siasis, hemolytic anemia (fervius) following consumption of broad beans, and stress oxidative hemolysis occasionally can cause severe hemolytic anemia following treatment with specific drugs or participated by infection. Aims. The aim of this study was to investigate the mutation spectrum and clinical significance of the G6PD gene among population in western Saudi Arabia. Methods. A total of 492 unrelated Saudi volunteers of both sexes (224 male, 268 female) were screened for G6PD deficiency by quantitative Methods. DNA was extracted from 42 G6PD-deficient Saudi subjects (36 males and 6 female). These subjects were screened for gene mutations using polymerase chain reaction/restiction fragment length polymorphism (PC- RFLP). Screening included Mediterranean563C>T, and Aures1437C and A1420G A. Results. G6PD Mediterranean mutation 563C>T accounts for most cases of G6PD deficiency in Saudi nationals followed by G6PD Aures1437C representing 38% and 17%, respectively. A new polymorphic variant (17%) has been identified during the course of this study although none of the samples showed A- mutation. Overall fre-
Prevalence of NMP1 Mutation in AML and MDS


Hospital Universitario Doctor Negrin, LAS PALMAS DE GRAN CANARIA, Spain

Background. A mutation on the nucleophosmin gene (NMP1) has been recently described on 25-35% of all AML patients. NMP1 is located on the 5q35 chromosomal region and this mutation, found on exon 12 of the NMP1 gene, causes the translocation of the NMP1 protein to the cytoplasm. This mutation is also linked to a wide spectrum of morphologic AML subtypes, normal karyotype, a better response to induction chemotherapy, and a higher prevalence of FLT3-ITD. Aims. To analyse the prevalence and prognostic value of the NMP1 mutation on AML and MDS patients.

Methods. A total of 55 patients with non-lymphoid AML were studied. These patients were previously examined for the FLT3-ITD mutation (5 M0, 8 M1, 10 M2, 12 M4, 4 M5, 1M6 and 15 not determined according to FAB criteria) and 12 MDS. The average age of the patients ranged from 18 to 95 years. Screening for NMP1 mutation was undertaken by the LightCycler system according to the Schnittger et al technique. (Blood 2005). Results. The NMP1 mutation was detected in 30.9% of patients with AML (15 out of 55), being this prevalence higher than that found for the FLT3-ITD, in the same population (23.8%). The 17 NMP1+ AML distributed as follows: 8 M1-M2 (44%), 4 M4-M5 (25%), and 5 not determined (28.5%). 68% of the NMP1+ patients had a normal karyotype, while 9% of them had cytogenetic anomalies. The FLT3-ITD mutation was found in 41.2% of the NMP1+ AML cases. The global mortality was analyzed with disregard to any risk factors, with a mortality of 67% in the NMP1+/FLT3-ITD- group standing in clear contrast to a 100% death rate in the NMP1+/FLT3-ITD+ group. Within the limits of the group studied, no significant prevalence of the NMP1 mutation was observed regarding the sex of the patient. Conclusions. 1) The prevalence of the NMP1 mutation in our LMA group was 30.9%, and contrary to what has been described in literature, a higher incidence on M4 and M5 subtypes was not found. As recently published by Thiede C et al., a higher incidence on M4 and M2 subtypes was found. 2) The NMP1 mutation prevalence was high among OS patients, which to our knowledge, had not been previously described in literature. 3) The other genetic anomaly most commonly associated to the NMP1 mutation was FLT3-ITD. 4) The screening for this mutation could be useful in the future when grouping patients with normal karyotype in a subgroup with better prognostic. 5) The high incidence of the NMP1 mutation in patients with MDS could suggest a role for this gene in the pathogenesis of this disease.
trast, BCL1 breakpoints were scattered within a 15 kb sequence at a distance of approximately 90 kb downstream of the MTC region. Two breakpoints localized within the promoter region of cyclin D1 at a distance of 1360 and 2870 bp from the transcription start site. In IGH, one translocation involved a D2-2/JH6 rearrangement, while the others showed involvement of either JH4 (4 cases) or JH6 (1 case). In summary, LDI-PCR was instrumental in the identification of an additional 3 breakpoints in BCL1 (80%) and 3 in BCL2 (50%) in patients who showed no breakpoints at the main cluster regions. Overall, BCL2-IGH fusion was detected in 17 patients with FL (85%) and BCL1-IGH fusion was found in 9 patients with MCL (82%). We conclude that M-PCR analysis for a fast determination of recurrent BCL1 and BCL2 breakpoints combined with LDI-PCR for assessment of variant breakpoint locations, is an effective approach for the diagnosis of t(11;14) and t(14;18) in non-Hodgkin lymphomas.

### Table 1. BCL1- and BCL2-IGH sequences identified by LDI-PCR.

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### Molecular targeting and gene therapy

#### 0065

**THE EFFECT OF HISTONE DEACETYLASE INHIBITORS ON B-CELL DIFFERENTIATION IS NOT UNIQUE FOR TEL-AML1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIAS**

M.L. Den Boer,1 W.A.G. Stams,1 H.B. Beverloo,2 E.R. Van Wering,2 G.E. Janka-Schaub,1 R. Pieters1

1Erasmus MC-Sophia Children’s Hospital, ROTTERDAM, Netherlands; 2Erasmus MC, ROTTERDAM, Netherlands; 3Dutch Childhood Oncology Group, THE HAGUE, Netherlands; 4COALL study group, HAMBURG, Germany

The TEL-AML1 fusion is the most frequently found translocation in childhood pediatric acute lymphoblastic leukemia (ALL). TEL-AML1-positivity is associated with L-asparaginase sensitivity and favorable clinical outcome in pediatric ALL. The fusion protein is thought to recruit co-repressors and histone deacetylases (HDACs), which in turn lead to transcriptional repression of AML1-responsive genes. FK228 (depsipeptide) is an HDAC inhibitor that affects the chromatin configuration and, as a consequence, affects gene transcription. We investigated whether HDAC inhibitors may be used to target TEL-AML1 positive ALL in children. To this aim, the in vitro cytotoxic effect of FK228 as single agent and in combination with L-asparaginase was tested in leukemic cells obtained from TEL-AML1 positive and negative children with ALL at initial diagnosis (by MTT-assay). In addition, the effect of FK228 on B-cell differentiation was analyzed by monitoring changes in differentiation marker expression using flow cytometry. Our data indicate that leukemic cells of 14 TEL-AML1 positive and 15 negative B-lineage ALL cases were both more in vitro sensitive to FK228 than normal bone marrow cells (p<0.05). FK228 exposure induced the differentiation of leukemic cells into more mature precursor B-cells. However, the in vitro cytotoxicity of FK228 did not differ between both ALL subtypes. FK228 had an additive but not a synergistic effect on in vitro sensitivity to L-asparaginase in both ALL subtypes. In conclusion, FK228 induces differentiation in children with B-lineage ALL, but its effect is not selective for TEL-AML1 rearranged B-lineage ALL only.

#### 0066

**IMATINIB MESYLATE CAN INDUCE MOLECULAR COMPLETE REMISSION IN IDIOPATHIC HYPEREOSINOPHILIC SYNDROME. A PHASE II MULTICENTRIC ITALIAN CLINICAL TRIAL**

M. Rondoni,1 D. Cilloni,1 E. Ottaviani,1 P. Piccaluga,1 M. Malagola,1 F. Messa,1 E. Gottardi,1 G. Saglio,2 S. Paolini,1 F. Pane,2 P. Puccini,2 M. Baccarani,1 G. Martelli1

1University of Bologna, BOLOGNA, Italy; 2Div. of Hematology, TURIN, Italy; 3Inst. of Hematology Seràgnoli, BOLOGNA, Italy; 4Ematologia, Università di Brescia, BRESCIA, Italy; 5Polichirico S. Matteo, Univ. di Pavia, PAVIA, Italy; 6University La Sapienza, ROME, Italy; 7Ceinge Biotecnologie Avanzate, NAPLES, Italy; 8Novartis Farma, ORIGGIO (VA), Italy

Idiopathic hyper-eosinophilic syndrome (HES) is a rare hematological disorder characterized by persistent peripheral blood greater than 1,500 cells/mL lasting for more than 6 months, in the absence of other apparent aetiologies for eosinophilia with signs and symptoms of organ involvement. HES may be a reactive condition or a chronic myeloproliferative disorder with evidence of clonal proliferation, in which latter case it is usually referred to as chronic eosinophilic leukemia (CEL). Patients with HES generally have a poor prognosis, but the course of the disease may be variable. Severe visceral complications, including cardiopathies, are common and are often fatal illness. Treatment of HES includes corticosteroids, chemotherapeutic agents and, more recently, interferon-α (IFN-α). Cools et al. reported the involvement of PDGFRα, fused with FIP1L1, in a number of HES patients responsive to imatinib therapy. We treated with imatinib mesylate (100 to 400 mg, daily) 59 patients affected by Hyper-Eosinophilic Syndrome (HES) enrolled in a multicentric Italian phase 2 clinical trial. All the patients were studied by molecular analysis for expression of FIP1L1-PDGFRα, TEL-PDGFRα, FGFR1-BCR and BCR-ABL chimerical transcripts. 23 patients (39%) were positive for the FIP1L1-PDGFRα rearrangement. Rapid, hematological complete responses (HCR) were recorded after one month of therapy in all FIP1L1-PDGFRα positive. In 36 patients resulted negative for FIP1L1-PDGFRα rearrangement we observed 8 (22%) hematological improvement (HI) and one HCR (HI-HCR 25%). Furthermore, a molecular complete remission (defined as the disappearance of FIP1L1-PDGFRα at qualitative RT-PCR evaluation) was recorded in all but one patients of the 23 valuable after three months of therapy, and we
obtained molecular remission in 18 out of 21 evaluable patients after six months of therapy. After one year of imatinib therapy, all 12 evaluable patients showed disappearance of the rearrangement. No significant toxicity was seen during the treatment. The median follow up was 7 months (range: 2-41). This is the largest series of HES patients treated with Imatinib with strong evidence of hematological and molecular effectiveness and absence of significant toxicity. This phase II study supports the use of Imatinib as first line therapy in FIP1L1-PDGFRA rearrangements positive HES patients. Acknowledgments. COFIN 2003 (Molecular therapy of Ph+ leukemias), by FIRB 2001, by the University of Bologna (60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R.), by Fondazione Del Monte of Bologna and Ravenna (Italy) and A.I.L. grants, LeukemiaNet grants.

0067

SIRNA VERSUS LOCKED NUCLEIC ACID RNA ANTAGONISTS: STAY SINGLE!


Sanitas Pharma, COPENHAGEN, Denmark

By the incorporation of Locked Nucleic Acid (LNA), a conformational analogue of RNA, single-stranded LNA/DNA oligonucleotides can be created which mimic RNA and have unrivalled gene silencing ability. Much discussion has centred on the utility and benefits of siRNA in both target validation and as a therapeutic option. This has been driven by significant publications including that of Soutcheck et al. (Nature 432, 173-177 2004) which demonstrated liver targeting as well as very high efficiency. Thus siRNA against ApoB was tethered to a cholesterol moiety. We therefore sought to compare single-stranded oligonucleotide antagonists containing LNA with siRNA against this target in both in vivo/in vitro settings. The same motif used in the Soutcheck study was targeted with the LNA molecule, and the activity of unmodified siRNA was compared to the cholesterol-linked and native LNA molecules in their ability to down-regulate ApoB expression. LNA (SPC3197) inhibited ApoB expression by 90% while at an equimolar concentration siRNA was ineffective in the liver and jejunum. Cholesterol linked siRNA was partially effective in the jejunum (50% reduction in mRNA). Only the LNA mediated inhibition of ApoB expression was paralleled by decreases in serum cholesterol in the host animal. In a second model, siRNA molecules targeting Hif-1α mRNA (Yu et al. Lab Invest 84, 553-561 2004) were compared to a single-stranded LNA/DNA mixture targeting Hif-1α, SPC2968. In vivo analyses of these 2 molecules were equally potent. However, in a murine model the increased half-life of the LNA molecules translated to a potent inhibition of Hif-1α as measured by QPCR. This effect was observed in jejunum and liver, and persisted for at least 4 days. Hif-1α inhibition mediated by siRNA was not seen in any tissue analysed. Overall we demonstrate clear superiority of single chain LNA based RNA antagonists over siRNA for the use as therapeutic molecules.

0068

DUAL SRC/ABL INHIBITOR SKI-606 BINDING MODE IN BCR-ABL KINASE HYPOTHESIZED ON THE BASIS OF MOLECULAR DOCKING STUDIES

S. Soverini,1 G. Tasco,2 T. Grafone,3 S. Colarossi,4 A. Gnani,1 G. Rosti,1 F. Castagnetti,4 F. Palandrini,1 M. Rondoni,1 A. Poro,1 I. Iacobucci,1 M. Ambable,1 M. Baccarani,1 R. Casadio,1 G. Martinelli1

1Universita di Bologna, BOLOGNA, Italy; 2Biscomputing Group, Univ. of Bologna, BOLOGNA, Italy; 3Inst of Hematology Seragnoli, BOLOGNA, Italy

Background. SKI-606 is a novel 4-anilino-3-quinolinecarbonitrile Src kinase inhibitor. SKI-606 has been shown to be a potent antiproliferative and proapoptotic agent when tested on Bcr-Abl-positive cell lines. The remarkable efficacy of SKI-606 against chronic myeloid leukemia (CML) cells in culture was mirrored by its activity in vivo against CML xenografts: K562 tumors regressed in nude mice when SKI-606 was administered per os once daily over a 5-day period. The crystal structures of the Bcr-Abl kinase domain complex with SKI-606, solved independently by our group and by the lab of H.A. Thiele, recently shed new light on the mode of binding of SKI-606. However, so far, the molecular details of this unique interaction and the mode of binding of the inhibitor to the Bcr-Abl kinase domain are not yet known. Moreover, there are currently no published data on the ability of SKI-606 to bind and efficiently inhibit the Bcr-Abl mutants known to confer resistance to imatinib. Aims. In this study, we used a molecular docking approach to a) determine SKI-606 binding mode to the wild-type (wt) form of the Bcr-Abl kinase; b) hypothesize SKI-606 binding mode to the more frequent, clinically relevant Bcr-Abl mutants known not to be inhibited by imatinib; c) predict which novel mutant forms might emerge and interfere with SKI-606 binding. Methods. Modelling of the human Abl kinase was performed with the program Modeller v7.7 (http://saliab.org/modeller/) adopting the highly related Mus musculus Abl homologue as a template structure (PDB: 1OJP, 0.175nm resolution). Chemsheet (http://www.acdlabs.com) was used to build a three-dimensional model of SKI-606. Flexible docking of the ligand to the protein was performed with Autodock v3.0 (http://www.scripps.edu/\~dbs/autodock.html). Results. SKI-606 binds the activation loop in the active (open) and inactive (closed) conformation (the latter is the one to which imatinib binds). According to our results, the interaction between SKI-606 and Bcr-Abl seems to be more stable when the activation loop is in the inactive conformation. The consequent structural study of SKI-606 modeled into wt-Bcr-Abl ATP binding site highlighted the variation located within a spherical environment of 0.5nm centered on SKI-606: Y253, T315 and F359 (residues numbered according to ABL exon 1 splice variant). The binding of SKI-606 to the eight Bcr-Abl mutants which are most frequently implicated in clinical resistance to imatinib mesylate was also studied: G250E, Y253H, E255K, T315I, M351T, F359V, H396R. Our results indicated that SKI-606 retains the ability of efficiently binding all the above mentioned Bcr-Abl variants with the exception of the T315I mutant. Finally, we identified six potential residues around SKI-606 that, if mutated, could potentially be able to interfere with the SKI-606/Bcr-Abl interaction: a) the charged residues K271, D281 and H304; b) the hydrophobic/alphahelical residues V299, A280 and M319. Conclusions. Pre-clinical data suggest that SKI-606 is a promising second-generation kinase inhibitor with potent antiproliferative and proapoptotic effects on CML cells. Our docking experiments indicate that SKI-606 may prove effective in imatinib-resistant patients since it is expected to retain the ability to bind several Bcr-Abl mutant forms. A trial is about to start with BCR-ABL and Philadelphia-positive acute lymphoblastic leukemia. Supported by European LeukemiaNet, COFIN 2003, FIRB 2001, AIRC, AIL, Fondazione del Monte di Bologna e Ravenna.

0069

EFFECTIVE INHIBITION OF BCR/ABL KINASE WITH TETRAMERIZATION DOMAIN DERIVED PEPTIDES MAPS TO COILED-COIL HELIX ALPHA-2

T. Beissert, V. Kaburova, A. Mian, O.G. Ottmann, D. Hoelzer, M. Ruthardt

University Clinic Frankfurt, FRANKFURT, Germany

Background. As a result of the (9,22), more than 95% of CMLs and 20-25% of adult ALLs express the p210(BCR-ABL) or the p185(BCR-ABL) fusion protein respectively. The BCR portion of the fusion protein harbors an N-terminal coiled-coil (CC) domain which induces tetramerization of BCR. The CC contains two α helical motifs - Helix α-1 and Helix α-2 - and assembles to dimers with antiparallel orientation which associate to form tetramers. Helix α-2 contributes the majority of the dimer and tetramer interface. 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. Targeted inhibition of BCR/ABL by the ABL kinase inhibitor Gleevec induces apoptosis in BCR-ABL transformed cells and leads to complete remission in CML and ALL patients. However, a large portion of ALL patients and CML patients in blast crisis relapse and acquire Gleevec resistant BCR/ABL mutations. It has been shown that Gleevec binds to the inactive conformation of the ABL-kinase which in case of the BCR/ABL fusion protein is present in monomers. We have previously shown that CC-derived peptides that interfere with BCR/ABL tetramer formation reduce the kinase activity and the transformation potential of BCR/ABL in vivo. Consequently the expression of CC-derived peptides results in an increased sensitivity of BCR/ABL expressing cells towards Gleevec by shifting the intracellular equilibrium towards BCR/ABL monomers. These CC-derived peptides provided a probe of concept that the tetramerization domain is a potential therapeutic target for BCR/ABL positive leukemia. Aims. Here we studied the inhibitory effects of the CC subdomain Helix α-2 which harbors the majority of the protein-protein interface. We aimed to i) reduce the molecular weight of the inhibitory peptides and ii) thereby to map the inhibitory effects of CC-derived peptides to CC-substructures. Methods. Helix-2-GFP fusion peptides were coexpressed with BCR/ABL in the IL-3 dependent cell line Ba/F3 using a bicistronic retroviral vector. The interaction of Helix-2 and BCR-ABL was checked by pull-down assays. The IL-3 independent proliferation of BCR/ABL expressing Ba/F3 cells in presence of Helix-2 was studied in presence and absence of Gleevec. Anti-phospho-ABL specific immunoblotting was used to reveal the BCR/ABL autophosphorylation in these cells. All studies were performed with the previously published CC-GFP fusion
peptide as control. Results. Here we report that i) Helix-α-2 interacts with BCR/ABL to the same extent as the complete CC domain; ii) Helix-2 like CC decreases the autophosphorylation of BCR/ABL in transduced Ba/F3 cells; iii) Helix-2 increases the sensitivity of IL-3 independent BCR/ABL expressing Ba/F3 cells towards Gleevec to the same extend as CC; iv) Helix-2 shows no inhibitory effects on IL-3 independent Ba/F3 cells expressing activated c-Kit. Conclusion. Taken together these results show that Helix-α-2 specifically targets the tetramerrization-interface of BCR/ABL. The peptides inhibit the ABL-kinase activity and enhance the inhibitory effects of Gleevec. This study provides important information for the use of Helix-α-2 as lead structure in the rationale design of small molecule inhibitors of BCR/ABL tetrarmerrization.

0070

SINGLE-AGENT SU11657, A NOVEL FLT3 INHIBITOR, SHOWS BIOLOGIC Activity IN ACUTE MYELOID LEUKEMIA CELLS IN VITRO

T. Grafoe,1 E. Ottaviani,2 M. Palmsano,3 M. Mancini,1 N. Testoni,1 M. Amabile,1 C. Terragna,1 A. Poero,1 M. Renzulli,1 S. Soverini,1 S. Colarossi,1 I. Iacobucci,1 M. Baccarani,2 G. Martinelli2

1Inst. of Hematology 'Seraglio', BOLOGNA, Italy; 2Inst. of Hematology 'Seraglio', Bologna, Italy

Background. Fms-related tyrosine kinase3 (FLT3) is one of the most commonly mutated gene in human acute myeloid leukemia (AML) and has implicated in its pathogenesis. Constitutive activation of the FLT3 receptor tyrosine kinase, has been linked either by internal tandem duplication (ITD) of the juxtamembrane region or by point mutation in the second tyrosine kinase domain (TKD). Aims. The purpose of the study was to evaluate, in vitro, the effect and the biological activity of SU11657 (Pfizer), a new compound FLT3 kinase inhibitor. SU11657 was investigated on human cell lines from AML patients (MV4-11 and HL-60) and blast samples from patients, using a wide range of concentrations (1nM-10mM). Methods. FLT3 expression levels were evaluated by flow cytometry. Furthermore, to evaluate the effect of SU11657 we analyzed the cytotoxicity, induction of apoptosis and inhibition of cell proliferation by flow cytometry. The antiproliferative and cytostatic effects of SU11657 were confirmed by analysis of signal transduction. HL-60 cell line served as a control it expresses a wild type receptor. MV4-11 is a cell line that expresses a naturally internal tandem duplication (ITD) in homozygous form. Results. In HL-60 does not show relevant effect after treatment with SU11657. Instead, in MV4-11 we observed a decrease dose-dependent in cell viability after treatment with SU11657. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) has been shown to induce apoptosis in a number of cell types, including cancer cells, normal cells, and immune cells. TRAIL is a member of the TNF superfamily and exerts its activity by engaging two cell surface receptors: two death receptors (DR4/TRAIL-R1 and DR5/TRAIL-R2) and three decoy receptors (DcR1/TRAIL-R3, DcR2/TRAIL-R4 and osteoprotegerin/OPG). Although these receptors are characterized by a high sequence homology in their extracellular domains, only DR4/TRAIL-R1 and DR5/TRAIL-R2 contain a functionally active cytoplasmic death domain that allows an apoptotic response to TRAIL stimulation. The biological significance of TRAIL as mediator of innate and specific immunity against transformed and virus-infected cells has been clearly documented by several reports. As membrane-bound TRAIL seems to have tumour-selective pro-apoptotic activity, the potential use of recombinant soluble forms of this molecule as cancer therapeutic is presently being exploited in pre-clinical and clinical studies. TRAIL has been shown to be effective in a wide range of xenograft and cell line models. The main advantages of using soluble TRAIL are that the drug is easily manufactured and has no effect on the body's own TRAIL receptors.

0071

POTENTIAL THERAPEUTIC APPROACH OF RECOMBINANT TRAIL IN CML IN BLAST CRISIS


Faculty of Medicine of Coimbra, COIMBRA, Portugal

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) has been shown to induce apoptosis in a number of tumour cell lines as well as in some primary tumours whereas cells from most normal tissues are highly resistant to TRAIL-induced apoptosis. TRAIL is a member of the TNF superfamily and exerts its activity by inducing two complementary death receptors: death receptors (DR4/TRAIL-R1 and DR5/TRAIL-R2) and three decoy receptors (DcR1/TRAIL-R3, DcR2/TRAIL-R4 and osteoprotegerin/OPG). Although these receptors are characterized by a high sequence homology in their extracellular domains, only DR4/TRAIL-R1 and DR5/TRAIL-R2 contain a functionally active cytoplasmic death domain that allows an apoptotic response to TRAIL stimulation. The biological significance of TRAIL as mediator of innate and specific immunity against transformed and virus-infected cells has been clearly documented by several reports. As membrane-bound TRAIL seems to have tumour-selective pro-apoptotic activity, the potential use of recombinant soluble forms of this molecule as cancer therapeutic is presently being exploited in pre-clinical and clinical studies. TRAIL has been shown to be effective in a wide range of xenograft and cell line models. The main advantages of using soluble TRAIL are that the drug is easily manufactured and has no effect on the body's own TRAIL receptors.
ing on the expression of the oncogenic Kras2LSL allele. We and others have bred the Kras2LSL mice with mice harboring an interferon-inducible Mx1-Cre transgene and found that induction of Cre by a single injection of an interferon-stimulant-activates the Kras2LSL allele in hematopoietic cells, causing a rapidly progressing, lethal MPD. We then bred Kras2LSL Mx1-Cre mice on a background of homozygosity for a condition (i.e., Rce1flx/flxKras2LSLMx1-Cre), in which induction of Cre simultaneously activated the Kras2LSL allele and inactivated Rce1. Controls were mice harboring a single conditional Rce1 allele (i.e., Rce1flx/+Kras2LSLMx1-Cre) in which Cre eliminated 50% of Rce1 expression. Results. As expected, expression of Cre in the control Rce1flx/+Kras2LSLMx1-Cre mice resulted in a rapidly progressing MPD with splenomegaly and overproduction of mature monocytes and granulocytes and an inability of hematopoietic cells to form colonies in methylcellulose in the absence of growth factors. In stark contrast, the inactivation of Rce1 in K-RasG12D-expressing cells accelerated the development of MPD, dramatically increased white blood cell counts and reduced survival. Moreover, Rce1-deficient K-RasG12D-expressing hematopoietic cells produced more and larger colonies in methylcellulose. The inactivation of Rce1 further resulted in a massive release of immature myeloid cells from the bone marrow which likely contributed to the early demise of the mice. Conclusions. Our hypothesis was that inhibition of Rce1 may be an effective strategy to block the development of hematologic malignancies. This hypothesis, which is based on a comprehensive in vitro studies, was dashed by the current experiments. We find that inhibition of Rce1 in vivo actually accelerates the development of K-Ras-induced MPD. Future studies will evaluate the mechanism behind this totally unexpected result.

0073

OPTIMIZING T CELL RECEPTOR GENE TRANSFER TO VIRUS-SPECIFIC T CELLS FOR CLINICAL APPLICATION

M. Griffioen, H.M. van Egmond, M.A.W.G. van der Hoon, R.S. Hagedoorn, M. Kester, R. Willemze, J.H.F. Falkenburg, M.H.M. Heemskerk
Leiden University Medical Center, LEIDEN, Netherlands

Patients with relapsed or resistant hematological malignancies after allogeneic stem cell transplantation (alloSCT) can be successfully treated by donor lymphocyte infusions (DLI). However, Graft-versus-Host Disease (GVHD) remains an important cause of morbidity and mortality. We previously showed that functional T cells with redirected anti-leukemic reactivity can be generated by transfer of T cell receptors (TCRs) specific for minor histocompatibility antigens (mHags) to virus-specific T cells. Adoptive transfer of virus-specific T cells to patients treated with alloSCT has a minimal risk for GVHD. The aim of this study is to develop a method for the generation of TCR-transduced virus-specific T cells for cellular immunotherapy of patients with relapsed hematological malignancies after alloSCT. Various single retroviral vectors were constructed containing the α and β chains of the HA-2 TCR linked by an IRES or 2A-like sequence. Introduction of a 2A-like sequence allows additional linkage of the human low affinity nerve growth factor receptor (NGFR) or CD20 selection marker genes by an IRES element. Inclusion of a selection marker gene allows purification of TCR-transduced cells, thereby reducing the risk for GVHD. The human CD20 gene also functions as suicide gene, allowing elimination of transduced cells in vivo when undesired side effects, like GVHD, occur. Since tetrameric complexes are currently not GMP-grade available, we explored the feasibility of using synthetic peptides for the generation of TCR-transduced virus-specific T cell lines. From various human individuals, CD8+ cells were isolated and stimulated with a mixture of CMV and EBV peptides. At day 3, CD8+ cells were transduced with the retroviral vectors encoding the HA-2 TCR. Due to selective expansion upon peptide stimulation, all cell lines were shown to contain high cumulative percentages of virus-specific T cells (20-80%) as well as TCR-transduced cells (10-50%) at day 8-12. Moreover, significant numbers of specific T cells were obtained, demonstrating that this strategy is feasible for adoptive cellular immunotherapy. Highest levels of TCR expression and HA-2-specific lysis were obtained with retroviral vectors containing two genes encoding the TCR α and β chains linked by an IRES or 2A-like sequence. Upon linkage of a third gene, TCR expression and HA-2-specific lysis were slightly (NGFR) or significantly (CD20) reduced. Since highly-enriched virus-specific T cells will be used for adoptive transfer, GVHD is not likely to develop and inclusion of a selection marker/suicide gene not strictly required. Therefore, for clinical applications, TCR-transduced virus-specific T cells, a retroviral vector containing two genes encoding the TCR α and β chains in the absence of a selection marker/suicide gene is preferable.

0074

DUAL SPECIFIC T CELLS CHANGE THEIR T CELL RECEPTOR (TCR) CELL SURFACE DISTRIBUTION UPON TCR TRIGGERING

J. Cavenagh, R.S. Hagedoorn, M. Hoogeboom, M.G.D. Kester, R. Willemze, J.H.F. Falkenburg, M.H.M. Heemskerk

St Bartholomew s Hospital, LONDON, United Kingdom; Leiden University Medical Center, LEIDEN, Netherlands

TCR transfer to engineer tumor specific T cells may be an alternative strategy for adoptive immunotherapy. When virus specific T cells are used for TCR transfer, we hypothesized that due to the latent presence of viral antigens the survival of TCR-transferred dual specific T cells will improve. However, repetitive stimulation of the endogenous TCR may lead to selection of dual specific T cells with high expression of the endogenous TCR and low expression of the introduced TCR. To address this issue, we used CMV-specific T cells that were transduced with the hematopoietic minor histocompatibility antigen HA-2 specific TCR. The dual specific T cells were repetitively stimulated either by their endogenous virus specific TCR or via the introduced HA-2 specific TCR and analysed by FACS after each stimulation. In time, the expression of the endogenous and introduced TCRA were measured with CMV and HA-2 tetrameric complexes converged. Repetitive stimulation of the endogenous TCR skewed the dual specific T cells towards a cell population that primarily expressed the endogenous TCR. In contrast, repetitive stimulation of the introduced TCR skewed the T cells towards T cells that primarily expressed the introduced TCR. However, this divergence in tetramer stainings appeared to revert quickly after stimulation via the other TCR, suggesting that this divergence was the result of a difference in TCR surface distribution and not of selective outgrowth of different T cells. To rule out that differences in tetramer stainings were the result of selective outgrowth, T cells were sorted after repetitive stimulation expressing primarily the endogenous or introduced TCR. These cells were subsequently stimulated on the endogenous or introduced TCR and analysed for TCR expression and functional activity. Results indicate that no selective outgrowth occurred, but that T cells change their TCR cell surface distribution dependent on which TCR is triggered. In conclusion, virus specific TCR-transferred T cells repetitively stimulated via their endogenous TCR phenotypically seemed to express primarily the virus specific TCR. However, when restimulated on the introduced TCR, T cells reverted into cells with high expression of the introduced TCR that exerted potent HA-2 specific anti-leukemic activity, indicating that these dual specific T cells are useful for clinical applications.

0075

RELEVANCE OF MEK/ERK ACTIVATION IN THE MAINTENANCE OF THE PML/RAR-ALPHA INTEGRITY IN ACUTE PROMYELOCYTIC LEUKEMIA

N. Barbarroja Puerto, E. Siendones Castillo, P. Buendia Bello, L.A. Torres Sanchez, F. Velasco Gimena, A. Torres Gomez, Ch. Lopez Pedrera
Hospital Universitario Reina Sofia, CORDOBA, Spain; Universidad Pablo de Olavide, SEVILLA, Spain

The mitogen-activated protein kinase MEK/ERK and phosphatidylinositol 3-kinase PI3K/Akt pathways are involved in proliferation, inhibition of apoptosis, differentiation and cell survival. These pathways are frequently activated in Acute Promyelocytic Leukemia (APL), and play a key role in the survival of neoplastic cells. The hallmark of APL is the t (15; 17), which leads to the expression of the PML/RARα fusion protein. PML/RARα is the central leukemia-inducing lesion in APL and is directly targeted by all-trans retinoic acid (ATRA) as well as by arsenic trioxide (ATO), both compounds able to induce clinical complete remissions. However, the precise intracellular mechanisms of action of ATRA and ATO remain unclear. The purpose of this study was to evaluate: 1) the effects induced by the downmodulation of MEK/ERK and PI3K/Akt pathways on the PML/RARα expression, and 2) the role of these pathways in the PML/RARα degradation induced by ATRA and low-dose ATO in APL cells. PML/RARα expression was analyzed by western blot after transient transfection of the promyelocytic cell line NB4 with the selective pharmacological inhibitors of MEK/ERK and PI3K/Akt pathways, PD98059 (20 μM) and LY294002 (20 μM) respectively, given either alone or in combination with ATRA (1μM) or ATO (0.5 μM). The inhibitor of the MEK/ERK pathway caused a significant degradation of PML/RARα, which was reversed after treatment with the specific caspase z-VAD-fmk (50 μM), indicating that PML/RARα degradation induced by downmodulation of MEK/ERK seems to be a mechanism dependent on caspase activation. In addition, the combined treatment with ATRA or
ATO and PD02059 further reinforced the PML/RARα degradation induced by ATRA or low doses of ATO, the other half of the cells were treated with arsenic trioxide and LY294002 reversed oncogene degradation induced by ATO alone, thus suggesting that the PI3K/Akt pathway might mediate the degradation of PML/RARα induced by low-dose ATO. Taken together our findings suggest that MEK/ERK activation might be responsible for the maintenance of the PML/RARα mRNA in APL cells. The present study supported the use of anti MEK/ERK reagents in clinical trials for the treatment of APL, either as single agents or in association with retinoids or arsenic compounds.

Supported by FIS 04/1291 and JA 0060/2005

**0076**

**TRANSCRIPTOME PROFILING OF PROTEASOME INHIBITOR-MEDIATED ANTITUMOR ACTIVITY IN EBSTEIN-BARR VIRUS-INFECTED NASAL NK LYMPHOMA CELLS**

J. Ohyashiki, Y. Zhang, R. Hamamura, N. Shimizu, K. Ohyashiki
Tokyo Medical University, TOKYO, Japan; Tokyo Medical and Dental University, TOKYO, Japan

**Background.** Natural killer (NK) cell lymphoma is frequently associated with Epstein-Barr virus (EBV), and activated NF-kappa B is a critical mechanism by which EBV-infected lymphoma cells are protected from apoptosis. The proteasome inhibitor, bortezomib, abrogates degradation of IkB, which blocks the transcriptional activity of NK-kB. This effect may account in part for anti-tumor effects in several types of cancer. Aim. To determine the precise link between inhibition of proteasomal degradation and induction of apoptosis on EBV-related NK cell malignancy. **Method.** By using DNA microarray (Affymetrix U133plus2.0 chip), we investigated the molecular pathway which may be linked to the anti-tumor effects of bortezomib on NK cell lymphoma cell line with EBV latency type II infection (designated as SNK-6). **Results.** Transcriptional profiling of bortezomib-treated SNK-6 cells involved downregulation of growth/survival signaling, up-regulation of molecules related to induction of apoptosis, as well as up-regulation of heat shock proteins and the ubiquitin/proteasome pathway, such as ubiquitin-specific peptidase 7 (USP7; herpes virus-associated). **Conclusion.** Our results demonstrated that proteasome inhibition elicits activation of multiple signaling pathways, and provides novel insight into the bortezomib mediated anti-tumor activity in EBV-associated NK lymphoma cells.

**0077**

**SIRNA-MEDIATED MLL-AF4 KNOCKDOWN AFFECTS TERT EXPRESSION**

J.G. Greil, M. Thomas, P. Geissner, O. Heidenreich
University of Heidelberg, HEIDELBERG, Germany; University of Tuebingen, TUEBINGEN, Germany

**Background.** The reciprocal chromosomal translocation t(4;11) (q21;q23) results in the expression of two fusion-proteins, MLL-AF4 and AF4-MLL, and marks a therapy-resistant infant acute lymphoblastic leukemia. Our results suggest that MLL-AF4 controls hTERT expression at least in part via HOXA7. The observed changes in methylation pattern of the hTERT promoter upon MLL-AF4 depletion supports a function of this leukemic fusion protein in the epigenetic control of gene expression. Analysis of intracellular pathways may not only elucidate the oncogenic action of MLL/AF4 but may also open the avenue for new treatment options by targeting MLL-AF4 key functions.

**This work was supported by the José Carreras Leukaemia Stiftung (DCLS-R03/10).**

**0078**

**PRENYLATION INHIBITORS MODULATE IL-6 AND IGF-1 DEPENDENT SIGNALING IN MULTIPLE MYELOMA CELLS**

M.A. Morgan, T. Sebil, D. Peest, A. Ganser, C.W.M. Reuter
Hannover Medical School, HANNOVER, Germany

**Background.** Multiple myeloma (MM) is a fatal hematologic malignancy associated with disruption of RAS-to MAP kinase (MAPK/ERK) signaling. IL-6 and IGF-1 promote malignant plasma cell proliferation through stimulation of MAPK and PI-3 kinase/akt signaling. Prenylation inhibitors such as farnesyltransferase inhibitors (FTIs), geranylgeranyl transferase inhibitors (GGTIs) and lovastatin block RAS post-translation-factor free medium (IC50s 1.3 µM, 1.8 µM and 4.2 µM, respectively). IL-6 moderately protected NCI-H929 cells from inhibitory effects of GGTI-2147, while IGF-1 had no effect (IC50s 1.1 µM and 0.5 µM, respectively). IL-6 and IGF-1 protected NCI-H929 cells from lovastatin-induced growth inhibition (IC50s 4.7 µM and 5.0 µM vs. 1.4 µM, respectively). Co-treating NCI-H929 cells with FTI L-744,832 and GGTI-2147 or IGF-1 proved synergistically in inhibiting cell proliferation in the presence of IL-6 or IGF-1. In primary MM cells (n = 7), FTI L-744,832 elicited anti-immunoyelma effects only at concentrations much higher than those found to inhibit healthy donor CD34+ cells (IC50s 51-396 µM vs. 8.2µM) and thus may be ineffective or cause non-specific toxicity when used as a single agent. However, GGTI-2147 andlovastatin induced specific anti-myeloma activity in some cases. Furthermore, combination of FTI with GGTI or lovastatin synergistically inhibited primary MM cell proliferation (IC50s 0.6-23.1 µM). Activating RAS mutations (4 K-RAS, 1 N-RAS; one sample had both K- and N-RAS mutations) were found in 4/7 (57%) MM patient samples, but anti-myeloma activity of prenylation inhibitors could not be correlated to RAS mutation status. Western blotting demonstrated that FTI/GGTI or FTI/lovastatin co-treatment more completely blocked activation of MEK-1/2 and MAPK-1/2 in NCI-H929 cells than treatment with any of the compounds alone. Furthermore, co-treatment elicited greater inhibition of IL-6 and IGF-1 induced MEK-1/2 and MAPK-1/2 activation in NCI-H929 cells. IL-6, IGF-1 and prenylation inhibitors did not affect AKT phosphorylation status in NCI-H929 cells. Summary/Conclusions. Our results support that inhibition of RAS down-stream signaling is a major mechanism through which FTI/GGTI and FTI/lovastatin co-treatment synergistically inhibit MM cell proliferation, even in the presence of cytokines and growth factors known to promote MM cell growth (e.g. IL-6 and IGF-1). Alternative prenylation of K- and N-RAS by GGTI was found in the presence of FTIs may explain the clinically observed incomplete response to FTI treatment. As the majority of RAS mutations in multiple myeloma occur in K- and N-RAS, FTI-resistance due to alternative geranylgeranylation may have therapeutic consequences in this disease.

**0079**

**EFFECTS OF BORTEZOMIB IN APOPTOSIS IN B-CLL CELLS INDEPENDENTLY OF PRIOR THALIDOMIDE TREATMENT: CHARACTERIZATION OF BIOCHEMICAL MECHANISMS ASSOCIATED WITH THE RESPONSE**

Faculty of Medicine, COIMBRA, Portugal; Clinical Hematology Service, University, COIMBRA, Portugal; Histocompatibility Center, COIMBRA, Portugal; Distal Hospital de Figueira da Foz, FICUEIRA DA FOZ, Portugal

B-cell lymphocytic leukemia (B-CLL) is the most common adult leukemia in the Western world. Although nucleoside analogues such as fludarabine and 2-chlorodeoxyadenosine (cladribine) have excellent activity in patients who have not received prior therapy, their impact on long-term survival is unclear and few agents display any activity in refractory disease. Nonetheless, the available evidence suggests that CLL emerges primarily as the result of deregulated apoptosis rather than unchecked
proliferation. Thus, agents that selectively target the survival pathway(s) active in B-CLL cells could reverse drug resistance. Proteasome plays a pivotal role in the control of many apoptotic and cell cycle-regulatory processes, become the focus of new approaches to the treatment of cancer, including B-cell malignancies. Bortezomib is the first proteasome inhibitor approved by FDA and EMEA for the treatment of refractory multiple myeloma. Extensive preclinical data is being developed to study the potential therapeutic of this new drug in other cancers. Our previous results show that BZ induces apoptosis as single agent, in a dose dependent manner, showing some selectivity for the transformed cells (EHA, 2005). Our preliminary results support the idea that the Bortezomib apoptotic effect may occur in a Bax dependent way (ASH, 2005). Here we examined the effects of bortezomib on apoptosis in peripheral blood mononuclear cell isolates from patients with CLL and characterized some of the biochemical mechanisms associated with the response. Mononuclear cells isolated from the blood of 24 CLL patients (14 without and 10 with prior conventional therapy, chlorambucil or fludarabine, 2 of those with refractory disease) were treated in vitro with bortezomib (ranging concentration from 0.1 nM to 10 µM), and evaluated for apoptosis by flow cytometry. Directly conjugated monoclonal antibodies to CD5 and CD19 were used to identify LLC-C cells. The expression of some proteins involved in mitochondria and membrane apoptotic pathways, namely the Bcl-2 proteins family, Bax and Bcl-2, the suppressor protein p53, the IAP proteins family, and heat shock proteins (i.e., HSPA, HSPCA), and oxygen stress (i.e., heme oxygenase-1). Since heme oxygenase-1 is believed to represent a key enzyme for the protection of cells against stress, it provides a growth advantage and contributes to cellular resistance against chemotherapy. Conclusion. Our results suggest that specific inhibition of heme oxygenase-1 expression in combination with proteasome inhibitor may be a new option in treating ATL patients and may be used as a sensitizer for chemotherapy.

**0080 NO EVIDENCE FOR CONSTITUTIVELY ACTIVATED FLT3 IN JUVENILE MYELO-MONOCYTIC LEUKEMIA**


_ErasmusMC-Sophia, ROTTERDAM, Netherlands; Dutch Childhood Oncology Group, THE HAGUE, Netherlands; University of Freiburg, FREIBURG, Germany; Children's Research Institute, VIENNA, Austria_

**Background.** Activating FLT3 mutations have been identified as prognostic factors in several myeloid malignancies. Recent studies have demonstrated that ligand-independent activation of FLT3 can also result from overexpression of wild-type FLT3. In addition, ligand-dependent activation has been observed in leukemic cells co-expressing FLT3 ligand (FLT3L), resulting in autocrine FLT3 signaling which is independent of FLT3 mutations. _Aims._ In Juvenile Myelo-Monocytic Leukemia (JMML), FLT3 internal tandem duplications (FLT3-ITDs) and mutations affecting the tyrosine kinase domain (TKD) are rare. However, no data are yet available on the frequency of expression of FLT3 and FLT3L in JMML. If activated FLT3 occurs in JMML these patients might benefit from treatment with small molecule FLT3 inhibitors, especially as the curative treatment of JMML is limited to allogeneic stem cell transplantation. _Methods._ The presence of activating FLT3/ITDs and FLT3/TKD mutations were screened in 51 JMML patients. In 21 patients FLT3 and FLT3L mRNA expression were assessed by real-time quantitative PCR (RT-qPCR). In MTT assays were performed to assess the sensitivity of JMML cells to the FLT3 inhibitor PKC412. _Results._ In none of the 51 JMML samples FLT3-ITDs or TKD mutations were found. FLT3 appeared to be expressed only at basal levels and FLT3L expression was very low. Consistent with the absence of mutations and lack of FLT3 and FLT3L expression, no PKC412 cytotoxicity was found in the JMML samples (n=13), in contrast to leukemic cells of infants with MLL-rearranged ALL which expressed activated FLT3. _Conclusions._ These data suggest that constitutively activated FLT3 does not occur in JMML. Therefore targeting FLT3 by tyrosine kinase inhibitors like PKC412 is unlikely to be effective in JMML.

**0081 MOLECULAR TARGETS OF THE PROTEASOME INHIBITOR, BORTEZOMIB, ON ADULT T-CELL LEUKEMIA CELLS**

R. Hamamura, J. Ohyashiki, T. Takaku, S. Honda, K. Ohyashiki
Tokyo Medical University, TOKYO, Japan

**Background.** The ubiquitin-proteasome system (UPS) is critical for regulation of fundamental cellular systems, such as cell cycle regulation, death, and immune response. The molecular mechanism of proteasome inhibitor-mediated anti-cancer activity has recently been extensively studied, and one of the major pathways is inhibition of the NF-κB cascade. Adult T-cell leukemia (ATL) is a fatal neoplasia derived from HTLV-1 infected T lymphocytes, and NF-κB activation is frequently associated with HTLV-1 infection. _Aims._ We therefore sought to determine the anti-tumor effect of the proteasome inhibitor, bortezomib in ATL cells, using gene expression profiling. _Methods_ and _Results._ Assessment of gene regulation by microarray analysis revealed that down-regulation of genes involved in anti-apoptosis (i.e., BCL2, and IAP5), up-regulation of genes related with apoptosis (i.e., FAF1 and TNFRSF10B), heat shock proteins (i.e., HSPA, HSPCA), and oxygen stress (i.e., heme oxygenase-1). Since heme oxygenase-1 is believed to represent a key enzyme for the protection of cells against stress, it provides a growth advantage and contributes to cellular resistance against chemotherapy. _Conclusion._ Our results suggest that specific inhibition of heme oxygenase-1 expression in combination with proteasome inhibitor may be a new option in treating ATL patients and may be used as a sensitizer for chemotherapy.

**0082 ANTISENSE THERAPY AGAINST MULTIDRUG RESISTANT GENE IN ACUTE MYELOBLASTIC LEUKEMIA CELL LINE**

F. Nadali, A. Pourfathollah, A. Ali-moghaddam, A. Dizaji, A. Somorodipour, E. Azizi, S. Rostmsi, A. Ghavamzadeh

_School of Medicine, ISFAHAN, Iran; Tarbiat Modares University, TEHRAN, Iran; Hematology-Oncology & BMT Research Center, TEHRAN, Iran; National Research Center For Genetic, TEHRAN, Iran; Toxicology Lab, Faculty of Pharmacy, TEHRAN UNIVERSITY OF MEDICAL SCIENCES, Iran_

**Background.** Acute myeloblastic leukemia (AML) is the most common leukemia in adults. Although the clinical outcome of acute leukemia has been proved by recent progress in chemotherapy, it is still a difficult disease to treat. One major problem is the emergence of leukemic blast cells that are resistant to anticancer drugs. This phenomenon is named multidrug resistance. A representative cause of MDR is the expression of the MDR1 gene and its product, P-glycoprotein (Pgp) on the cell surface membrane. Expression of Pgp is associated with its resistance to several types of antineoplastic agents such as anthracyclines, taxans, epipodophytoxines and vinca alkaloids. _Aim._ In this study we tried to reverse MDR phenotype in leukemic cells by antisense in complex to nanoparticle (PEI) against MDR1 gene. _Methods._ In the present study, the Pgp expressing cell line was established from parental K562 cell line with increasing concentrations of Doxorubicin until the KDI/20. In order to reverse the MDR phenotype due to Pgp expression different sequences of sense, antisense and one random sequence with phosphorothioate (PTO) modification (PS-ODN) against MDR1/mRNA was synthesized. They were treated on the KDI/20 in combination with two nonviral vectors: 1) Fugene 6 transfection reagent (cationic lipid) and 2) polyethylenimine (a cationic polymer, nanoparticle). The effect of PS-ODN was assessed at the cellular level by flowcytometry for Pgp detection, rhodamin 123 assay for functional assessment of Pgp, RT-PCR at the molecular level for MDR1/mRNA and MTT assay in order to assess the sensitivity of cells to Doxorubicin. _Results._ The results showed a decrease in the percentage of Pgp protein and MDR1/mRNA expression and an increase in the accumulation of Rh 123 and drug sensitivity of cells to Doxorubicin by antisense I and III. _Summary._ The results showed that antisense can reverse MDR phenotype at transcription level and the PEI vector is more efficient than cationic lipid.
M. Mayerhofer, M. Mayerhofer, E. Selzer, K.V. Gleixner,
We found EphA3 overexpression in Ph- myeloproliferative
W. Pickl, A. Gruze, P. Valent, V. Wacheck, V. Gleixner,
Eph receptors tyrosine kinase and their ephrin ligands,
As assessed by Northern blotting and RT-PCR, the human MC
EphA3 mRNA expression was analyzed, using Real
time PCR, in 266 samples obtained from CMPD patients (138 PB and 133 BM), 48 with a diagnosis of PV, 55 ET, 20 IM, 24 CML, 4 HES, 50 CML in chronic phase and 90 patients with a diagnosis of Ph-CMPD. 38 normal controls (18 PB and 20 BM) were also evaluated. Moreover, we investigated the expression level of EphA3 in 35 sample of B-CLL, 39 AML, 27 ALL and in 7 cell lines (Jurkat, K562, HL-60, MEL, NIH3T3, 293T, COS-7). Protein expression and localization were examined using Western Blot. Immunoprecipitation and Immunofluorescence analysis with appropriate antibodies. Transient transfection was performed in 293T e COS EphA3- cells using EphA3 plasmid. Nucleotide sequencing of tyrosine kinase catalytic domain was performed in 45 EphA3+ patients and in Jurkat cells. BMS incubation of normal/pathological samples and cell lines was performed (5,10,20 nM). Cells proliferation was evaluated using MTT assay; apoptosis rate was analyzed by FACS (Annexin V) and colony growth was examined on methylcellulose cul-
ture. Results. We found EphA3 overexpression in Ph- myeloproliferative patients (45%) compared to normal controls (5%) [p=0.004 in the PB e p=0.005 in the BM], with a significantly difference in the amount of transcript. 14% of B-CLL, 40% of ALL, 80% of AML and 8% of CML were positive. The overexpression was observed more frequently in BM as compared to PB (51,5% vs 22,8%). No expression difference was not-
ed among the Ph-CMPD. Western Blot analysis confirmed protein expression in EphA3+ samples and revealed receptor phosphorylation. Dasatinib led to significant dose-dependent inhibition of EphA3 phospho-
ylation. Moreover, BMS induced significant apoptosis (mean val-
ue 32%), colony growth reduction (mean value of 54,2 vs 76,5) and pro-
iferation rate inhibition (48%) of EphA3+ cells compared to normal controls. Immunofluorescence assay showed transmembrane localization of EphA3 receptor and revealed cells projections reduction, cell repulsion and cell rounding only in EphA3+ transfected cells. No kinase domain mutations were found in EphA3 overexpressing patients and Jurkat cells studied. Conclusion: EphA3 is abnormally expressed in different hema-
tological malignancies with a significant overexpression in CMPD as compared to normal controls. EphA3 phosphorylation blocking induced by BMS-554285 results in growth arrest and apoptosis of EphA3 over-
expressing cells. Therefore, EphA3 may represent a potential candidate for targeted signal transduction therapy.

Chronic myeloproliferative disorders I

0084
EPHA3 TYROSINE KINASE RECEPTORS AS TARGETS IN CHRONIC MYELOPROLIFERATIVE DISEASES

A. Chiarenza, F. Messa, S. Carturan, R. Catalano, F. Arruga, L. De Filippis, V. Rosso, D. Di Giovanni, P. Nicoli, A. Morotti, E. Messa, A. Serra, E. Bracco, G. Saglio, D. Cilloni

University of Turin, TURIN, Italy

Background. Eph receptors tyrosine kinase and their ephrin ligands, highly expressed during embryogenesis are involved in many key develop-
mental processes. Eph/ephrin interaction triggers a bidirectional signals transduction cascade that regulates morphogenesis and cell-cell interaction. Although Ephs receptors are not detectable in normal adult tissues, they are overexpressed in many tumors, suggesting a possible role of these PTKs in oncogenesis. Activation of tyrosine kinases and cell-
signal transduction pathways are of increasing interest in the pathogen-
esis of chronic myeloproliferative disorders (CMPD). Dasatinib (BMS-
354825), a novel BCR-ABL inhibitor exhibits an interesting inhibitory activity on some tyrosine kinases. Aim. The aim of this study was to comprehensively evaluate the expression of EphA3 overexpressing cell lines, investigating the possibility of exploiting EphA3 as a therapeutic target of BMS-
354825. Methods. EphA3 mRNA expression was analyzed, using Real
Time PCR, in 266 samples obtained from CMPD patients (138 PB and 133 BM), 48 with a diagnosis of PV, 55 ET, 20 IM, 24 CML, 4 HES, 50 CML in chronic phase and 90 patients with a diagnosis of Ph-CMPD. 38 normal controls (18 PB and 20 BM) were also evaluated. Moreover, we investigated the expression level of EphA3 in 35 sample of B-CLL, 39 AML, 27 ALL and in 7 cell lines (Jurkat, K562, HL-60, MEL, NIH3T3, 293T, COS-7). Protein expression and localization were examined using Western Blot. Immunoprecipitation and Immunofluorescence analysis with appropriate antibodies. Transient transfection was performed in 293T e COS EphA3- cells using EphA3 plasmid. Nucleotide sequencing of tyrosine kinase catalytic domain was performed in 45 EphA3+ patients and in Jurkat cells. BMS incubation of normal/pathological samples and cell lines was performed (5,10,20 nM). Cells proliferation was evaluated using MTT assay; apoptosis rate was analyzed by FACS (Annexin V) and colony growth was examined on methylcellulose cul-
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tological malignancies with a significant overexpression in CMPD as compared to normal controls. EphA3 phosphorylation blocking induced by BMS-554285 results in growth arrest and apoptosis of EphA3 over-
expressing cells. Therefore, EphA3 may represent a potential candidate for targeted signal transduction therapy.

0085
IDENTIFICATION OF MCL-1 AS A NOVEL TARGET IN NEOPLASTIC HUMAN MAST CELLS: EVIDENCE FOR COOPERATIVE GROWTH-INHIBITORY EFFECTS OF MCL-1 ANTISENSE OLIGONUCLEOTIDES, PKC412, AND AMN107


Medical University of Vienna, VIENNA, Austria; Institute of Immunology, VIENNA, Austria; Department of Clinical Pharmacology, VIENNA, Austria; Department of Radiation Therapy, VIENNA, Austria; Department of Internal Medicine I, VIENNA, Austria

Background. Mcl-1 is a Bcl-2 family-member that has been described to act anti-apoptotic in various myeloid neoplasms and therefore has been

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proposed as a potential therapeutic target. Systemic mastocytosis (SM) is a myeloid neoplasm involving myelomastocytic progenitors. Aims. We examined the expression and functional role of Mcl-1 in neoplastic mast cells (MC), to determine whether Mcl-1 could serve as a target in MC neoplasms. Methods. As assessed by RT-PCR and immunohistochemistry, primary neoplastic MC were found to express Mcl-1 mRNA and the Mcl-1 protein in a constitutive manner in all patients analyzed. Moreover, the human MC-leukemia cell line HMC-1 was found to express Mcl-1. Transfection of these cells with Mcl-1-specific antisense oligonucleotides (ASO) or an mcl-1-specific siRNA using lipofectin resulted in a reduced survival and increased percentage of apoptotic cells compared to controls. Results. The effects of mcl-1 ASO were seen with the HMC-1 subclone carrying the G506V c-kit mutation (mcl-1 ASO, 250 nM: 49 ± 4% apoptotic cells compared to control: 3 ± 2%, p < 0.05; mcl-1 siRNA: 41 ± 5% vs control: 5 ± 3%, p < 0.05) as well as with HMC-1.2 cells carrying both the G506V c-kit mutation and the D816V c-kit mutation (mcl-1 ASO, 250 nM: 56 ± 2% apoptotic cells compared to control: 61 ± 1%, p < 0.05; mcl-1 siRNA: 80 ± 6% vs control: 5 ± 2%, p < 0.05). Moreover, mcl-1 ASO were found to cooperate with the tyrosine kinase inhibitors (Novartis Pharma AG) imatinib, AMN107, and PKC412 in producing growth inhibition in HMC-1.2 cells. Summary. Together, these data show that Mcl-1 is a novel survival factor and attractive target in neoplastic human MC. Whether the Mcl-1-targeting concept can be developed far enough to reach clinical application remains to be elucidated.

0086
ANAGRELIDE: STUDIES ON ITS MODE OF ACTION USING DIFFERENT MODEL SYSTEMS OF MEGAKARYOCYTE DIFFERENTIATION
J. D. Erusalimsky, A. Gutierrez del Arroyo, J. Hernandez, G. Wang, Y. Hong
University College London, LONDON, United Kingdom

Background and Aims. Anagrelide is a potent and selective inhibitor of megakaryopoiesis used for the treatment of essential thrombocythaemia. Although the effectiveness of this drug in lowering platelet counts is now firmly established, its primary mechanism of action remains elusive. We have previously demonstrated that anagrelide inhibits the development of megakaryocytes from isolated CD34 positive hematopoietic progenitors (Wang et al. Br J. Pharmacol. 2005;146:324). Given that the use of cell lines could facilitate the identification of the molecular target of anagrelide, in this study we have compared the effects of the drug on the proliferation and differentiation of CD34 positive cells with those observed in UT7/ml, a growth factor-dependent haematopoietic cell line engineered to express the human thrombopoietin (TPO) receptor MPL. Methods. CD34 positive cells were purified from human umbilical cord blood and cultured for 12 days in IMDM-based medium supplemented with 20 ng/mL TPO. UT7/ml cells were maintained in exponential growth in α-MEM containing 418 and 2 ng/mL GM-CSF. To induce megakaryocytic differentiation in UT7/ml cells GM-CSF was replaced by 20-100 ng/mL TPO; alternatively, cells were transfected in the presence of GM-CSF with 10 nM phorbol myristyl-acetate (PMA). Results. Culture of UT7/ml for 5 days with GM-CSF or TPO led to >10-fold cell expansion. Addition of anagrelide at 1 µM, a concentration which causes maximal inhibition of megakaryocytogenesis in CD34 positive cell cultures and corresponds to >10-fold its IC50 in that system, caused only a slight and non-consistent inhibition of UT7/ml cell expansion (10-26% in cells grown with GM-CSF and -8 to +17% in cells grown with TPO). In addition, flow cytometric analysis showed that, unlike its effect in CD34 postive cell cultures, in UT7/ml cells anagrelide did not inhibit TPO-induced expression of the megakaryocytic differentiation marker CD61. Furthermore, the lack of anagrelide activity was unrelated to the concentration of TPO used. Since UT7/ml cells undergo megakaryocytic differentiation also when treated with phorbol esters, the activity of anagrelide against this class of agents was also tested. PMA completely inhibited UT7/ml cell growth, caused a marked enlargement in cell size and induced a >5-fold increase in the expression of CD61. Addition of 1 µM anagrelide had no significant effect on any of these parameters. Conclusions. These findings indicate that UT7/ml cells cannot replace normal haematopoietic progenitors as an in vitro model system to study the mechanism by which therapeutic doses of anagrelide inhibit megakaryocytic differentiation. In contrast, our study suggests that the molecular target of anagrelide lies further down stream from the ligand binding site of MPL. Since PMA induces megakaryocytic differentiation through activation of protein kinase C, our study further suggests that this pathway is not a target of anagrelide.
No differences in platelet and leukocyte activation were observed according to the JAK2 genotype. "Interpretation and Conclusion. Patients with myelofibrosis show platelet, leukocyte and coagulation activation patterns similar to those found in PV and ET.

0089
EPIGENETIC ALTERATIONS AND MUTATION OF JAK2 TYROSINE KINASE IN PATIENTS WITH BCR/ABL NEGATIVE MYELOPROLIFERATIVE DISORDERS

UK Aachen, AACHEN, Germany; Oncology Practice, WRSSELEN, Germany; St Nikolaus Krankenhaus, EUPEN, Belgium; Sidney Kimmel Comprehensive Canc, BALTIC, USA

Background. Bcr/abl negative myeloproliferative disorders (MPD) are a group of clonal stem cell diseases and comprise traditionally essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis with myeloid metaplasia (MMM). The recent discovery of the autoactivating mutation with a V617F amino acid substitution in the JAK2 tyrosine kinase has been a great step forward in understanding of the pathophysiology of MPD. However, this mutation is found only in about half of the patients with MPD. Aims. Hypermethylation of CpG islands within gene promoter associated regions is associated with transcriptional inactivation and represents an important mechanism of gene silencing in the pathogenesis of human cancer. In this study, we sought to determine the potential role of DNA methylation changes in the context of JAK2 mutation in MPD. Methods. We analysed the JAK2 mutational status by direct sequencing and the methylation patterns of 12 cancer-related genes by methylation specific polymerase chain reaction in bone marrow and blood specimens from 83 patients with MPD. Genes analysed were SOCS-1, E-cadherin, MGMT, TIMP-2, TIMP-3, p15, p16, p53, DAPK1, RASSF1, RAR, 2 and MLH1. Results. The frequency of aberrant methylation was 4/22 for SOCS-1, 1/22 for p15, TIMP-2 and E-cad in patients with MMM, 1/7 for SOCS-1 and MGMT in PV and 1/4 for SOCS-1 and DAPK1 in ET. We detected at least one hypermethylated gene in 11/33 patient samples. The JAK2V617F mutation was found in 9/22 patients with MMM, 4/7 for PV and 1/4 with ET. Our data indicate that hypermethylation of tumour suppressor genes can be considered as a common phenomenon in bcr/abl negative MPD in addition to the JAK2V617F mutation. We found concomitant heterogeneous mutation of JAK2 and hypermethylation of the cyclin dependent kinase 5 (CDK5) in SOCS-1 in two patients. The cell adhesion gene E-cadherin was methylated in one patient with MMM and one patient with ET, while the patient with PV was detected to carry both mutation of JAK2 and hypermethylation of SOCS-1. However, in most patient samples, we found either JAK2V617F somatic mutation without CpG island hypermethylation or altered methylation patterns without genetic aberration of JAK2. Summary. In conclusion, we detected in MPD, in addition to the recently discovered activating mutation of JAK2, CpG island hypermethylation of cancer-related genes, especially SOCS-1, a negative regulator of JAK2. These results suggest, that epigenetic changes may, in addition to the well defined JAK2 activating mutation, contribute to the pathogenesis of bcr/abl negative MPD and thus can be considered as a potential therapeutic target for demethylating agents.

0090
CLINICOPATHOLOGIC HETEROGENEITY OF CHRONIC HYPEREOSINOPHILIC SYNDROMES LONG-TERM EXPERIENCE ON 32 PATIENTS

Silesian Medical University, KATOWICE, Poland; *Outpatient Haematologic Institute, KATOWICE, Poland

Background. Chronic hypereosinophilic syndromes (CHS) comprise a wide spectrum of indolent to aggressive diseases characterized by prolonged, unexplained hypereosinophilia. Aims. A study was planned to evaluate clinical and pathologic features in patients with chronic, non-reactive hypereosinophilia. Material: 32 patients (pts) observed between 1990-2005 with absolute eosinophilia count (AEC) of higher than 0.7×10^9/L, for at least 3 months were included. Results. There were 13 males and 19 females, aged 18-76 yrs (median 52 yrs). The diagnosis was following: Hypereosinophilic Syndrome (HES, n=16), Chronic Idiopathic Eosinophilia (CIE, n=12), T-cell mediated HES (n=1), Chronic Eosinophilic Leukemia (CEL, n=1) and CEL PDGFRα+ (n=2). Organ involvement included: heart (n=9), lungs (n=5), spleen (n=7), liver (n=4), lymph nodes (n=4), skin (n=3), peripheral nerves (n=2) and gut (n=1). Median white blood cell (WBC) count at diagnosis was 12.6×10^9/L (range 5.2-81.6), with AEC of 4.32×10^9/L (0.9-32.9) and bone marrow eosinophilic infiltration of 30% (8.0-55.0). Median IgE level was 92.5 IU (range 0.1-13966), vitamin B12 concentration-250pg/ml (range 60-3559). Only one patient revealed a slight increase in spindle-shaped mast cells on bone marrow exam, but there were no c-kit D816V and JAK2V617F-PDGFRα mutations in this case. On cytogenetic evaluation normal karyotype was present in 13/14. One patient with CD3+CD4+CD8- T-cells and TCRβ rearrangement showed t(6;11)(p21;q23). BCR/ABL was undetectable in 19/19. RT-PCR for JAK2V617F-PDGFRα was detectable in 2 of 19 pts at diagnosis (11%). The first-line therapy consisted of steroids and interferon. In 5/21 pts with MMM the treated HES cells received CHOP regimen and one patient with CEL in accelerated phase was given induction therapy (Har-hydroxyurea, adryanycin, ara-c). Majority of pts with HES and CIE responded promptly to low dose of prednisone (10-20 mg/day), but eosinophilia recurred shortly after prednisone tapering or discontinuation. Eight patients due to resistance to prior therapy were administered imatinib at initial dose of 100 mg daily. A complete remission was documented in 3/8 (37%). Two out of three, who achieved complete hematologic remission in a median time of 14 days (range 13-65), were FIPI11-PDGFRα positive at diagnosis. One out of two FIP positive patients showed leukocytosis, but improved at six months. Eight patients attempted to discontinue imatinib, but relapsed promptly. Imatinib was resumed at 100 mg daily with rapid eosinophilia resolution. Patient with CEL underwent allogeneic bone marrow transplantation from his brother and currently is disease-free. Patient with T-cell mediated HES was placed on autologous stem cell transplantation. We have discovered molecular remission while eosinophilia persisted. One patient developed pure red cell aplasia during the disease course and it results from the prior hydroxurea treatment. Current status of pts included to the study is following: complete remission in 17 pts, partial response 4 pts, non-responders- 10 pts, 1 death due to cardiac insufficiency. Conclusions. Our study showed that majority of pts with CHS has a benign disease course and steroids are sufficient to control the eosinophil count. We confirmed the high efficacy of low dose of imatinib in patients carrying the JAK2V617F-PDGFRα mutation. Discontinuation both steroids and imatinib was followed by rapid eosinophilia recurrence.

0091
APPLICATION OF PRV-1 MRNA EXPRESSION LEVEL AND JAK2V617F MUTATION FOR DIFFERENTIAL DIAGNOSTICS BETWEEN POLYCYTHEMIA VERA AND SECONDARY ERYTHROCYTOSIS. INFLUENCE OF INTERFERON THERAPY ON PRV-1 EXPRESSION

V.V. Tuvataeva, M.A. Sokolova, N.V. Tsvateeva, E.A. Semenova, V.L. Ivanova, T.I. Kolosheinova, A.V. Misirun, N.D. Khoroshko
Hematology Research Centre RAAMS, MOSCOW, Russian Federation

Background. Polycythemia vera (PV) is a clonal myeloproliferative disorder (MPD) lacking specific biological markers. Recent discovery of PRV-1 mRNA overexpression and JAK2V617F mutation as a significant difference in molecular markers in patients of PV can facilitate differential diagnosis between PV and non-malignant disease - Secondary Erythrocytosis (SE). Furthermore, recently established influence of interferon (IFN) therapy on PRV-1 expression might reflect treatment efficiency. Aims. Confirm PRV-1 mRNA overexpression and JAK2 mutation in the group of patients diagnosed with PV and absence of these markers in the group of patients diagnosed with SE. Investigate influence of Interferon therapy on PRV-1 expression levels in patients with PV. Methods. We studied 46 patients (22 M/24 F) diagnosed with polycythemia vera based on PVSG criteria. For 39 patients diagnosis was confirmed by histological studies. Average patient age was 54 years, average time from diagnosis was 6.8 years. At the time of study out of 46 patients 9 were naive and did not receive cytostatic treatment, 25 were pretreated with Hydroxyurea (HU) and 12 were pretreated with IFN. PRV-1 expression level was determined twice for 7 patients continuously receiving 5 millions ME daily 8 month after first analysis. Also we studied 14 patients with secondary erythrocytosis. Total duration of Interferon treatment for these patients was 23 month. Control group includes fifteen normal donors. PRV-1 expression level was determined by reverse transcription and quantitative PCR (iCycler IQ, BioRad). Normalization to β2 microglobulin expression level was used for comparison between different samples. Jak2V617F mutation was determined by PCR. Results. In the first analysis PRV-1 expression level was 1.17×10^-4 ± 2.17×10^-4. PRV-1 mRNA overexpression was observed in 8 out of 9 naive patients without cytostatic treatment, in 21 out of 25 patients pretreated with HU and in 8 out of 12 patients.
pretreated with IFN. Overall we found PRV-1 overexpression in 37 out of 46 patients in the study (80%). We did not find PRV-1 overexpression in patients diagnosed with SE (Figure, left panel). Sequencing for determination of Jak-2 mutations was performed for 22 patients diagnosed with PV. From this group 5 were naïve, 9 pretreated HU, 7 pretreated with IFN. Also 6 patients with SE and 3 healthy donors were examined. Mutation was found in all patients with PV and was not found in patients with SE and healthy donors. After 8 month of prolonging treatment with IFN PRV-1 overexpression level was decreased strongly (p=0.04) in all 7 examined patients (Figure, right panel). Six patients from this group archived remission at the time of second analysis. Criteria’s of remission were reduction of platelet level down to 400-600x10^12/L, leukocytes to 10-12x10^9/L, and erythrocytes to 6x10^12/L.

Conclusions. We show high sensitivity specificity and utility of PRV-1 expression level and especially Jak2V617F mutation for differential diagnosis between PV and SE. Decrease of prv-1 expression levels in the group of patients receiving Interferon might be designated in the future as a molecular marker of treatment efficiency.

0093

V617F JAK2 MUTATION IN CHILDREN WITH ESSENTIAL THROMBOCYTENOMA (ET)

M.L. Randi, M.C. Putti, M. Scapin, E. Pacquola, F. Fabris
University of Padua, PADOVA, Italy

Background. The diagnosis of ET is usually made by excluding other primitive myeloproliferative disorders (MPD) and reactive thrombocytosis, since no specific biological marker is available. Recently, a somatic mutation V617F of janus kinasis 2 [Jak2] has been found in virtually all adults patients affected by polycythemia vera and in about 35-50% of ET patients. On the other hand no informations are available about the occurrence of jak2 mutation in the rare cases of ET in children. Aim. To evaluate the occurrence of V617F Jak2 in pediatric ET. Methods. We searched V617F Jak2 mutation with sequencing test and allele-specific PCR, in 20 children (15 females and 5 males, median follow up 7.5 years) diagnosed to be affected by ET in agreement with the PVSG criteria and they were compared to 47 consecutive adult ET (36 females and 11 males, median follow-up 9.2 years) younger than 65. The comparison between categorical variables was performed by chi-square statistic test with Yates variable and p-value <0.05 were considered statistically significant. Results. Heterozygous V617F Jak2 was found in 4 children and 28 adults. The correlation between mutated jak2 and thrombotic complications occurring both at diagnosis or during follow-up is summarized in the following Table: The occurrence of V617F Jak2 resulted significantly less frequent in children than in adults (p=0.0069) while the results on our adult patients are strongly consistent with the data reported in other papers. Conclusions. Recently, a link between age and V617F Jak2 mutation incidence has been surmised, possibly related to the influence of age on genetic instability. At present we cannot exclude that our children with ET will develop V617F Jak2 mutation. However, on the basis of the present data, we conclude that in ET, V617F Jak2 mutation is less frequent in children than in adults. The V617F jak2 mutation is common both in pediatric and adult ET patients presenting with unusual thrombosis, namely sovra-hepatic, portal and cerebral veins thrombosis.
0094
PREVIOUS HISTORY OF THROMBOSTATIC COMPLICATION IS THE MAIN RISK FACTOR THAT INCREASES THE INCIDENCE OF THROMBOTIC EVENTS IN ESSENTIAL THROMBOCYThEMIA PATIENTS

F. Radaelli,1 M. Colombi,2 V.R. Zilioli,3 S. Bramanti,4 A. Iurlo,5 A. Zanel6
1Ospedale Maggiore Policlinico, Mangiagual, MILANO, Italy; 2Ospedale Maggiore Policlinico, MILANO, Italy

Background and Aims. Thrombotic and hemorrhagic complications are the main causes of morbidity in essential thrombocytopenia (ET). We investigated the clinical and laboratory characteristics associated with the occurrence of thrombotic events, with the aim of identifying subgroups of patients who would benefit from antiaggregant and/or anticoagulant therapy. Methods. 306 consecutive ET patients followed between January 1979 and December 2002 (median age 58 years, male/female ratio 0.55, median follow-up 96 months) were included in this study. In order to identify the possible predictive factors of thrombotic risk, the following variables were considered: age, gender, platelet count at diagnosis and at the time of thrombotic event, previous history of thrombotic and/or hemorrhagic complications, disease duration and cardiovascular risk factors (arterial hypertension, hypercholesterolemia, diabetes, smoking, obesity and a familial history of thrombosis). Results. 46 patients (15%) had a previous thrombotic event (blood clotting, myocardial ischemia, pulmonary embolism, retinal artery occlusion, deep venous thrombosis and pulmonary embolism). Age, gender, platelet count at diagnosis and at the time of thrombotic event, and disease duration did not appear in our series to significantly increase the incidence of thrombotic complications. These events occurred in 26/64 (40.6%) patients with a previous history of thrombosis and in 20/242 (8.3%) without a previous history of disease. Major thrombotic complications included stroke, transient ischemic attack, myocardial infarction, angioplast, peripheral arteriothrombosis, retinal artery occlusion, deep venous thrombosis and pulmonary embolism. Age, gender, platelet count at diagnosis and at the time of thrombotic event, and disease duration did not appear in our series to significantly increase the incidence of thrombotic complications. These events occurred in 26/64 (40.6%) patients with a previous history of thrombosis and in 20/242 (8.3%) without a previous history of thrombosis (p=0.0001 Fisher’s exact test, odd ratio 7.6). When patients with no previous history of thrombosis were stratified according to the number of cardiovascular risk factors (five or more vs. five or less), we observed a significant correlation with the occurrence of thrombotic events (p<0.05). 31 patients (10%) experienced major hemorrhagic complications, mainly gastrointestinal tract bleeding: three of them had a positive and 28 a negative history of hemorrhagic events (p=0.052). Major bleeding was defined as an event that threatened life or organ function, or required a transfusion of red blood cells. Conclusions. This study was based on a large cohort of patients followed for many years at a single institution and confirmed that a previous history of thrombosis is the main risk factor for developing further thrombotic events during the follow up. Age and platelet count, generally accepted as very important risk factors for thrombosis, did not appear in our series associated with an increased risk for thrombosis. Asymptomatic patients with a negative history of thrombosis and without any cardiovascular risk factors can be considered at low risk and therefore should not be considered for treatment - regardless of platelet count, age, sex and the disease duration. For patients with a negative history of thrombosis, but with a cardiovascular risk factor, it is essentially to consider them and further evaluate the opportuneness of an antiaggregant and/or cytotherapeutic therapy.

0095
RATIONAL AND DESIGN OF A DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL TO EVALUATE THE CITICOSTEROID-SPARING EFFECTS OF ANTI-IL5 MONOCLONAL ANTIBODY (MEPOLIZUMAB) IN SUBJECTS WITH HYPEREOSINOPHILIC SYNDROME

X. Parkin,1 M. Rothenberg,2 F. Roufosse,3 F.C. Lambert,3 A. Klion,3 M. Colombi,4 V.R. Zilioli,3 S. Bramanti,4 A. Iurlo,5 A. Zanel6
1GlassSmithKline, MIDDLESEX, United Kingdom; 2Children’s Hosp Med Ctr, U of Cincinnati, CINCINNATI OH, USA; 3Hôpital Erasme ULB, BRUSSELS, Belgium; 4Lab of Parasitic Diseases, NIH, BETHESDA MD, USA; 5McMaster University, HAMILTON, ONTARIO, Canada; 6Vanderbilt Univ. Dept of Medicine, NASHVILLE TN, USA

Background. Hypereosinophilic syndrome (HES) is comprised of a group of rare hematological disorders characterized by sustained eosinophil overproduction. Clinical manifestations result from damage to multiple organs associated with local release of toxic granule products by infiltrating eosinophils. Management is currently based on corticosteroid, interferon-α, and/or cytotoxic therapies, each of which is associated with significant toxicity and tolerability issues. Mepolizumab is a humanized anti-IL-5 monoclonal antibody that blocks the actions of IL-5, the major hematopoietin responsible for eosinophil production, differentiation, and survival. Preliminary data from a small number of patients with HES, asthma, and hypereosinophilic syndrome demonstrated that treatment with intravenous mepolizumab was associated with reduced blood eosinophils and was well-tolerated. Due to the variability in clinical presentation and lack of validated disease indices for HES, clinical parameters alone may not provide a sensitive and precise measure of drug efficacy. However, the ability of a treatment to enable corticosteroid-sparing effects of mepolizumab treatment in patients with HES. Aims. The primary objective of this ongoing study is to assess the effect of mepolizumab versus placebo on reducing corticosteroid requirements in patients with HES requiring 20-60 mg/day of prednisone to maintain eosinophils at <1500cells/µL. The primary endpoint is the proportion of subjects requiring ≥10 mg/day prednisone for at least 8 consecutive weeks during the 32 week treatment period. Methods. This multicentre (30 sites worldwide), randomized, double-blind, placebo-controlled, parallel-group study recruited patients 18/85 years of age with HES (blood eosinophil count >1500/µL) and a previous history of organ involvement or dysfunction related to eosinophilia, without any other cause of eosinophilia, who were steroid-responsive and tested negative for the FIP1L1-PDGFαR gene rearrangement. Eligible patients were stabilized on prednisone monotherapy (20-60 mg/day) during a 6-week run-in period and then randomly assigned to receive intravenous mepolizumab 750 mg or saline (placebo) every 4 weeks. Prednisone was tapered at weekly intervals following the first infusion according to a pre-specified algorithm. HES-related end-organ involvement was monitored using concomitant assessments, echocardiograms, computed tomography scans of the lung, abdomen and maxillary sinus, pulmonary function tests, and esophagogastroduodenoscopy. Patients’ perceptions of HES symptom bother, health status, and limitations of daily living were determined using quality of life questionnaires. Blood samples were collected to characterize mepolizumab pharmacokinetics. Subjects completing the trial, or who withdrew due to lack of efficacy, could enter an open-label extension study to evaluate the long-term safety, efficacy and optimal dosing frequency of intravenous mepolizumab. Results. This trial, initiated in March 2004, was fully enrolled by May 2005 with 85 patients started on study medication. Summary/Conclusions. This ongoing study is the largest trial to be conducted to date in patients with HES, and the only placebo-controlled trial in this population. Findings from this study will provide importantly important information on the treatment of HES with mepolizumab, and enable better understanding of this rare condition.

0096
FIP1L1-PDGFRA HYPEREOSINOPHILIA: HOW MANY DISEASES?

F.C. Lambert,1 L. De Leval,2 C. Herens,2 A. Chriot,2 V. Bours1
1University of Liège, LIGE, Belgium; 2Surgical Pathology, LIGE, Belgium

Aims. The Hyper eosinophilic Syndrome (HES) has remained for a long time a problematic diagnosis. WHO criteria relies on identification of rare signs of clonality which allow differential diagnosis between chronic eosinophilic leukemia (CEL) and true idiopathic states (HES). Recently, a new mechanism of mutation was described: a cryptic interstitial microdeletion at chromosome band 4q12 generating a FIP1L1-PDGFαR (F/P) fusion gene (FG). According to the WHO guidelines, this clonal abnormality has been proposed as a new surrogate marker for CEL. Subsequently, the F/P FG was reported in patients with hyper eosinophilia and atypical bone marrow (BM) mast cells (MC), suggesting a new hypothetical systemic mast cell disorder with hyper eosinophilia subgroup (F/P+ SMCD-eos). Unfortunately, these SMCD-eos diagnoses were mainly based on histologic criteria (i.e., the major and the first minor WHO criteria) which are essentially subjective. The leukemic stem cell where the F/P deletion arises as well as the specificity of the loosely aggregates of tryptase positive mast cells in CEL remain to be identified before the relationship between these two clinical entities could be elucidated. It is the regard of WHO guidelines for SMCD-eos differential diagnosis may deserve to be updated. Aims. We stressed out the potential subjective bias in WHO criteria interpretation and questioned the relationship between FIP1L1-PDGFαR+ CEL and SMCD-eos.
Specificity of tryptase expression in the context of hypereosinophilic diseases was assessed at the protein and mRNA level.

Table 1. Clinical, cytogenetic and molecular characteristics of the patients.

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<th>Patient # 3</th>
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tion was performed and test controls were prepared by using the Spectral Chip 2600TA (Spectral Genomics), an array consisting of 2,621 BAC clones at an average of 1-2 Mbp resolution, according the manufacturer’s specifications. Fluorescent images were obtained using an Agilent G2565BA scanner and quantified using GenePix 6.0 software (Axon, Molecular Devices) using the irregular feature finding option. Extracted raw data was filtered and normalized using Bacanal (Lozano et al., unpublished), an in-house implementation of the Limma package developed within the Bioconductor project in the R statistical programming environment. Results. Among the 20 analyzed patients, in two cases a genotypic copy number change was detected. Case 2 showed a gain of 3p24-p24.3 (RP11-2455E5, RP11-208G16) and case 6 presented a gain of 6p23.2 (RP11-121I7, RP11-111F7), a region that encodes the CSMD1 gene. In addition, two patients (cases 11 and 12) showed a variation copy number polymorphisms in 16p11.1-p11.2 (RP1-488201, variation 0196 and RP1-80F22, variation 0197) and in 2q37.3 (R5F-1011017, variation 0032 where FLJ40712 and FLJ1327 are located and CTR-17211S), respectively. The gains and losses detected in these patients were not detected in the remaining comments. Patients treated with hydroxyurea either alone or in combination the time of analysis was 51 (range 1-192) months. Most of the patients were treated with hydroxyurea either alone or in combination for at least 2 years and 21 were followed for at least 4 years. The mean follow-up of the patients was 5.2 years. Among the 20 analyzed patients, in two cases a genotypic copy number change was detected. Case 2 showed a gain of 3p24-p24.3 (RP11-2455E5, RP11-208G16) and case 6 presented a gain of 6p23.2 (RP11-121I7, RP11-111F7), a region that encodes the CSMD1 gene. In addition, two patients (cases 11 and 12) showed a variation copy number polymorphisms in 16p11.1-p11.2 (RP1-488201, variation 0196 and RP1-80F22, variation 0197) and in 2q37.3 (R5F-1011017, variation 0032 where FLJ40712 and FLJ1327 are located and CTR-17211S), respectively. The gains and losses detected in these patients were not detected in the remaining patients. Results. Array CGH reveals an absence of recurrent genomic copy number changes in essential thrombocytemia. FISH studies with the affected BAC clones will be performed to confirm these results. Acknowledgments. Grants FIS PI030345, C08/07 and C08/10 from the Spanish Ministry of Health.

0099
THE INCIDENCE OF DEL20Q12 BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION (I-FISH) IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS
L. Pantelidou,1 I. Bazdari,2 G. Tryptisian,3 D. Margaritis,4 A. M. Vannucchi5 1Democritus University of Thrace, ALEXANDROUPOLIS, Greece; 2Aristotle University of Thessaloniki, THESSALONIKI, Greece

Background. Chronic Myeloproliferative Disorders (CMPD), including Polycythemia Vera (PV), Essential Thrombocytemia (ET), Idiopathic Myelofibrosis (IMF) and Unclassified CMPD, are acquired diseases of the hematopoietic stem cell, characterized by clonal proliferation of one or more cell lines. Chromosomal abnormalities have been reported in less than 20% of CMPD patients at the time of diagnosis and in higher frequencies during the course of the disease.

Aim. The aim of the present study was to determine by I-FISH the incidence of 20q12 deletion (del20q12) in bone marrow samples from CMPD patients either at diagnosis or during the course of the disease and to assess its clinical utility.

Patients and Methods. Eighty four samples from 38 men and 40 women with median age 63 (range 17-84) years were studied using I-FISH, utilizing a locus specific probe for 20q12 (D20S108). 38 patients were diagnosed with ET, 34 with PV and 3 with IMF and 3 with unclassified CMPD. Bone marrow samples from 12 healthy volunteers were used as assay controls. Del 20q12 was detected in 1 out of 84 samples (23%). The chromosomal aberration was revealed in 7/84 (21%) PV patients, in 9/88 (24%) ET patients and in 2/5 (67%) Unclassified CMPD. Sequential I-FISH studies were performed in six patients. In one PV patient, although the initial study was normal, the del20q12 was developed after a period of 21 months. The del20q12 was significantly associated with treatment failure (p=0.012) and inferior outcome of the disease. The five years survival without disease progression (myelofibrosis, secondary leukemia or myelodysplastic syndromes and death) was 62±15 months vs 89±5 months in patients with or without del20q12 respectively. Thus, patients with del20q12 were associated with significant increased odds of having disease progression (OR=3.1, 95% CI=1.17-8.6). Nevertheless, there was no correlation between the presence of del20q12 and age, sex, therapy duration as well as the interval between the presentation of the disease and I-FISH analysis. Conclusion. Our experience, in concordance with other studies, showed that the del20q12 can be revealed by I-FISH in approximately 20% of CMPD patients. Poor prognosis and treatment failure were statistically associated with the del20q12. Larger and prospective series are needed to ascertain the possible clinical implications of del20q12 in CMPD patients.
ment of diagnostic parameters characterizing polycythemia vera, e.g., the identification and allele specific examination of JAK2 mutation V617F and PRV-1-mRNA quantification; the screening for hemoglobin variants with high oxygen affinity and for familial 2,3-BPG deficiencies; the molecular analysis of factors related to disorders of erythropoietin synthesis (e.g. von-Hippel-Lindau gene mutations) and signaling (e.g. erythropoietin-resistant mutations). The database is located on www.erythropoiesis.org and can be accessed by any physician after online registration. Patients’ identification is codified. Registration and data collection, after patient informed consent, include the clinical and family history, evolution, complications and treatment and the hematological, biochemical and molecular biology studies. At any time data can be updated and follow-up information can be included. Patient’s members can be genealogically connected. Among other potentialities, the database is prepared to generate statistical information, query based data exports in standard formats and dynamical patients profiles ready for checking the database for matches. Discussion involving registered doctors is a simple procedure. Conclusion. This European online database will be a powerful instrument to obtain systematic data on the clinical presentation, on results of various diagnostic procedures and the clinical evolution of patients with congenital primary and secondary erythrocytosis and of young patients with PV.

0102

ERYTHROCYTOSIS AND THROMBOCYTOSIS IN CHILDHOOD: THE EXPERIENCE OF A SINGLE CENTRE

U. Ramenghi, 1 P. Quarello, 1 D. Renga, 1 A. Doria, 1 S. Martino, 1 L. Foglia, 1 L. Alba, 1 S. Carturan, 1 E. Sterpone, 1 E. D’Aracco, 1 L. Garbanni, 1 L. Parma, 2

1 University of Turin, TORINO, Italy; 2 University of Turin, Clinical Science, ORBASSANO, TO, Italy

Background. Erythrocytosis (E) and thrombocytosis (T) in childhood are mainly secondary forms and result from several causes. The diagnosis of Polycythemia Vera (PV) and Essential Thrombocythemia (ET) can often be difficult and currently relies on clinical and biological criteria defined by WHO. Recently, an activating somatic point mutation of jak2 has been described in the vast majority of patients with PV as well as in sub-sets of patients with ET. Presence of this mutation is highly correlated with PV-V1 expression, another molecular marker of MPD. Aims: The aims of this report is to describe the clinical, biological and molecular features of 24 paediatric patients affected by E or T, focusing on primary forms. Methods. We conducted a retrospective study on all patients affected by E or T (including criteria: Hct> +2DS of the expected value and platelet count ≥1x10^11/L) referred to our Centre between 1st January 2000 and 31st December 2005. Results. Thirteen patients with E and eleven patients with T (M/F: 10/3 and 4/7; median age at diagnosis: 88 and 14 months, respectively) were investigated. 4/13 E resulted secondary to congenital heart disease, 1/13 secondary to persistent obstructive sleep apnea, 2/13 familiar forms and 6/13 primary erythrocytosis (PE). E was diagnosed in 32 patients according to WHO criteria; he showed thrombotic complications (a cerebral ischemia and a splenic infarction) and is currently treated with oral anticoagulant therapy, low-dose aspirin, hydroxyurea and regularly undergoes to phlebotomy. Reactive thrombocytosis (RT) was diagnosed in 6 out 11 T, associated with bacterial or viral infections (mean duration = 4 months) while ET was diagnosed in 5 (mean duration = 45 months) according to WHO criteria (see clinical features of PE and ET in Table 1).

Conclusions. 1. Primary erythrocytosis (PE) in children is mainly secondary to congenital heart disease, while ET was diagnosed in 5 children. 2. Molecular markers showed significant differences between ET and PE: JAK2 mutation V617F was in 6/5 ET and in 0/11 PE. 3. Thrombotic complications were more frequent in PE than ET. 4. Treatment of PE is mainly phlebotomy, while ET was treated with low-dose aspirin and hydroxyurea. 5. JAK2 mutation V617F was in 0/5 ET and in 6/6 PE. 6. JAK2 mutation was not present in any patient with PV. 7. JAK2 mutation was present in 56% of ET patients with thrombosis and in 13% of PE patients. 8. JAK2 mutation is associated with higher platelet counts and higher white cell counts (p<0.01).

0103

PREDICTIVE VALUE OF ALTERATIONS OF COAGULATION AND THE JAK2 MUTATION ON THE RISK OF THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA

C.B. Burgala, 1 D. de Miguel, 1 M. Lopez Rubio, 1 C.B. Besses, 1 B.B. Bellosillo, 1 T.P. Pascual, 1 B. Paramio, 1 J. García Suárez, 1 H.B. Bañas, 1 C.P. Perez Calvo 2

1 Hospital U. Príncep de Asturias, ALCALÁ DE HENARES, Spain; 2 Hospital del Mar, BARCELONA, Spain; 3 Príncipe de Astur, ALCALA, Spain; 4 Hospital Príncipe de Asturias, ALCALA DE HENARES, Spain, “Clínica la Concepción, MADRID, Spain

Background. Essential thrombocytemia (ET) is a heterogeneous disorder in which thromboembolism remains the major cause of morbidity and mortality. Previous studies have shown high frequency of thrombosis in patients with ET. In the present study we investigated alterations of coagulation and the JAK2 mutation in ET patients and its relation with haematological parameters and risk of thrombosis. Methods. We have studied 58 patients previously diagnosed of ET. Sex distribution was 25 males and 33 females. Age ranged from 20 to 86 years (mean 59±15). Patients follow up was from six to 253 months (mean 89±54). In all patients, the presence of thrombophilia alterations was studied. The studies included: APTT, von Willebrand factor antigen C, Protein C, Protein S, Antithrombin III, Leiden factor V, prothrombin 20210A, Factor V Leiden, Prothrombin 20210A and MTHFR mutation. JAK2 mutation was analyzed in all patients. The significance of this mutation was evaluated in relation with, haemoglobin, leukocytes and platelets levels, and its relation with haematological parameters and risk of thrombosis. Results. To 1st objective: 17 patients (29%) develop thrombosis, 7 before diagnosis and 10 during evolution. 59% of thrombosis were in patients older than 60 years, mean age of thrombosis was 61 years old. 50% of patients with thrombosis showed high expression of von Willebrand antigen factor, Results in both groups of patients were (Mean±SE: 159±51 and 128±48) respectivly. To 2nd objective: JAK2 mutation was present in 56% of the ET patients. Patients with the mutation presented higher hematocrit values (44±5 vs 40±3; p=0.04), higher white blood cell counts 8951 vs 7304; p=0.05) and higher mean number of platelets (908.000 and 685.000 respectively; p=0.01). JAK2 Mutation was present in 5 from 8 patients with thrombosis and in 13 from 24 who do not develop thrombosis. Conclusions. 1. Thrombosis risk in ET is increased in patients with alterations of coagulation, however none of these alterations shows independent predictive value. 2. Results from our serie confirm the presence of JAK2 mutation in more than 50% of ET patients and shows relation of this mutation with hematocrit value and higher white cell and platelets counts. 3. Relation of JAK2 mutation and thrombosis cannot be established at the present time out of prospective studies.
Acute myeloid leukemia I

0104

MOLECULAR PROFILING USING ARRAY-CGH AND GENE EXPRESSION PROFILING REVEALS NEW CANCER GENES IN AML WITH NORMAL KARYOTYPE

F.G. Rücker,1 L. Bullinger,1 H.A. Kestler,2 P. Lichter,2 K. Döhner,2 A.A. Quinino,3 H. Döhner4
1University of Ulm, ULM, Germany; 2Department of Internal Medicine III, ULM, Germany; 3Division of Neuroinformatics, ULM, Germany; 4PO San Gennaro, NAPLES, Italy; SDFKZ, Heidelberg, Germany

Clonal chromosome abnormalities represent one of the most important prognostic factors in adult acute myeloid leukemia (AML), and cytogenetic data are used for risk-adapted treatment strategies. By conventional cytogenetic analysis, approximately 50% of patients lack clonal chromosome aberrations, and normal cytogenetics are associated with an intermediate clinical outcome. This clinically heterogeneous group seems to be in part characterized by molecular markers, such as MLL, FLT3, CEBPA, and NPM1 mutations. In order to identify novel candidate regions of genomic imbalances, we applied comparative genomic hybridization to microarrays (array-CGH). Using this high-resolution genome-wide screening approach, we analyzed 49 normal karyotype AML cases characterized for the most commonly relevant clinical molecular markers (MLL-PTD n=13, FLT3-ITD n=7, FLT3-ITD/NPM1 n=4, MLL-PTD/FLT3-ITD n=3, CEBPA n=12, CEBPA+FLT3-ITD n=1; CEBPA+/NPM1 n=1; no molecular markers n=8) with a microarray platform consisting of 2799 different BAC or PAC clones. In addition to known copy number polymorphisms in 5q11, 7q22, 7q35, 14q32, and 15q15, we were able to disclose copy number alternations (CNAs) in terms of gains in 9p, 11q, 13q and losses in 5p, 9p, 11q, 12p, 13q, and 16p. In a subset of cases we profiled global gene expression and the correlation of array-CGH findings with global gene expression profiles allowed the identification of candidate genes, e.g., FOXP1 and RYBP in 5p13 and MLL and DDX6 in 1q25. Furthermore, we applied two-class supervised analyses using the significance analysis of microarrays (SAM) method identified for the MLL-PTD cases a gain of a single clone harbouring the MLL gene underlying the power of array-CGH detecting small genomic aberrations. While the significance of these findings, which were already in part validated using fluorescence in-situ hybridization (FISH), still remains to be determined, our preliminary results demonstrate the power and reliability of this microarray-based technique allowing genome-wide screens of genomic imbalances. Furthermore, ongoing correlation of high-resolution genomic profiling with global gene expression studies will help to disclose pathways underlying normal karyotype AML, thereby leading to new insights of leukemogenesis.

0105

CO-EXPRESSION OF CD34, MDR1 AND BCRP INDICATES A CLINICALLY RESISTANT PHENOTYPE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA OF OLDER AGE

M.M. van den Heuvel-Eibrink,1 B. van der Holt,1 P.J.M. Vosselbedt,2 A.K. Burnett,1 W.U. Kraau,2 P.P. Piccaluga,1 M.E. Fey,3 G.E.G. Verheo,2 E. Vellenga2, G.J. Ossenkoppele,3,4 B. Löwenberg,1 P. Sonneveld1
1Erasmus MC-Sophia, ROTTERDAM, Netherlands; 2Erasmus MC-Daniel, ROTTERDAM, Netherlands; 3Erasmus MC, ROTTERDAM, Netherlands; 4Wales School of Medicine, Cardiff Univ., WALES, United Kingdom; 5Univ. Hosp. Benjamin Franklin, BERLIN, Germany; 6S Orsola-Malpighi Hosp., BOLOGNA, Italy; 7Inselspital, BERN, Switzerland; 8Univ. Hosp. Gastrohuis- berg, LEUVEN, Belgium; 9Univ. Med. Center Groningen, GRONINGEN, Netherlands; 10VU Medical Center, AMSTERDAM, Netherlands

Clinical resistance to chemotherapy in acute myeloid leukemia (AML) is often associated with the expression of the multidrug resistance (MDR) proteins P-glycoprotein (P-gp), encoded by the MDR1/ABC1 gene, multidrug resistant-related protein (MRP1/ABCC1), the lung resistance-related protein (LRP), or major vault protein (MVP), and the breast cancer resistance protein (BCRP/ABCG2). The clinical value of MDR1, MRP1, LRP/MVP and BCRP mRNA expression was prospectively studied in 154 newly diagnosed AML patients aged ≥60 years, who were treated in a randomized clinical trial. Expression of MDR1 and BCRP showed a negative while MRP1 and LRP showed a positive correlation with the global copy number loss of respective transcripts, p<0.01. Higher BCRP mRNA was associated with secondary AML (p<0.05). A strong correlation between MDR1 and BCRP mRNA expression was observed (p<0.001), while also MRP1 and LRP mRNA co-expression was found (p<0.001). High MDR1 but not MRP1, LRP or BCRP mRNA expression was associated with a lower complete remission rate and with worse event-free survival (EFS) and overall survival (OS), using univariate analysis. Although CD34 expression overruled all other prognostic factors, co-expression of MDR1 and BCRP identified a clinically resistant subgroup of elderly AML patients with a low CR rate and poor EFS and OS (p-values respectively: 0.01, 0.01 and 0.05).

0106

REPLICATIVE SENESCENCE INDUCTION IN GOOD-RISK ACUTE MYELOID LEUKEMIA

J-M. J. M. Zijlmaas,5 S.J.J. Swiggers,6 H.B. Beverloo,6 B. Löwenberg,6 ErasmusMC Rotterdam, ROTTERDAM, Netherlands

Immortal cell growth is considered the hallmark of tumor cells. In contrast, normal cells have a limited proliferative capacity of 40-60 cell divisions, also known as the Hayflick limit. The limited proliferative capacity of normal cells relates to gradual shortening of telomeric DNA as a consequence of the end-replication problem. Upon critical shortening of telomeric DNA, cells enter a non-replicative but viable state referred to as replicative senescence. These replicative senescent cells stain blue in a β-Galactosidase assay. Human fibroblast models have shown that escape from senescence results from loss of p53 and Rb function. Escape is associated with activation of a telomere maintenance mechanism (fully or at least partially). This model accounts for the limited replicative capacity of normal cells and most tumor cells, allowing for immortal cell growth. Recently, we demonstrated relatively low levels of telomerase in AML patients with t(8;21) or inv(16) (Swiggers et al., GCC 2006). Interestingly, levels of telomerase in similar samples were similar to levels of telomerase in normal bone marrow progenitor cells. We hypothesized that these AML cells, where telomerase is not reactivated to high levels, may not have inactivated the senescence pathways that limit the proliferative capacity of normal cells. This hypothesis was addressed by studying AML patient samples with t(8;21), t(15;17) or inv(16) in vitro (long-term cell cultures in presence of growth factors) and in vivo (following transplantation in NOD-SCID mice and in patients at time of relapse) for cells with all characteristics of replicative senescence, i.e., viable, non-proliferating, blue-coloring in β-Gal assay, and critical short telomeres. AML cells with all characteristics of replicative senescence were clearly observed in AML samples with either t(8;21), t(15;17) or inv(16). Gradual telomere shortening was observed in these AML cells in vitro and on long-term culture, in vivo following transplantation in NOD-SCID mice and in vivo in patients at relapse, indicating that these AML cells do not have an adequate telomere maintenance mechanism. We included in the study a control group of AML that is characterized by telomerase reactivation to high levels (complex karyotype group, n=8). Cells with characteristics of replicative senescence were not induced in vitro or in vivo in any of the samples of this AML control group. We conclude that AML cells with t(8;21), t(15;17) or inv(16) are characterized by intact pathways of replicative senescence. Intact pathways that limit proliferative lifespan may be critical to the high cure rates following chemotherapy treatment of patients with good-risk AML.

0107

PROGNOSTIC IMPACT OF FLOW CYTOMETRICALLY DETERMINED MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

W. Kern,1 D. Voskova,2 C. Schoch,2 S. Schnittger,3 T. Haferlach2
1MILL Munich Leukemia Laboratory, MUNICH, Germany; 2Erasmus MC Rotterdam, MUNICH, Germany; 3Leukaemia Service, BETH ISRAEL DEACONESS MEDICAL CENTER, BOSTON, USA

Background. Adaption of treatment in acute myeloid leukemia (AML) is based on individual risk profiles. Significant prognostic information may be provided by the level of minimal residual disease (MRD). Aims. To assess the prognostic impact of flow cytometrically quantified MRD levels in AML in a multivariate analysis. Methods. We applied multiparameter flow cytometry (triple staining) to highly sensitively quantify MRD in patients with AML. A total of 586 patients receiving standardized intensive antileukemic treatment was analyzed at different check-points: CP1 (up to day 28, n=156), CP2 (day 28-60, n=122), CP3 (day 61-120, n=195), CP4 (day 121-365, n=172), and CP5 (after day 365, n=73). Leukemic cells were identified by their individually defined leukemia-associated aberrant immunophenotypes (LAIPs). MRD levels were calculated as the logarithmic difference (LD) between LAIP-positive cells at diagnosis and LAIP-positive cells at follow-up assessment. Results. The median LD amounted to 2.18 (range, -0.03 to 4.17) at CP2, 2.49 (0.11 to 4.17) at CP3, 2.58 (-0.28 to 4.28) at CP4, and 2.87 (0.46 to 4.02) at CP5. A higher LD (continuous variable) was associated with shorter overall survival (OS) and event-free survival (EFS) as well as the risk of relapse. The median LD at CP1, CP2, CP3 and CP4 increased progressively with worse EFS and OS (p<0.001). In a multivariate analysis, LD at CP1 was independently associated with inferior OS (hazard ratio = 2.87, 95% confidence interval [1.36-5.25], p=0.005). Conclusions. The level of minimal residual disease (MRD) during treatment may serve as an additional prognostic parameter in AML patients. Further studies are required to determine the optimal cut-off level to achieve significant clinical impact.
related to a better event-free survival (EFS; CP1, \(p=0.0002\); CP2, \(p=0.00001\); CP3, \(p=0.0012\); CP4, \(p=0.0001\); CP5, \(p=0.00007\)) and to a better overall survival (OS; CP1, \(p=0.004\); CP2, \(p=0.001\); CP3, \(p=0.021\); CP4, \(p=0.00006\)). Other parameters related to EFS and OS were age and cytogenetics in the present series. The prognostic impact of MRD levels on outcome was independent of cytogenetics and age for EFS (CP2 to CP5) and OS (CP3 and CP4). MRD was the most important prognostic parameter at CP4 and CP5. Separation of patients into two groups, respectively, by the median LD resulted in significant differences in EFS at all CPs and in OS at CP1 to CP4. The largest difference was observed at CP4; median EFS, 57.1 vs. 17.5 months, \(p=0.00001\); 3-year-OS, 95% vs. 65%, \(p=0.0005\). Summary. A highly powerful and independent prognostic parameter is provided by the MRD levels determined by multivariate analysis of flow cytometry which is applicable to the total of an AML population. It should be evaluated as a stratification parameter in clinical trials.

0109

**EXPRESSION OF TUMOR-ASSOCIATED ANTIGENS IN ACUTE MYELOID LEUKEMIA AND THEIR CORRELATION WITH SURVIVAL**


University of Ulm, ULM, Germany, Stanford University, STANFORD, USA

Background. The expression of tumor-associated antigens (TAAs) might play a critical role in the control of minimal residual disease (MRD) in acute myeloid leukemia (AML). Here, we investigated whether TAAs were associated with clinical outcome in AML. Methods. A DNA-microarray analysis of 116 AML samples as well as ELISPOT, FACS and chromium release assays were performed to assess TAA-specific T cell responses in these patients. Results. A significant correlation of high mRNA expression of G250/CA9 with a longer overall survival \((p=0.022)\), a trend for better outcome in patients with high expression levels of PRAME \((p=0.105)\), and a hint for RHAMM/HMMR. In contrast, for other TAAs like WT1, TERT, FRTN3, BCL2, and LAMR1 we found no correlation with clinical outcome. Interestingly, the co-expression of RHAMM/HMMR, PRAME and G250/CA9 provided a favorable prognostic effect \((p=0.006)\). We also observed specific T cell responses at high frequency for these antigens. Positive immune reactions were detected in 9/17 (47%) AML patients for RHAMM/HMMR-derived, in 5/17 (29%) for PRAME-derived, and in 6/17 (35%) for G250/CA9-G2-derived peptides. Furthermore, we could demonstrate specific lysis of T2 cells presenting these epitope peptides. Conclusion. The expression of the TAAs RHAMM/HMMR, PRAME and G250/CA9 induced strong anti-leukemic immune responses possibly enabling the control of MRD in AML patients. Thus, these TAAs represent interesting targets for polyvalent immunotherapeutic approaches.

0110

**PROGNOSIS OF ACUTE MYELOID LEUKEMIA PATIENTS < 60 YEARS WITH TRISOMY 8 AS A SOLE ABERATION: POOLED DATA ANALYSIS OF THE GERMAN ACUTE MYELOID LEUKEMIA INTERGROUP**


Universitätsklinikum C.C.Carus, DRESDEN, Germany, Medizinische Klinik I, UNIVERSITÄTSKLINIKUM ULM, Germany, Klinik für Hämatologie/Onkologie, UNIVERSITÄTSKLINIKUM HANNOVER, Germany, Medizinische Klinik V, Germany, Abteilung für Medizinische Informatik, UNIVERSITAT MÜNSTER, Germany, Medizinische Klinik A, UNIVERSITÄTSKLINIKUM MÜNSTER, Germany

Background. Trisomy 8 (\(+8\)) occurs in about 8-13% of patients with acute myeloid leukemia (AML). However, so far the prognostic impact and best consolidation strategy of this recurrent aberration is unclear. Thus, additional prognostic factors are needed to further classify these patients and to deliver appropriate treatment. Methods. Pooled data analysis was performed on 198 adult patients (median age 49 (17-60) years) with \(+8\) treated between 1995 and 2002 in eight prospective German AML treatment trials. Patients with \(t(8;21)\), inv(16) and an additional \(+8\) were not included in the study. Clinical, diagnostic and laboratory data were reviewed for consistency and completeness before analysis by a central coordination center. Results. Ninety-two (46%) patients had \(+8\) as a sole aberration, 59 (20%) had one additional secondary aberration and 67 (34%) had \(+8\) within complex karyotypes with at least three independent abnormalities. Patients with \(+8\) as a sole aberration had a 3-year overall (OS) and relapse-free survival (RFS) of 27% (95%-CI 18%-36%) and 31% (95%-CI 18%-43%), respectively. Multivariate analysis including standard clinical and laboratory data, as well as percentage of \(+8\) positive metaphases and FLT3 status revealed extramedullary disease at diagnosis as a significant prognostic variable for worse survival (HR 2.56 (95%-CI 1.34-4.87); \(p=0.06\)) whereas post-remission therapy (i.e. high-dose cytaraabine vs. autologous vs. allogeneic stem cell transplantation) did not influence survival. Conclusion. AML patients with \(+8\) as a sole aberration can be stratified by extramedullary disease at diagnosis into two prognostic groups. However, alternative treatment approaches are needed to achieve more durable remissions in this AML entity in the future.
0111

PROGNOSTIC IMPACT OF THE NPM1/FLT3 ITD MUTATION STATUS IN ELDERLY PATIENTS >60 YEARS OF AGE WITH NORMAL KARYOTYPE AML: RESULTS OF AMLSG TREATMENT TRIAL AML HD98B

K. Döhner, A. Corbacioglu, B. Krebs, S. Hein, L. Bullinger, S. Frohling, H. Döhner, R.E. Schlenk
University of Ulm, ULM, Germany

Background. NPM1 mutations have been identified in approximately 50% of patients with normal karyotype acute myeloid leukemia (AML) and thus represent the most frequent genetic alteration in this subset of patients. In three recent studies mainly involving younger adults, statistical analysis revealed a significant interaction between NPM1 mutations and FLT3 internal tandem duplications (ITD). Only patients with NPM1 mutation in the absence of FLT3 ITD had a significantly better relapse free survival (RFS) and overall survival (OS). Aims. To evaluate the prognostic impact of the combined NPM1/FLT3 ITD mutation status on response to therapy, RFS and OS in elderly AML (> 60 years) with normal karyotype, with a favorable outcome. These data are most relevant when aiming at selecting those elderly patients who may benefit from intensive therapy and those who will not.

0112

PROGNOSTIC DETERMINANTS IN ADULT AML PATIENTS WITH INTERMEDIATE RISK KARYOTYPE

F. Buccisano,1 L. Maurillo,1 G. Del Poeta,1 M.I. Del Principe,1 P. Panetta,1 D. Renzi,2 C. Sarlo,1 L. Franceschini,1 V. Gattei,1 F. Lo Coco,1 S. Amadori,1 A. Venditti1
1Tor Vergata University Hospital, ROME, Italy; 2Centro Riferimento Oncologi-co, AVIANO (PN), Italy

Background. According to the prognostic classifications of karyotypic abnormalities of AML (MRC and SWOG), the intermediate group includes patients either lacking good and poor karyotype or with normal karyotype. Therefore, it represents, by definition, a miscellaneous group for which the evaluation of the better treatment strategy is difficult due to its heterogeneity. Moreover, patients belonging to this intermediate group account for the large majority of AML cases enrolled into clinical trials. Aims. The aim of our study was to analyze the factors specifically affecting the outcome of patients bearing intermediate karyotypic abnormalities in a group of 94 AML cases entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61yrs) or AML13/AML15 (age >61yrs), consisting in intensive induction and consolidation cycles. Methods. The clinico-biological variables evaluated in our model included age, FAB, WBC count, MDR1 phenotype, FLT3 mutations and level of post-consolidation bone marrow residual leukemic cells (BMRLC) assessed by multiparametric flow-cytometry (MPFC). By applying the maximally selected log-rank statistics, a threshold discriminating MDR negative from positive cases was set at 3.5×10^4 BMRLC, a level that allowed the identification of distinct subgroups of MDR- and MDR+ patients, both at post-Ind and post-Cons time-points. Results. Patients with <3.5×10^4 BMRLC at the end of consolidation therapy were considered MDR- and showed a better outcome, patients whose level of MDR were >3.5×10^4 at the end of consolidation were considered MDR+ and showed a poor prognosis. Using the MRC classification, 14/94 patients (15%) had a good-risk cytogenetics, 74/94 (79%) an intermediate-risk and 6/94 (6%) a poor-risk. When we restricted the analysis to cases with intermediate-risk karyotype we found that: 1) patients in the MDR- and MDR+ group differed significantly in terms of relapse free survival (RFS), overall survival (OS) and relapse rate (p<0.001, 0.006 and <0.001, respectively); 2) MDR- patients had an outcome slightly better than those bearing good risk karyotype; 3) MDR+ patients showed a dismal outcome comparable to poor-risk cytogenetic patients. Conclusions. These results suggest that the inclusion of the MDR assessment of MRD in patients with intermediate risk karyotype may be particularly useful in discriminating subgroups with different outcomes, in a group of AML where karyotype does not represent a clear prognosticator, allowing clinicians to design risk-based therapeutic programs.

0113

ROLE OF DNA METHYLTRANSFERASES (DNMTs) AND POLYCOMB GROUP OF PROTEINS IN LEUKEMIA

L. Di Croce
Centre for Genomic Regulation, BARCELONA, Spain

PML-RAR induces a block of hematopoietic differentiation and acute promyelocytic leukemia. This block is based on its capacity to inactivate the terminal maturation program of promyelocytic leukemia. In analogy to the studies performed in younger AML, statistical analysis revealed a significant interaction of NPM1 and FLT3 ITD mutations. Only the NPM1+/FLT3 ITD- genotype predicted for high response to induction therapy and better survival probabilities. Complete remission rate (CR) of this subgroup was significantly better than that in the other three subgroups (NPM1-/FLT3 ITD-, NPM1+/FLT3 ITD+, NPM1-/FLT3 ITD+). Treatment failure in the three latter groups was due to a higher degree of refractory leukemias (55% vs. 20.5%). The higher response to therapy translated into significant better RFS (p=0.001) and OS (p=0.01) probabilities in the NPM1+/FLT3 ITD- compared to the three other genotypes. Conclusions. The NPM1+/FLT3 ITD- genotype defines a distinct subset of elderly AML. These data are most relevant when aiming at selecting those elderly patients who may benefit from intensive therapy and those who will not.

Reference


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**0114**

**TRISOMY 8 AS SOLE ANOMALY OR WITH OTHER CLONAL ABERRATIONS IN ACUTE MYELOID LEUKEMIA: IMPACT OF CLINICAL PRESENTATION AND TREATMENT OUTCOME**

X. Thomas, N. Jaff, Y. Chelghoum, M. Elhamri, I. Tiguad, M. Michallet
Edouard Herriot Hospital, LYON, France

**Background.** Trisomy 8 is the most frequent numerical aberration in acute myeloid leukemia (AML). It occurs either as the sole anomaly or together with other clonal chromosome aberrations. Only few data are available regarding their prognostic significance. Aims. In order to investigate whether accompanying chromosome anomalies influence the clinical outcome of patients with trisomy 8, we assessed clinical and biological characteristics, and response to therapy, of an unselected group of patients with previously untreated AML, presenting with trisomy 8 either alone or with other chromosomal aberrations. Methods. One hundred and fifty-four cases (median age: 65 years) were diagnosed in our institution between 1981 and 2005 including 47 patients (31%) with trisomy 8 as the sole aberration, 107 patients (69%) associated with other cytogenetic abnormalities (13% with favorable risk, 54 with intermediate risk, and 40 with unfavorable risk cytogenetics). Results. Twenty-eight patients only received symptomatic therapy or died before any chemotherapy could be given. All other patients received induction treatment according to different protocols used during the period of study. Overall complete remission (CR) proportion was 48% (95% confidence interval (CI): 40% - 56%). Sixty-six patients achieved CR after one course of chemotherapy and 8 patients after salvage therapy. Median disease-free survival (DFS) of the entire cohort was 7.8 months (95% CI: 6.5-9.9 months) and median overall survival (OS) was 8.3 months (95% CI: 5.2 - 9.8 months). In multivariate analysis, age more than 60 years and trisomy 8 associated with unfavorable chromosomal aberrations were of poor prognostic value for CR achievement. Age more than 60 years and antecedents of dysmyelopoiesis were of prognostic value for DFS and OS. Patients with trisomy 8 alone did not show a significant difference in terms of outcome as compared with those in whom trisomy 8 was associated to intermediate risk chromosomal aberrations. Patients with trisomy 8 in addition to favorable chromosome aberrations maintained a good clinical outcome, while those with trisomy 8 in addition to unfavorable karyotypes showed the worst prognosis. Conclusions. Trisomy 8 as a whole has poor survival, which is largely attributable to worsened outcomes among patients whose trisomy 8 was associated with unfavorable cytogenetic abnormalities. A particular poor outcome was observed in patients presenting trisomy 8 with antecedents of myelodysplasia.

**0115**

**ANALYSIS OF 1458 ACUTE MYELOID LEUKEMIA FROM THE ALERT PROJECT (ACUTE LEUKEMIA CLINICAL REGISTER) IN THE CZECH REPUBLIC IN 1996-2006**

I.K. Indrak,1 D.M. Doubek,2 V.J. Voglová, K.V. Koza, K.T. Kozák,1 S.T. Szotkowski,1 M.J. Mayer,1 Z.P. Žák,1 C.P. Cetkovský,3 M.K. Michalova,3 J.M. Jarosova,3 S.K. Steinerova,3 M.J. Muzik,3 D.I. Dusek,3 M.J. Maaloufova4
1University Hospital, OLOMOUC, Czech Republic; 2Institute Hematol. and Blood Transf, PRAGUE, Czech Republic; 3Center of Biostatistics and Analysis MU, BRNO, Czech Republic; 4Institute Hematol. and Blood Transf., PRAGUE, Czech Republic

Project ALERT was initiated 9 years ago as population registry for acute leukemias, based on the cooperation of large haematological centres in the Czech Republic. Although the primary aims were rather clinical than epidemiological, the growing database tends to become representative for the Czech population, at least in the category of intensively cured AML patients. The database is improved to provide parametric collection of data gathered in accord with the therapeutic protocols and is full-scale record of prognostic markers including cytogenetic maps. The now presented and analysed cohort consists of 1458 AML patients registered in ALERT from January 1996 to December 2005. Median age of the patients was 58 years (52-74 year: 10.9% kvalnt limit), 636 (44%) patients were ≥55y and 822 (56%) older than 55y. The patients were observed with median 2y (0-5.5 y 5-9.5y kvalnt limit). 90.4% of younger group pats were treated by intensive remission (CR rate 76%, median OS 17 months, DFS 22m and OS of the patients that achieved CR was 47.5 m). No differences were found between different induction intensity regimens. In the older group out of 56% of patients that received induction treatment 53% pats achieved CR with median OS 9m (DFS 11.2 m and OS of the patients achieved CR was 20 m). Out of 435 younger pats 18.5% were treated with standard dose consolidation, 58% with intermediate or HDCH and 39% with SCT (31% Auto SCT, 20% MUD SCT and 49% sibling donor SCT). In the older group out of 254 patients treated with consolidation treatment 37% were treated with standard dose consolidation CH, 45% with ID or HDCH and 10% only with SCT. The differences for OS and DFS with different consolidation treatment regimens will be given. The analysis of prognostic significance of age confirmed age over 55 as poor prognostic factor. The cytogenetic results of 758 intensively treated patients were studied. These include 106 PML pts. More than 80% of these patients live in molecular CR. The stratification of cytogenetic data for very good prognosis (AML, good prognosis, standard prognosis and poor prognostic confirmed their prognostic significance for OS, but surprisingly there were not found prognostic differences for DFS between standard and good prognostic group. Very important result revealed the analysis of centers showing comparable therapeutic results in all centers treating AML in the Czech Republic, even if they do not use the same therapeutic protocols for the treatment of AML. More detailed data will be given in the presentation.

**0116**

**CLORETAZINE (VNP40101M) HAS SIGNIFICANT ACTIVITY AS INDUCTION THERAPY FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA OR ADVANCED MYELODYSPLASTIC SYNDROME**

J. Giles,1 D. Rizzieri,2 K. D. Khan,1 M. Guft,3 G. Verhoef,1 E. Wijermans,1 A. Advani,4 V. Karsten,3 R. Geller,4 S. O’Brien,4 N. Vey,5 MD Anderson Cancer Center, HOUSTON, USA; 1Duke University Medical Center, DURHAM, NC, USA; 2Cancer Research UK, LONDON, UK; 3King’s College, LONDON, UK; 4University Medical Center, Utrecht, THE NETHERLANDS; 5Institut Paoli-Calmettes, MARSEILLE, France

**Background.** The incidence of AML increases with age at a median age of 68 years at diagnosis. Elderly pts with AML are more likely to have adverse prognostic factors related to the biology of the disease (adverse cytogenetics, history of MDS or prior exposure to cytotoxic agents, increased expression of multi-drug resistance) and medical comorbidities. Little progress has been made in improving outcomes in older pts. Despite variations on the 7+3 regimen, most elderly pts do not receive cytotoxic chemotherapy; pts who are treated with current regimens have lower response rates and overall survival, as well as poorer tolerance of side effects, compared to younger pts. New agents are required to increase complete remission (CR) rate and duration with improved safety in this population. Cloretazine, is a novel alkylating agent that has shown significant anti-leukemia activity in vitro and in vivo models. Aims. A multi-center Phase II study was conducted to investigate activity and safety of Cloretazine, in pts ≥60 years old with newly diagnosed AML or high risk MDS. Methods. Cloretazine, was administered IV at 600 mg/m² as a single 80-60 min. IV infusion. Second induction was allowed for patients who showed improvement. Patients who achieved CR or CRp could receive a consolidation course of 400 mg/m².
an overall response rate of 31%. Response in 45 de novo AML, 45 sec-ondary AML and 39 high risk MDS was 30%, 42% and 42% respec-tively. Response by cytogenetics was 42% in 57 intermediate pts and 22% in 41 unfavorable pts. The CR rates achieved with Cloretazine are consist-ent despite increasing age and declining performance status. Severe drug-related non-hematologic toxicity was rare. Nineteen (18%) pts died within 30 days of receiving Cloretazine. As demonstrated in the figure below, however, 1-year survival for all treated pts (N=105) was 12% and 28% for pts with CR (N=53). Patients with de novo AML who achieved CR had a median survival of 5 months and a 1-year survival of 52% (N=22). Conclusion: Cloretazine, is well tolerated and has sig-nificant activity in an elderly patient population with AML or MDS. The encouraging activity in patients with de novo AML warrants further evaluation.

**0117**
**DOsing of TroxATylin (TROXACitABine, SXG-145) BASED on renAL FUNCTION**

M.J. Kelner,¹ J. Giles,¹ J. Feldman,¹ J. Roboz,¹ J. DeAngelo,¹ A. Lokker,¹ K. Burley¹

¹University of California, San Diego, SAN DIEGO, USA; ²M.D.Anderson, HOUSTON, USA; ³New York Presbyterian Hospital, NEW YORK, USA; ⁴Dana-Farber Cancer Institute, BOSTON, USA, ⁵SGX Pharmaceuticals, Inc., SAN DIEGO, USA

**Background.** Troxatyl™ (troxacitabine, SXG-145) is a novel L-configuration nucleoside analogue with unique mechanistic and cytotoxic properties. Troxatibine is clinically active in patients with relapsed or refractory acute myelogenous leukemia (AML), including those who have failed bone marrow transplantation. Troxatibine has also demonstrated clinical activity against chronic myeloid leukemia, myelodysplastic syndromes, renal cell carcinoma, and pancreatic cancer. An international (European and North American) multi-center Phase 2/3 clinical trial is currently underway to evaluate the safety and efficacy of troxatibine continuous IV infusion treatment in second salvage AML (SDP758-216; www.clinicaltrials.gov). The major route of elimination of troxatibine is renal excretion as unchanged drug (~70%) and there is no detectable protein binding. These results suggest that renal function may play a significant role in determining the troxatibine blood concentration in an individual patient. Aims. The aims of this study were to determine the influence of renal function on troxatibine steady-state plasma concentrations (Css), and to determine if a correlation exists between troxatibine CcSs and clinical response. Methods. Pharmacoki-netic and toxicity data from multiple AML clinical trials (enrolling >200 patients) are being analyzed to (1) Determine the relationship between troxatibine CcSs and estimated creatinine clearance; (2) Identify the minimum troxatibine Ccss required to achieve a clinical response (Cr or CRp); (3) Define an upper limit of troxatibine Css for adverse risk; and (4) Develop a dose nomogram or equation to prospectively adjust troxatibine dosing, based on patient renal function, which will allow avoidance of excessive toxicity while still achieving therapeutic blood levels. Results. Initial results indicate that in order to induce remis-sion in relapsed or refractory AML patients, troxatibine Ccss values must ≥ 80 ng/mL. High troxatibine Ccss values may correlate with increased toxicity, although an upper limit for adverse risk has not yet been defined. For patients with normal to mildly impaired renal function (creatinine clearance > 45 but < 125 mL/min), a Calvert style formula, based on estimated creatinine clearance, has been developed to define the minimum dose required to achieve the target troxatibine Ccss of > 80 ng/mL. Both linear and non-linear nomogram models are being developed to adjust troxatibine dosage for patients with moderate impairment of renal function (creatinine clearance < 45 mL/min) or for patients with higher glomerular filtration rates (creatinine clearance > 125 mL/min). Summary. A firm relationship exists between renal function and troxatibine Ccss values, and between troxatibine Ccss values and clinical response in patients with relapsed or refractory AML. These results indicate that developing a dosing strategy, based on patient renal function, may be warranted to obtain optimal troxatibine Ccss values with minimal toxicity.

**0118**
**CYTogenetic RESPONSES in oLDER PATIENTS witH AML and COMPLEX karyotype TREATED wITh low-Dose 5-Aza-2-deoxycytidine (DECITABINE)**

B. Rüter,¹ S. Knipp,¹ U. Gernig,¹ M. Lübbert¹

¹University of Freiburg, FREIBURG, Germany; ²University of Düsseldorf, DÜSSELDORF, Germany

**Background.** The demethylating agent Decitabine (DAC) induces hematologic and cytogenetic remissions in older patients (pts) with MDS and AML (Ruter et al. Int J Hematol. 2004; 80: 128-35). Cytogenetic nor-malization was more frequently seen in pts with poor-risk cytogenetics (mostly complex karyotype) compared to intermediate-risk (Lübbert et al Br J Haematol 2001; 114: 349). Aim. Prospective analysis of induction of cytogenetic and hematologic responses in AML pts aged > 60 (meli-odysplastic, high risk MDS with AML) treated with DAC (15mg/m² total) over 8 weeks. Methods. We systematically evaluated sequential cytogenetics in 39 consecutive pts (median age 73 years, range 65-86) treated at two study centers. Pts aged > 60 years diagnosed with AML (according to FAB, >30% blasts) were treated with DAC (15mg/m² total) over 8 weeks or cycles over a median of 6 weeks (range 2-4) during course 2 in DAC-sensitive pts. For pts with successfully comple-mented 4 courses a maintenance treatment with 20 mg/m² DAC i.v. over 1 hour on 3 days (total dose 60 mg/m², outpatient administration) every 4 weeks was offered. Cytogenetics were performed before course 1-3 and at the end of course 4. Results. Of 35/39 successfully karyotyped pts 23 had chromosomal abnormalities prior to treatment. Cytogenetic sub-groups were: good risk: 0; intermediate risk: 21 pts (12 with normal karyotype); poor risk: 14 pts. Pts received a median of 2 DAC courses (range 1-4) and 10 pts a median of 2 maintenance courses (range 1-9). 21/35 pts received ≥ 2 courses DAC (17 also ATRA) and were evaluable for cytogenetic remissions. 13 of the 21 pts (62%) had chromosomal abnormalities at diagnosis (9 with complex karyotype). 3/13 pts had a complete cytogenetic normalization, all with a complex karyotype at time of diagnosis (aberrations of chromosome 5 in 3, of chromosome 7 in 2). The cytogenetic response occurred after a median of 3 cycles (range 1-5). Overall hematologic response in pts with intermediate-to-treat (ITT) was 43% (17/39) with 6 CR (15%), 4 PR (10%) and 7 antileukemic effect (AL) (18%). Conclusions. Low-dose DAC is active, by ITT, in inducing hematologic response in 45% and cytogenetic nor-malization in 15% of older AML pts (according to FAB), all 3 with com-plex karyotype. In MDS pts cytogenetic normalization (after a median of 4 courses) had been seen frequently (31%). AML pts received a median of 2 courses which could explain at least in part the lower cytogenetic response rate.

**0119**
**SINGLE POINT MUTATIONS in the juxtAMembrane domaIN of FLt3 ARE BARELY FOUNd in aLM PATIENTS and DEFINE the THIRD CLASS of ActivATING MUTATIONS**

C. Reinid,¹ K. Bagrintseva,² S. Vempati,² S. Schnittert,² J.W. Ellwart,² K. Wenig,² K.P. Hopfner,² H. Hiddemann,³ K. Spiekermann³

¹University of Düsseldorf, DÜSSELDORF, Germany, ² claw, University of California, San Diego, SAN DIEGO, USA; ³University of Munich, MUNICH, Germany

**Background.** FLT3 is a receptor tyrosine kinase involved in granulocytic/myeloid differentiation. Up to now, FLT3 mutations have been classified in two groups: internal tandem duplications (FLT3-ITD) and point mutations (FLT3-TKD) in the juxtamembrane (JM) domain, which occur in approximately 30% and 15% of patients, respectively. FLT3-ITD is found in 20-25% of patients with high risk MDS and 5-10% of patients with AML. Our aim was to analyze the FLT3 gene for activating point mutations in patients with MDS or AML. Methods. The FLT3 gene was amplified and analyzed by direct sequencing and by an allele specific PCR assay. Results. FLT3 point mutations in the juxtamembrane domain were found in 10% of patients with MDS and 5% of patients with AML. Conclusion. FLT3 point mutations are rare in MDS and AML. However, the presence of activating FLT3 mutations in AML and MDS may have important therapeutic implications. Other potential roles of FLT3 point mutations in the JM domain of FLT3 could be FLT3 activation by ligand-dependent and ligand-independent mechanisms. Further studies, including functional analysis, are required to evaluate the impact of FLT3 point mutations in the JM domain of FLT3.
showed a weaker gain-of-function phenotype in terms of proliferation, anti-apoptosis, activation of FLT3 and the downstream target STAT5. As the FLT3-JM-PM cluster at a core interaction site of the JM domain with the remainder of the molecule we hypothesized that the oncogenic potential results from the perturbation of the autoinhibitory mechanism. Mapping of the FLT3-JM-PM on the crystal structure of FLT3 (figure) could show that these mutations probably reduce the stability of the autoinhibitory JM domain. In comparison to FLT3-ITD mutations, that increase the length of JM, the FLT3-JM-PM induce a considerably weaker perturbation and this might provide a structural basis for the weaker (FLT3-JM-PM) versus stronger (FLT3-ITD) transforming capacity of these mutations. **Summary.** A third class of activating FLT3 mutations exists in 2% of AML patients, point mutations in the structurally important JM domain (FLT3-JM-PM). Patients carrying FLT3-JM-PM might benefit from the treatment with selective FLT3 inhibitors. FLT3-JM-PM provide a remarkable example how mutations disturbing the autoinhibitory JM domain of class III RTK can contribute to the pathogenesis of cancer.

**0120**

**ORIGINAL DATA BASED META-ANALYSIS ON PATIENTS >60 YEARS OF AGE WITH ACUTE MYELOID LEUKEMIA RESULTS OF THE GERMAN AML-INTERGROUP**

R.F. Schlenk,1 B. Krahl,1 C. Sauerland,1 T. Büchner,1 M. Schaal,2 G. Ehninger,1 A. Ganser,3 K. Döhner,1 H. Döhner,1 D. Niederwieser4 1University of Ulm, ULM, Germany; 2University of Leipzig, LEIPZIG, Germany; 3University of Münster, MÜNSTER, Germany; 4University of Dresden, DRESDEN, Germany; Hannover Medical School, HANNOVER, Germany

**Background.** Acute myeloid leukaemias exhibiting t(8;21) or inv(16) karyotypes are referred to as core binding factor (CBF) acute myeloid leukaemia (AML). In younger patients with CBF-AML relapse free survival (RFS) was markedly improved following dose-intensive cytarabine for consolidation therapy. However, in older patients (>60 years) the clinical course is characterized by significantly inferior outcome and the value of dose-intensive cytarabine remains unclear. **Aims.** To review the clinical course of CBF-AML in a large cohort of elderly patients and to define the relative value of different treatment strategies. **Methods.** We performed a meta-analysis on 65 patients with t(8;21) and on 51 patients with inv(16) from 4 German leukaemia study groups. The patients were treated in 2 different prospective multicenter treatment trials between 1995 and 2004 (DSII, n=36, AMLSG n=26, AMLCG n=34, OSHO n=20). Induction therapy consisted of standard dose cytarabine (ARAC) combined with etoposide/idarubicin or mitoxantrone, or dose-intensified cytarabine in combination with idarubicin or mitoxantrone; postremission therapy consisted of intensive chemotherapy followed by maintenance therapy in two trials. **Results.** The median age was 66 years (range 61-85) and median follow up time was 55 months. Response to induction therapy for t(8;21) and inv(16) was as follows: complete remission (CR) 72.5% and 86%, refractory disease (RD) 12% and 10%, early/hypoplastic deaths 15.5% and 4%, respectively. RFS and overall survival (OS) after 4 years were for t(8;21)-AML 20% (95%-CI 11-38%) and 20% (95%-CI 11-35%) and for inv(16)-AML 35% (95%-CI 23-54%) and 33% (95%-CI 22-51%), respectively. To evaluate the impact of dosage of cytarabine on outcome patients were categorized into the HiDAC-group if they received at least one cycle of high-dose cytarabine with a cumulative dosage of 26g/m² (n=35) or otherwise into the STANDARD-dose cytarabine group (n=54). RFS was significantly (p=0.002) better in the HiDAC-group (44%, 95%-CI 30-65%) compared to the STANDARD-group (16%, 95%-CI 7-35%). **Conclusion.** Elderly patients with CBF-AML seem to benefit from dose-intensification of cytarabine above or equal to 6g/m² in at least one treatment cycle.

**0121**

**THE KINETICS OF REDUCTION OF MINIMAL RESIDUAL DISEASE IMPACTS ON DURATION OF RESPONSE AND SURVIVAL OF PATIENTS WITH ACUTE MYELOID LEUKEMIA**

F. Bucossi,1 L. Maurilli,2 G. Del Poeta,1 M.I. Del Principe,1 P. Panetta,1 N. Cristofari,1 D. Fraboni,2 G. De Angelis,1 M. Postorino,1 M. Ales,1 R. Cerretti,1 M. Rizzo,1 V. Gatti,1 F. Lo Coco,1 S. Amadori,1 A. Venditti1 1For Vergata University Hospital, ROME, Italy; 2Centro Riferimento Oncologico, AVIANO (PN), Italy

**Background.** We assessed by multiparametric flow-cytometry the levels of minimal residual disease (MRD) in 100 adult patients with acute myelogenous leukaemia (AML) achieving complete remission (CR) after standard dose-intensive chemotherapy. **Aims.** The aim of the present study was to determine the optimal threshold level, in term of residual leukemic cells (RLC), and the time-point of choice, i.e. post-induction (post-Ind) or post-consolidation (post-Cons), capable to better predict outcome of AML patients. **Methods.** By applying the maximally selected log-rank statistics, the threshold discriminating MRD negative from positive cases was set at 3.5×10⁴ RLC, a level that allowed the identification of distinct subgroups of MRD⁺ and MRD+ patients, both at post-Ind and post-Cons time-points. **Results.** Post-Cons MRD⁺ patients had a superior outcome in terms of relapse rate, OS and RFS (p<0.001, for all comparisons), regardless of the MRD status after induction. In particular, patients entering MRD negativity only after consolidation showed the same outcome as those achieving early negativity after induction. Multivariate analysis including karyotype, age, MDRI phenotype, post induction and post-consolidation MRD levels indicated that the post-consolidation MRD status was an independent factor affecting relapse rate, OS and RFS (p<0.001, for all comparisons). **Conclusions.** 1) the threshold of 3.5×10⁴ is valid in discriminating risk categories in adult AML; 2) MRD assessment at post-consolidation time-point is critical to predict disease outcome.

**0122**

**CLOFARABINE AS FIRST-LINE TREATMENT OF ELDERLY (>65 YRS) AML PATIENTS WITH AN UNFAVOURABLE CYTOGENETIC PROFILE WHO ARE UNSUITABLE FOR STANDARD TREATMENT**

A.K. Burnett,1 M. Baccarani,2 P. Johnson,3 J. Yin,4 N. Russell5 1University of Wales College of Medicine, CARDIFF, United Kingdom; 2Institute of Haematology & Oncology, BOLOGNA, Italy; 3Western General Hospital, EDINBURGH, United Kingdom; 4Manchester Royal Infirmary, MANCHESTER, United Kingdom; 5Nottingham City Hospital, NOTTINGHAM, United Kingdom

**Background.** BIOW-121 is a phase II non-randomised trial of clofarabine, a next generation purine nucleoside analogue, in older patients (≥65 yrs) with previously untreated acute myeloid leukaemia (AML) who are unsuitable for standard (5+7) chemotherapy. **Aims.** The primary endpoint of study BIOW-121 is to determine the overall response rate (ORR) of clofarabine in this elderly (≥65 yrs) AML population who are unsuitable for standard treatment. ORR is defined as the sum of the number of patients who achieve a complete response (CR), a complete response with incomplete peripheral count recovery (CRi) and a partial response (PR) according to the international working guidelines. Secondary endpoints include duration of remission, time to progression, and the safety and tolerability of clofarabine in this patient population. **Methods.** 66 patients aged ≥65 yrs with untreated AML defined by the WHO classification were enrolled in BIOW-121. All patients were considered unsuit- able for standard treatment based primarily on age (≥65 yrs) and/or performance status. All patients received clofarabine 30mg/m² for 5 days repeated every 28 days (1 course). A preliminary analysis was conducted for patients with an unfavourable cytogenetic profile (16/68), for whom data was available, and for patients >70 years of age (36/66). Unfavourable cytogenetic profile was defined as the presence of a com-
plex karyotype, monosomies of chromosome 5 (del[5q] or 5q-), or 3q abnormalities as reported by Grimwade et al (U.K. MRC, AML 10 trial). Results. Of 63 patients, 25% (16/63) had an unfavourable cytogenetic profile. The median number of clonafarin courses administered was one. With a median age of 66 years the overall response rate (ORR) in this unfavourable cytogenetic patient group was 50% (8/16) with a 44% complete response (CR or CRi). 55% (36/66) of patients were aged >70 years. The ORR and CR rate was 56% and 44% respectively in patients aged > 70 years. The safety profile of clonafarin was acceptable and manageable in this elderly AML population considered unsuitable for standard treatment. Summary/Conclusions. Clonafarin demonstrates efficacy as first-line treatment of elderly (> 65 yrs) AML patients with an unfavourable cytogenetic profile, considered unsuitable for standard (3+7) chemotherapy.

### 0123

**ABCB1 (PGP) and ABCG2 (BCRP) PROTEINS ARE INDEPENDENT PROGNOSTIC FACTORS FOR COMPLETE REMISSION AND RELAPSE RISK, RESPECTIVELY, IN NORMAL KARYOTYPE ADULT DE NOVO ACUTE MYELOID LEUKEMIA**

M. Tiribelli, D. Damiani, E. Calistri, A. Geromin, A. Chiarvesio, A. Michelutti, M. Cavallin, R. Fanin

Division of Hematology and BMT, UDINE, Italy

**Background.** Multidrug resistance (MDR) is a major cause of failure in acute myeloid leukaemia (AML). P-glycoprotein (PGP) has demonstrated a high prognostic power, as a negative correlation between PGP over-expression, remission rate and survival has been observed in different studies on AML. Recently a new ATP-binding cassette protein, the breast cancer resistance protein (BCRP), has been identified. In cell lines BCRP confers resistance to many different compounds and plays an important role in affecting drug disposition, while its effective role in vivo is much less defined. Aims. We have compared the expression of BCRP and PGP in 73 consecutive cases of normal cytogenetic AML, in the attempt to identify another prognostic factor potentially useful to design a risk-adapted therapy. Methods. Seventy-three patients with a diagnosis of de novo AML with normal karyotype were included in our study. Median age was 55 years (range: 15-76) and 35 patients (48%) were older than 55. Patients were homogeneously treated, with an induction regimen containing fludarabine, cytara-bine and idarubicin, and a consolidation course with high-dose cytarabine and idarubicin. Complete remission (CR) was defined after two courses of therapy, according to the published criteria. Twenty-five patients considered at high risk of relapse and with an identical donor underwent allogeneic stem cell transplantation (SCT). Results. BCRP protein was over-expressed in 24/73 (33%) patients. BCRP positive cases showed a higher expression of CD56 antigen (11/24, 46%) compared to BCRP negative patients (10/47, 21%) (p=0.03). Similarly, PGP was more frequently over-expressed in patients with a concomitant expression of BCRP (15/24, 54%) than in BCRP-negative cases (11/49, 22%) (p=0.006). CR was obtained in 85/78 (75%) patients. Only advanced age, high level of PGP and CD34 expression affected remission rate in the univariate analysis. The first two factors retained their statistical significance also in the multivariate analysis, whereas CD34-positivity showed a strong trend toward significance (p=0.06). On the contrary, BCRP expression was not associated with CR obtaining. However a significantly higher probability of relapse was observed in patients with high BCRP expression: 14 out of 18 (78%) BCRP-positive patients relapsed, compared to only 14/37 (38%) in the BCRP-negative group (p=0.005). No other parameters were associated with an increased relapse risk. BCRP over-expression affected also disease free survival (8 vs 27 months, p=0.027). PGP expression, which is one of the strongest predictors of remission, did not influence DFS. Finally, a shorter survival was associated with response to induction therapy (CR or not, p=0.02), CD56 positivity (p=0.03) and by the expression of at least one MDR-associated protein (p=0.05). Summary/Conclusions. BCRP was over-expressed in a significant percentage of AML patients with normal karyotype. BCRP over-expression did not influence achievement of remission, as PGP did, but significantly affected CR duration. BCRP-positive patients displayed a significantly higher relapse rate. BCRP may be therefore regarded as an easy evaluable prognostic factor in AML with normal karyotype, and should help in the design of a risk-adapted post remission therapy.

### 0124

**IMMUNOPHENOTYPIC PATTERN OF HIGH RISK KARYOTYPE ACUTE MYELOID LEUKEMIA IS CHARACTERIZED BY EXPRESSION OF CD34 AND LACK OF MPO**


Hospital Clínico U. Salamanca, SALAMANCA, Spain; Hospital N. Sra de Sousos, AVILA, Spain; Hospital Clínico Universitario, VALLADOLID, Spain; Complejo Hospitalario, LEON, Spain; Hospital Virgen de la Concha, ZAMORA, Spain; Hospital del Bierzo, PONFERRADA, Spain

**Background.** Cytogenetic is nowadays the most remarkable prognostic factor in acute myeloid leukaemia (AML), and immunophenotypic analysis may help to identify some of the most frequent cytogenetic abnormalities in this disease. Aim. To identify immunophenotypic pattern present in AML with adverse cytogenetics. Methods. Samples from a total number of 185 de novo AML patients (median age: 54 years, range 10-91; 56% male and 44% female) were immunophenotypically analyzed by multiparametric flow cytometry, using a large panel of 25 monoclonal antibodies. A cut-off point of 20% of the total blast cell population was used to define a marker as positive or negative. Chromosome analysis was done on bone marrow cells, and the presence of an abnormal karyotype was confirmed using short-term (24-48 h) unstimulated cultures. At least 20 metaphases were analyzed. Definitions of cytogenetic clonality and karyotypic descriptions were in accordance with ISCN guidelines (1995). Results. Cases with abnormalities in chromosome 3, 5, 7 or complex karyotype (8 or more abnormalities) were considered as adverse prognosis. Among the AML cases classified as high risk karyotype, 32/59 (69%) showed a constant immunophenotypic pattern in blast cells characterized by the CD34 expression and lack of expression of cytoplasmatic myeloperoxidase. In contrast, this pattern was observed in 4/61 (9%) and 10/51 (22%) of good and intermediate cytogenetic risk group respectively (p<0.001). Moreover, when patients with chromosome 3 abnormalities were considered as an isolated group (n=9), this pattern was present in 89% of cases, while AML with -5/5q (n=10) and -7/7q (n=19) showed this immunophenotypic pattern in 50% and 53% of cases, respectively. By contrast, AML with good risk karyotypes (n=61) only showed this immunophenotypic pattern in 2/31 (6%) and 5/21 (24%) of inv(16) and no cases with t(15;17). Therefore, once the CD34+/MPO- immunophenotypic pattern is identified, the relative risk of bearing a cytogenetic abnormality belonging to the adverse cytogenetic group in de novo AML patients is 5.2 times when compared to the immunophenotypic profiles. Conclusions. The immunophenotypic pattern characterized by the expression of CD34 and lack of expression of MPO (CD34+ MPO-) is associated with adverse prognosis karyotype. Additional cytogenetic studies (e.g. FISH) should be performed in cases with normal or unsuccessful cytogenetics analysis in order to identify possible overlooked abnormalities.

### 0125

**ABNORMALITIES IN P53 AND P14ARF IN DE NOVO AML PREDICTS A VERY SHORT OVER-ALL SURVIVAL AND IN VITRO DRUG-RESISTANCE.**

H. Nahi, M. Merup, S. Lehmann, S. Bengtzen, M. Jansson, L. Möllgärd, C. Paul

Karolinska University Hospital, STOCKHOLM, Sweden

The purpose of this study was to correlate the karyotype of myeloblast to long-term overall survival and in vitro cytotoxicity of conventional antileukemic drugs in patients with de novo AML. Abnormalities in chromosome 17, which are associated with p53 mutations, and 9p, the locus that encodes among others p14ARF which binds and inactivates the HDM-2, which in turn targets p53 for degradation. Thus deletions in 9p21 resulting in inhibitory effects on p53 protein were focused on. Methods. Blast cells were isolated from 361 patients diagnosed with de novo AML during the last 20 years at our clinic. Chromosomal analysis was successful in 318 cases. All samples were tested for in vitro cytotoxicity for fludarabine, AMSA, mitoxantrone, vesepside, daunorubicine and Ara-C after 4days culture, using the ATP assay, in vitro cytotoxicity was correlated to chromosomal aberrations. In the 318 patients, five main groups were identified: cases with monosomy 7 or deletion 7q (n=52), complex karyotype (n=50), normal karyotype (n=114), abnormal chromosome 17 (n=20) or abnormal 9p (n=13). Complex karyotype and the chromosome 7 abnormalities are well known markers for poor prog-
noss, long term outcome and in vitro drug resistance. The first three groups were compared to patient's samples with abnormal chromosome 17/9p. Results. Abnormalities of chromosome 17 indicate a significantly higher drug resistance for all drugs tested and a significant shorter overall survival compared to patients with normal chromosome 17, but the differences was not significant. All patients with abnormalities on chromosome 17 died within eleven months after diagnosis. Patients with abnormal 9p had a shorter overall survival but did not differ in in vitro drug resistance compared to patients presented with normal karyotype. Conclusions. Abnormalities in chromosome 17 appears to be a strong marker for both in vitro drug resistance and adverse outcome, even when compared to other high risk karyotypes in AML. Patients with abnormalities in 9p, which affect p53 protein pathway and degradation, showed a shorter overall survival but less obvious drug resistance.

**0126**

**LEUKEMIA IN UKRAINIAN CLEAN-UP WORKERS OF THE CHORNObYl ACCIDENT: EPIDEMIOLOGIC AND HEMATOLOGIC ASPECTS**

D. Bazyka, A. Romanenko, D. Bazyka, V. Bebeshko
Research Center for Radiation Medicine, KYIV, Ukraine

**Background.** Leukemia holds a special place in the study of radiation-related cancer because bone marrow is one of the tissues most sensitive to the carcinogenic effect of ionizing radiation, radiogenic leukemia has the shortest latent period among radiation-induced cancers, and its appearance suggests that solid tumors may follow. U.S. National Cancer Institute (study team - A Bouville, M.Hatch, G.Howe, N.Luckyanov, I.Masnyk, I.Zablotska) and Research Center for Radiation Medicine from Ukraine (study team NG Bakhina, E Bakhanova, EL Bomko, Yu Byelyayev, V Chumak, I Dyagil, NA Gudzenko, TF Lubarets) have initiated a study of radiogenic leukemia. Aims. The main objective is to test the hypothesis that exposure to radiation during cleanup operations following the Chornobyl accident led to an increase in leukemia among male cleanup workers from Ukraine. Results. A retrospective case-control study of ionizing radiation and leukemia was conducted in a cohort of 110,645 male Ukrainian liquidators involved in cleanup work following the accident at the Chornobyl nuclear power plant in northern Ukraine which occurred on April 26, 1986. The cohort includes 46% of clean-up workers in Ukraine. Information on all cases from 1986 to 2000 was collected in the hematological, pathological departments of local hospitals and registries of radiation exposed after Chornobyl in 5 target areas of Ukraine and Kyiv city including clinical records and blood smears, bone marrow slides, cytochemical and histological preparations, immunophenotype. Cases were evaluated by the international diagnostic review from 5 pathologists (2-USA, 2 - Ukraine, 1- United Kingdom). Annual case distribution showed a marked tendency to increase with age after the exposure. To assess the influence of ionizing radiation exposure analysis was performed of observed and expected (spontaneous) leukemia numbers at the cohort basing on the data on male population of Ukraine. The doses in cases obtained by RADRUE retrospective dosimetry were higher than in controls. Risk estimates were performed. Summary: Obtained data show the elevation of cases number and radiation risks in Chornobyl clean-up workers in Ukraine 8-14 years after the radiation exposure. Further follow-up of the cohort is performed.

**0127**

**QUANTITATIVE ASSESSMENT OF AML/ETO FUSION TRANSCRIPT AS A USEFUL TOOL FOR MINIMAL RESIDUAL DISEASE DETECTION AND OUTCOME PREDICTION IN T(8;21) AML**

A. Poerio,1 D. Cilloni,2 E. Ottaviani,1 M. Fava,1 E. Gottardi,1 F. Messa,1 F. Arruga,1 I. Defilippi,1 P. Nicoli,1 S. Carturan,1 A. Guerrasio,2 G. Martinelli,1 G. Saglio1
1Inst. of Hematology ‘Seràgnoli’, BOLOGNA, Italy; 2University of Turin, ORBASSANO, Italy; 3University of Bologna, BOLOGNA, Italy

**Background.** The t(8;21) translocation derives from the fusion of AML1 on chromosome 21 and ETO on chromosome 8. It is associated with FAB subtype M2 acute myeloid leukemia (AML). In order to predict relapse, qualitative PCR has a limited value since a positive PCR can be observed for a long follow-up period, even during continuous complete remission (CCR). From preliminary studies, quantitative RT-PCR seems to be a good candidate to predict clinical outcome of patients presenting AML1-ETO rearrangement. Aims. To test the usefulness of quantitative RT-PCR assay in detecting minimal residual disease and in predicting relapse in patients affected by t(8;21) AML. Methods. We analyzed in a retrospec-

**0128**

**PROGNOSTIC SIGNIFICANCE OF BAALC EXPRESSION IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE: FROM MICROARRAY TO RQ-PCR ASSAY**

N. Maroz,1 O. Biglia,1 V. Forestier,1 A. Martinez,2 N. Borie,1 N. Carbuccia,1 N. Vey,2 D. Birnbaum,3 M. Mozzi,2 F. Hermite1
1Inserm, MARSEILLE, France; 2Inserm-UERRR 194, MARSEILLE, France; 3Institut Pauli-Cabinettes, MARSEILLE, France

**Background.** Clonal cytogenic abnormalities are one of the most important factors predicting clinical outcome in Acute Myeloid Leukemia (AML) and are used to guide risk-adapted treatment strategies. However, approximately 50% of de novo AML have normal karyotype and therefore lack informative chromosome markers. The identification of relevant genetic features as well as the discrimination between different subsets of patients within this group remain major challenges. BAALC high expression in pre-treatment blood samples (peripheral blast) was proposed to be an independent adverse prognosis factor for Overall Survival (OS) Event Free Survival (EFS) and Disease Free Survival (DFS). Aims. The goals of this study were to determine if expression of BAALC was a prognostic factor in AML by using a new specific quantitative PCR (RQ-PCR) assay and to compare these results with data obtained on microarrays. Methods. We developed a new specific RQ-PCR assay for quantification of BAALC transcripts in human cells. This assay is based on the plasmid technology which allows the precise control and normalisation of RQ-PCR results and is highly reproducible. The ratio of BAALC transcript to endogenous ABL transcript provides a normalised quantification of BAALC independent of the cell count and RT efficiency. We previously profiled on a 9000-cDNA microarray 61 adult AML samples with normal cytogenetic at diagnosis. Prognostic significance of BAALC and EVI1 expression levels was analysed using univariate Cox proportional hazards regression analyses. Results. Microarray data showed that BAALC expression values were associated with outcome (p=0.07). In addition, we could note that when combined with EVI1 expression values, results were even better. We thus could determine the expression of the ELN1 gene as a prognostically correlate (CCR patients: group 1: p=0.03); (ii) BAALC+ and BAALC+/EVI1- patients correspond to a poor prognosis class and; (iii) BAALC- and EVI1+ to a good prognosis class (increased OS, p=0.0076) (Figure 1). RQ-PCR assay analytical validation showed a reproducible sensitivity greater than 10-5 (less than 100 BAALC positive KG1a cells in haematologica/the hematology journal | 2006; 91(s1) | 47
5.106 BAALC negative K562 cells or 50pg of KG1a RNA in 1μg of MV4-11 RNA). We determined BAALC levels in normal peripheral blood samples (n=26). The median normalised copy number (NCN) of BAALC was 2210 and ranged between 129 and 6427. Pathological values were assessed on a subset of AML samples (n=24) already used for microarray analysis. The median NCN was 1693, and ranged between 61 and 51850. The RQ-PCR results demonstrate a clear direct correlation (p=0.0057) between OS and expression of BAALC alone in normal karyotype AML patients and identified a BAALC - / EVI1 + class with favourable outcome. The sensitivity and dynamic range of the BAALC RQ-PCR assay may contribute to treatment option decisions at diagnosis. Larger patient cohort and clinical trials will evaluate the impact of this new molecular tool on patient care. Further RQ-PCR studies will be performed to accurately assess the importance of EVI1 expression correlation with outcome in BAALC - group.

A MULTICENTER PROSPECTIVE RANDOMIZED STUDY OF LENOGRASTIM IN CONSERVATION CHEMOTHERAPY FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (JALSG GML200 S STUDY)

S. Ohtake,1 F. Yasagaki,1 S. Miyawaki,2 M. Matsuda,1 H. Taguchi,1 T. Matsushima,2 K. Toba,1 M. Yoshida,2 T. Naoe,1 R. Ohno1
1Kanazawa University, KANAZAWA, Japan; 2Saitama Medical School, SAITAMA, Japan; 3Saiseikai Maebashi Hospital, MAEBASHI, Japan; 4Kochi University, KOCHI, Japan; 5Gunma University, MAEBASHI, Japan; 6Niigata University, NIGATA, Japan; 7Japan Adult Leukemia Study Group, HAMAMATSU, Japan

Background. Although G-CSF administration after consolidation chemotherapy is recommended in some clinical guidelines, effective use of G-CSF administration has been under investigation. Aims. We conducted a multicenter prospective randomized study to compare two different administration schedules of lenograstim after consolidation chemotherapy for elderly AML patients. The primary endpoint of this randomized study is to compare the duration of fever (above 38°C). Methods. Pathologically diagnosed untreated de novo AML older than 64 years of age who attained complete remission (CR) were eligible for this study. After consolidation chemotherapy with daunorubicin (200 mg/m², d1-5) and mitoxantrone (age<70 years; 7 mg/m², d1-5, age ≥70; 5 mg/m²; d1-5), patients randomly assigned to receive lenograstim (5 μg/kg, 30 min i.v.) either after absolute neutrophil count (ANC) less than 1000/μL, (Arm A, prophylactic administration) or after ANC less than 1000/μL with fever above 37.5°C (Arm B: therapeutic administration) until neutrophil recovery. Results. Between August 2000 and March 2005, 110 evaluable patients were registered. 54 patients were in Arm A and 56 in Arm B, and the median age of both groups was 71 years old. There were no significant differences in patient characteristics. All patients received lenograstim in Arm A. Twenty-nine patients (51.8%) received lenograstim in Arm B, because 27 patients did not experience fever above 37.5°C. The duration of fever was not significantly different between Arm A and Arm B (Mean±SD: 1.2±2.1 vs. 1.4±2.1 days, respectively, p=0.54). The duration of febrile neutropenia (ANC< 500/μL and temperature >37.5°C) was significantly shorter in Arm A (Mean±SD: 0.8±1.4, as compared with 2.0±2.5 in Arm B, p=0.052). The duration of infectious complications was slightly shorter in Arm A (p=0.10). The recovery of neutropenia was more rapid in Arm A compared to Arm B (ANC>500/μL: median 5.0 vs. 9.0 days, p=0.0001; ANC>1000/μL: median 8.5 vs. 14.5 days, p=0.0001). There was no difference of development of lenograstim-related severe toxicity between Arm A and Arm B. Conclusions. Prophylactic administration of lenograstim after consolidation chemotherapy obtained better clinical benefits, especially for those who received more intensive chemotherapy.

INHIBITION OF FLT3-ACTIVATING MUTATIONS MAY NOT PREVENT CONSTITUTIVE ACTIVATION OF ERK/AKT/STAT PATHWAYS IN SOME AML SAMPLES: A POSSIBLE CAUSE FOR THE LIMITED EFFECTIVENESS OF THERAPY WITH SMALL-MOLECULE INHIBITORS

C.H. Lopez-Pedrera,1 E. Siendones,2 N. Barbarroja,3 P. Buendia,1 A. Torres,1 F. Velasco1
1Hospital Reina Sofia, CORDOBA, Spain; 2CentroAndaluz Biol Desar U Pablo Olavide, SEVILLA, Spain; 3Hospital Reina Sofia, CORDOBA, Spain

AML cells are characterized by genetic alterations involved in the expression of transcriptional regulators that are critical for normal hematopoietic development and differentiation. Activating mutations on Flt3 receptors are the most common genetic alterations in AML, conferring a poor prognosis and decreased overall survival. Thus, Flt3 is now considered as a major target for therapeutic intervention, and a group of new small molecule inhibitors targeting the Flt3 RTK are currently being evaluated in clinical trials. However, clinical responses in relapsed or refractory AML are limited and transient. This study investigates the rele-

Figure 1. Microarray data analysis, OS and DFS.

Figure 2. RQ-PCR data analysis.
vance of activating mutations of Flt3 on the constitutive activation of intracellular pathways abnormally active in AML, and their effect on blast cell survival. A total of 28 patients with acute myeloid leukaemia (AML) diagnosed according to the classification of the French-American-British (FAB) committee, as well as ten healthy controls, were entered into this study after informed consent. Blast cells were obtained either from peripheral blood or bone marrow aspirate and, whenever possible, isolated by gradient centrifugation using Ficoll-Hypaque. Flt3 gene mutations were identified by allele-specific polymerase chain reaction (PCR) amplification from Flt3 cDNA, followed by agarose gel electrophoresis analysis. The status of activation of the MAPK/ERK/STAT pathways was analyzed by both Western blot and electrophoretic mobility shifts assays. A high resolution bone marrow aspirate flow cytometry panel was used to assess the simultaneous expression of different AML subtypes analyzed, and even between the different AML samples belonging to the same AML subtype. Contrary to previous reports, we found that inhibition of mutant Flt3 phosphorylation did not prevent phosphorylation of ERK, STAT5 or Akt in some AML samples, suggesting the implication of Flt3-activating mutations and other cytoskeletal or molecular unknown events. On the other hand, resistance to spontaneous apoptosis was found to be related to the simultaneous activation of several intracellular signals. Moreover, antiapoptotic routes regulated by the mutant and the wild-type Flt3 diverged in some subsets of primary AML blasts. Taken together our results showed differential activation of multiple pathways even in the same AML subtype, and not related to activating mutations of the Flt3 receptor. This suggests the existence of distinct mechanisms of activation or even other mutant components with constitutive activation that might silence the effect of specific kinase-inhibitors. Thus, a simultaneous intervention, adequately targeting the signaling pathways altered in each AML patient, may provide a more effective approach to reverse leukemogenesis. Supported by FIS 080916, FIS 041291 and JA 002/2005.

The presence of mutations in the juxtamembrane domain of the tyrosine kinase receptor gene fetal liver tyrosine kinase 3 (FLT3) has emerged as a powerful prognostic indicator in acute myeloid leukaemia (AML). The presence of FLT3 internal tandem duplications (ITDs) has been associated with poor clinical outcomes in AML, including shorter overall survival and disease-free survival rates. In this study, we analyzed the relevance of FLT3 mutations (ITDs and point mutations) on the clinical outcome of AML patients. FLT3 mutations were identified by allele-specific polymerase chain reaction (PCR) amplification from Flt3 cDNA, followed by agarose gel electrophoresis analysis. The status of activation of the MAPK/ERK/STAT pathways was analyzed by both Western blot and electrophoretic mobility shifts assays. A high resolution bone marrow aspirate flow cytometry panel was used to assess the simultaneous expression of different AML subtypes analyzed, and even between the different AML samples belonging to the same AML subtype. Contrary to previous reports, we found that inhibition of mutant Flt3 phosphorylation did not prevent phosphorylation of ERK, STAT5 or Akt in some AML samples, suggesting the implication of Flt3-activating mutations and other cytoskeletal or molecular unknown events. On the other hand, resistance to spontaneous apoptosis was found to be related to the simultaneous activation of several intracellular signals. Moreover, antiapoptotic routes regulated by the mutant and the wild-type Flt3 diverged in some subsets of primary AML blasts. Taken together our results showed differential activation of multiple pathways even in the same AML subtype, and not related to activating mutations of the Flt3 receptor. This suggests the existence of distinct mechanisms of activation or even other mutant components with constitutive activation that might silence the effect of specific kinase-inhibitors. Thus, a simultaneous intervention, adequately targeting the signaling pathways altered in each AML patient, may provide a more effective approach to reverse leukemogenesis. Supported by FIS 080916, FIS 041291 and JA 002/2005.

0132
THE PRESENCE OF FLT3 MUTATIONS DOES NOT IMPAIR STEM CELL MOBILIZATION AND FEASIBILITY OF AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKAEMIA


‘Cardarelli Hospital, NAPOLI, Italy; ‘Federico II University, CEINGE, NAPOLI, Italy

Background. The presence of mutations in the juxtamembrane domain of the tyrosine kinase receptor gene fetal liver tyrosine kinase 3 (FLT3) has emerged as a powerful prognostic indicator in acute myeloid leukaemia (AML). The presence of FLT3 internal tandem duplications (ITDs) has been associated with poor clinical outcomes in AML, including shorter overall survival and disease-free survival rates. In this study, we analyzed the relevance of FLT3 mutations on mobilization and collection of CD34+ cells as well as on feasibility of PBASCT in AML patients with normal cytogenotype.

0133
MYELOABLATIVE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL INFUSION MAY OVERCOME THE ADVERSE PROGNOSTIC IMPACT OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKAEMIA


‘Cardarelli Hospital, NAPOLI, Italy; ‘Florenc University, FIRENZE, Italy; ‘Federico II University, CEINGE, NAPOLI, Italy

Background. Within the past few years the presence of an activating internal tandem duplication (ITD) in the juxtamembrane domain of the tyrosine kinase receptor gene fetal liver tyrosine kinase 3 (FLT3) has emerged as a powerful prognostic indicator, ahead of cytogenetics, predicting for relapse from complete remission (CR) in acute myeloid leukaemia (AML). In particular, patients with activating FLT3 mutations, either in the form of an internal tandem duplication (ITD) or a point mutation in the activation loop [point mutations of Asp835 (FLT3/D835 mutations)] have a significantly higher risk of relapse, namely in intermediate risk AML, in which most patients present with normal cytogenotype. To analyze the prognostic relevance of FLT3 mutations in 73 patients with acute myeloid leukaemia with normal cytogenotype, who survived induction and consolidation and received autologous stem cell transplantation (ASCT) after successful mobilization of peripheral blood stem cell (PBSC) in CR1. Patients and Methods. There were 44 males and 29 females with a median age of 54 years (range 20-77). Overall, 16 out of 73 autografted patients (22%) had FLT3 mutations. More in detail, FLT3/ITDs were detected in 10 out of 73 patients (14%), while FLT3 D835 mutations were detected in 5 cases (7%). One patient (1%) was found as having both abnormalities. Pre-transplant therapy consisted of ICE as induction (idarubicin + cytarabine + etoposide) followed by NOVIA (mitoxantrone + intermediate dose cytarabine) as consolidation and mobilizing regimen for patients aged up to 60 years, and of continuous infusion (c.i.) of fludarabine and cytarabine (cIFLA) as induction and consolidation for patients >60 years. Conditioning regimen was a combination of 5 days c.i. idarubicin plus busulphan for 4 days (i-Bu) for 56 patients (reduced by one day for both drugs for patients >60 years), and classical Bu-Cy for the remaining 17 cases. Results. Analysis of basal characteristics of the patients showed that white blood cell count (p<0.009), serum concentration of lactate dehydrogenase (p<0.01), and percentages of peripheral blood (p<0.002) and bone marrow blasts (p<0.05) were significantly higher in patients positive for FLT3 mutations. On the contrary, overall survival and disease-free survival were similar between patients with or without FLT3 mutations (p=0.73 and 0.78, respectively). Conclusions. Our data suggest that myeloablative chemotherapy supported by auto-PBSC may overcome the adverse prognostic implications of FLT3 mutations in AML. However, it is to consider that autografted patients are highly selected for best response to induction, consolidation and mobilization as well as for minor non-haematologic toxicity.

0134
PEDIATRIC RELAPSED ACUTE MYELOID LEUKAEMIA IN THE NETHERLANDS FROM 1980 UNTIL 2000

B.F. Goemans, K. Corbijn, R.J.Y. Tammings, K. Hahlen, G.J.L. Kaspers

‘VU University Medical Center, AMSTERDAM, Netherlands; ‘DCOG, THE HAGUE, Netherlands; Beatrix Children’s Hospital, UMCG, GRONINGEN, Netherlands; ‘Sophia Children’s Hospital, Erasmus mc, ROTTERDAM, Netherlands

The prognosis of pediatric AML has improved considerably over the past decades, with overall long-term survival rates up to 60%. However, relapse remains the major cause of treatment failure, occurring in 30-40% of patients. Patients with relapsed AML have a poor prognosis. Studies from different groups have shown survival rates of 15-50%. The outcome of Dutch pediatric relapsed AML patients is unknown. We therefore studied all pediatric de novo AML patients initially diagnosed autografting the remaining 21 patients (29%) included early relapse (n=10), toxicity after consolidation (n=5) and failure to mobilize PBSC (n=6). Of note, early relapse rate was higher for FLT3- patients (5 out of 7, or 71%) as opposed to 5 out of 14 (36%) for the group of FLT3- patients, p=0.27. Conclusions: the analysis of our data demonstrate that the presence of FLT3 mutations has no influence on mobilization and collection of CD34+ cells as well as on overall feasibility of PBASCT in AML patients with normal cytogenotype.

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between 1980 and 1998 who suffered from a relapse. Most were initially treated on the subsequent Dutch Childhood Oncology Group (DCOG) studies ANLL 80, 83, 87 and 94. Data were collected from the central data collection center of the DCOG. From 1980-1998 354 patients were diagnosed with de novo AML in the Netherlands. 113 patients (52%) relapsed between 1980 and 2000. Most (65%) relapsed within a year after reaching first complete remission (CR1) (median time to relapse was 11 months for 56% of patients). 80% (N=90) of patients were treated with curative intent. Patients were treated with different treatment regimens as during most of this period no uniform treatment protocols were available. CR2 was achieved in 84% of patients after a median of 2 courses of chemotherapy. Stem cell transplantation (SCT) was performed in 22 patients after achieving CR2. Overall, probability of 10 year overall survival (10-year OS) for AML patients with relapsed AML was 0.16 (SE=0.04). Patients that relapsed early (CR1±1 year) were significantly less likely to survive than patients that suffered from a late relapse (5-year OS 0.12 vs. 0.29, p=0.0001). One third (8/22) underwent an autologous and two-thirds (14/22) an allogeneic SCT. We performed multivariate analysis, including SCT (as a time-dependent variable) and CR1 duration (CR1±1 year). Both SCT and CR1 duration were significantly correlated to survival. Patients that received a SCT in CR2 had a significantly improved survival (RR=0.43, p=0.008), while a CR1 duration ≥1 year resulted in a significantly poorer survival (RR=2.7, p=0.0001). In conclusion, relapse rate between AML patients are limited to survivors. Patients with an early relapse do worse compared to patients with a late relapse, confirming results from other study groups. There is a survival benefit for SCT after relapse. We recently opened an international randomized phase III trial for children with relapsed/refractory AML (I-BFM/DCOG Relapsed AML 2001/01), randomizing FLAG (flu-darabine, cytosine arabinoside, prednisone, L-asparaginase) versus chemotherapy with survival of CR2 patients. This ongoing trial enables internationally uniform treatment of the rare cases of relapsed AML in children and will show us if the addition of liposomal daunorubicin is of benefit for these children.

0135 PROTEIN KINASE CK2α, AS AN INDEPENDENT PROGNOSTIC MARKER AND A NOVEL THERAPEUTIC TARGET IN ACUTE MYELOID LEUKEMIA
Yonsei University College of Medicine, SEOUL, South-Korea; ‘Clinical Cancer Research, YUMC, SEOUL, South-Korea

Background. Protein kinase CK2 (formerly casein kinase II) is a highly conserved, and ubiquitously expressed protein serine/threonine kinase implicated in various cellular processes including proliferation, differentiation, and transformation. However, the biological significances of CK2α have not been elucidated in acute myeloid leukemia (AML). Aims. We tried to evaluate the clinical and biological significances of CK2α in AML. Methods. We first analyzed the expression and activity of catalytic subunit of CK2 (CK2α) by Western blot and its association with clinical outcomes in consecutive 59 AML patients with normal karyotype. In the context of clinical outcome, CK2α expression was observed in 30 (50.8%) cases. Constitutive expression of CK2α was not demonstrated in bone marrow samples obtained from healthy volunteers. Levels of CK2α expression were highly correlated with CK2α catalytic activity (r=0.0001) in primary AML cells. Kaplan-Meier analysis showed that the disease-free survival (DFS) and overall survival (OS) rate were significantly lower in the CK2α-positive cases compared to the CK2α-negative cases (p=0.05 and p=0.01, respectively). Multivariate analysis revealed that CK2α expression was an independent prognostic factor in the DFS (p=0.002) and OS (p=0.003). Treatment of U937 leukemia cell line with a CK2-selective inhibitor, apigenin, for 24 h potentially reduced the expression levels of phosphorylated PTEN, phosphorylated Akt/PKB and Ake/PKB downstream molecules in a dose-dependent manner. In contrast, an induced overexpression of CK2α increased the levels of anti-apoptotic proteins including Bcl-2, Bcl-xL, Mcl-1, survivin and XIAP in U937 cells. Although apigenin did not potentially reduce cell death in U937 cells, an induced overexpression of CK2α remarkably enhanced the sensitivity of the cells to the-apigenin-induced cell death. Interestingly, apigenin-induced cell death was remarkably higher in the CK2α-positive primary AML samples (82±4%) compared to the CK2α-negative AML samples (41±1%, p=0.001), or normal BM samples (5±2%, p=0.001). Conclusions. These results strongly suggest that protein kinase CK2α is an independent prognostic marker in AML with normal karyotype. In addition, our finding that CK2 inhibitor effectively induces cell death preferentially in the CK2α-positive AML provides a novel approach to the targeted therapy for AML.

0136 INTERLEUKIN-2 CAN BE SAFELY ADMINISTERED TO AML PATIENTS IN 1ST CR AFTER AN AUTOGLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION
S.M. Trisolini, S. Capria, L. Cardarelli, V. Gianfelici, G.A. Natale, M. Ribersani, F. Mandelli, R. Foà, G. Meloni
La Sapienza University, ROMA, Italy

Interleukin-2 (IL2) is a cytokine with anti-tumor activity. When administered after autologous stem cell transplantation, it appears to reproduce the graft versus leukemia effect of allogeneic transplant and possibly prolong disease-free survival (DFS). Since 1999, at the Hematology Department of La Sapienza University in Rome, 24 AML patients in first CR underwent immunotherapy with IL2. All patients received hydroxyurea followed by HD or SD-AraC plus Daunorubicin and Etoposide as induction treatment, and Daunorubicin plus ID-AraC as consolidation. Subsequently, an allogeneic or autologous peripheral blood stem cell transplantation (PBSCT) was planned according to donor availability. IL2 was administered following PBSCT after BU-CY conditioning regimen in 18/24 patients, and after consolidation in 6 patients not eligible for an autograft because of infections (2 pts) or mobilization failure (4 pts). IL2 therapy was started after a median of 4 months from autograft (range 1-7) and a median of 7.5 months from consolidation therapy (range 4-14). An absolute neutrophil count higher than 1x10^9/L, or neutrophil count greater than 50% of total blood cell count were required for starting active infections were required to start the treatment. IL2 was administered subcutaneously on 5 consecutive days, on a monthly basis, for 1 year or until relapse. The dosage of IL2 was 4x10^6 IU on day 1, followed by 8x10^6 IU on days 2 through 5. All patients received paracetamol and prophylactic trimethoprim/sulfamethoxazole to prevent bacterial infections during IL2 therapy. No patient required treatment discontinuation because of a grade of 4 toxicity according to NCI-CTC criteria. Fever (grade 1-2) was observed in all patients 4-6 hours after IL2 administration, with grade 1 arthralgia in 15 of them. The majority of patients showed gastrointestinal toxicity (grade 1-2) in the form of nausea and vomiting (21/24), diarreah (4/24) and transient transaminase increase (7/24). Skin toxicity (grade 1-2) was observed as desquamation (7/24), rash and pruritus which required systemic measures (4/24) and injection site reactions (16/24). With regard to hematological toxicity, grade 3 thrombocytopenia requiring a 50% dose reduction was observed in 2 patients. Concerning neurological toxicity, only 2 patients showed irritability and insomnia during IL2 administration, not requiring dose modification. No patient showed infective complications. In all cases, toxicity completely recovered within 48 hours from IL2 discontinuation. Five patients relapsed on therapy, after 2 (CNS relapse), 3, 4, 6 and 11 months from the start of IL2, while 2 patients relapsed after 5 and 15 months from treatment discontinuation. One patient is still on therapy and 16 are in CCR after a median of 18 months (range 1-63) from IL2 discontinuation. The eighty-months projected probability of DFS for the 24 patients is 55%, the median has not been reached. Based on our experience, it appears that IL2 therapy is a feasible approach devoid of serious toxicity also after an autograft procedure. A randomized trial is currently ongoing to compare a CORT/CIME/AML12 protocol to document whether or not IL2 is capable to enhancing the likelihood of disease-free survival.

0137 STEM CELL DETECTION IN AML PATIENTS UNDER REMISSION CONDITIONS: A NEW ERA IN MINIMAL RESIDUAL DISEASE DETECTION
VU University Medical Center, AMSTERDAM, Netherlands

Background. In CD34-positive acute myeloid leukemia (AML), the leukemia-initiating event likely occurs in the CD34+CD38- stem cell compartment. Survival of these cells after chemotherapy hypothetically leads to minimal residual disease (MRD) and relapse. Aims. In the present study we investigated, using 4-colour flow cytometry, whether aberrant antigen expression at diagnosis can be used to identify AML CD34+CD38- cells in remission bone marrow. Such would offer opportunities for stem cell MRD detection and for future patient risk stratification and guidance of therapeutic intervention. Methods. FACS analysis was performed on fresh bone marrow CD34+CD38- cells of AML samples at diagnosis and after chemotherapy and of normal and regenerating bone marrow samples. Antibody combinations always consisted of anti-CD34FITC, anti-CD45PerCP and anti-CD38APC together with different PE-labelled antibodies against CD-1, CD2, CD3, CD7, CD8, CD20, CD33 and CD34.
CD11b, CD19 and CD56, with the exception of CD1-1 all derived from leukemia-associated phenotypes, LAP’s, used in immunophenotypic MRD detection (Feller et al., Leukemia 8:1380, 2004). Expression on CD34+CD38- cells was scored as <50% or >50%. At least 50% expression at diagnosis is needed for accurate measurements of residual malignant cells after chemotherapy. Results. We found that at diagnosis (60/77) AML stem cell MRD was detectable in >1% CD34+ cells and were considered as CD34-positive. We were able to measure a reliable number of CD34+CD38- events (>20) in 56/60 cases. CLL-1 expression was >50% in 15/60 cases, LAP expression in 9/60 cases and both CLL-1 and LAP in 8/60 cases. Altogether in 32/60 CD34-positive cases, AML stem cell MRD was possible. In normal bone marrow (n=4) as well as in regenerating bone marrow, the CD34+CD38- cells did show low (<2%) CLL-1 expression (n=6) and low LAP expression (n=2, for all antigens). Therefore, under MRD-conditions CLL-1 and/or LAP staining might enable to accurately discriminate between normal and malignant CD34+CD38- cells in part of AML patients. In agreement with this, the different ratios of malignant and normal CD34+CD38- cells, that were found in a number of patients in follow-up material parallelled clinical outcome. For comparison, whole blast MRD measurements using LAP was possible in 51/60 CD34-positive patients. In 3 patients without whole blast MRD possible, stem cell MRD could be used. The higher success rates have been used to assess clonality of various tumours and X-linked disorders. Conflicting results have been published on the frequency of clonal patterns in female patients with Acute Myeloid Leukemia (AML). Previous studies have used DNA methylation to measure X inactivation, but aberrant methylation is known to occur in some situations. Aim. The aim of this study was to evaluate the patterns of X-chromosome inactivation in AML patients at the RNA level. Materials and Methods. Two hundred normal females and 45 patients with AML at remission were selected. A non radioactive reverse transcription polymerase chain reaction (RT PCR) method was used to study the expression of the polymorphisms of G6PD, iduronate 2 sulfatase (IDS) and palmitoylated membrane protein (P55) genes at the RNA level. Results. The frequency of heterozygosity was found to be 48.5% (119/245) for F55 gene. Forty percent (95/245) were heterozygous for IDS and only 28.9% (71/245) of individuals showed polymorphism at nt.1511 C/T for G6PD gene. Some individuals were heterozygous for more than one gene polymorphism. 92/100 (92%) normal female individuals showed a polyclonal X-chromosome inactivation pattern in lymphocytes (L) and granulocytes (G). Clonal patterns were observed in lymphocytes and granulocytes of 44/45 (96%) de novo AML patients at presentation, a significantly higher proportion than in controls (6%) (p<0.01). 25/27 (85.2%) of patients at remission had a clonal X-chromosome inactivation pattern in both G and L cells. 4/27 (15%) patients showed polyclonal patterns. Ten patients were available for a longer follow up. A clonal pattern was observed in G, L and T cells of seven patients. Three patients converted from clonal to polyclonal and showed a polyclonal X-chromosome inactivation pattern in G, B and T lymphocytes. The incidence of clonal patterns is generally associated with good prognosis, but the studies concerning their exact role are hampered by the low number of cases. Aims. To assess the incidence of eosinophilia and of the inv(16) on the prognosis of acute myelomonocytic leukemia (M4) and acute myelomonocytic leukemia with abnormal eosinophilia (M4EO). Methods. In a non concurrent-prospective setting, we analyzed patients with AML-M4 consecutively enrolled in two GIMEMA clinical trials, in 35 Italian hematological divisions. Results. Between December 1993 and December 2002, 1656 valuable adult patients over 1702 with a diagnosis of AML, were consecutively admitted to the EORTC-GIMEMA AML10 and AML 99p trials; among these, 400 patients (355 M4 and 45 M4Eo) were studied. The diagnosis of M4 and M4Eo was first established at each institution and subsequently centrally reviewed at the time of study entry. The following parameters were evaluated: morphology, immunophenotype, cytogenetics performed at the onset of the disease, complete remission achievement and duration, overall survival (OS) and disease-free survival (DFS) from AML diagnosis. Cytogenetic analysis failed or was not carried out in 40% of cases, while it was successfully analyzed in 240 cases; inv(16) was found in 17% of them. Patients with M4Eo were younger and more frequently associated with inv(16) compared to M4. Concerning the probability of obtaining a CR after standard treatment, at univariate analysis M4Eo had a trend significant advantage compared to M4, while presence of inv(16) was significantly correlated to a higher CR probability; the proportion of patients with resistant disease was higher in patients with M4 morphology compared to M4Eo. Fitting a statistical model for the analysis of factors including interactions, the multivariate analysis showed a significant advantage only of M4Eo + inv(16) compared to M4 without eosinophilia and without inv(16). DFS was not different in univariate analysis between patients carrying or not inv(16), while a borderline advantage of M4Eo was observed with respect of M4, not confirmed at multivariate analysis. OS curves showed at univariate analysis a significant advantage between patients with the presence of eosinophilia (p<0.004) and of inv(16) (p<0.01); at multivariate analysis, patients with M4Eo + inv(16) had a highly significant advantage compared to M4 without eosinophilia and without inv(16) (p<0.004), but also compared to M4 + inv(16) (p=0.043), and M4Eo-without inv(16) (p=0.076). Finally OS and DFS of the 400 patients with M4and M4Eo was compared to the general AML population with the same age range (median age was 19.0 years) and gender ratio was respectively for OS (p=0.17) and 19.7 and 16.4 months for DFS (p=0.51) in M4 versus other AML. Conclusions. AML-M4 with or without eosinophilia represent 28.7% of AML. The presence of eosinophilia and of inv(16)(t;16;16) can be both considered favourable prognostic factors; however, only the association of both factors is strongly significantly associated with a highly significant advantage in terms of CR and OS. Concerning OS the combination of both is also significant vs. the presence of one of them. Polymorphisms in the RAD51 and XRCC-3 genes increase the risk of developing acute myeloid leukaemia J. Ciudad, E. Fabiani, F. Guidi, F. D’Alò, M. Giachella, G. Dumiéro, G. Leone, M.T. Voso Centro de Investigación del Cáncer, SALAMANCA, Spain; Universidad Católica San Carlo, ROME, Italy Background. DNA is at constant risk from damage from both endogenous and exogenous sources and this damage causes chromosomal instability leading to oncogenesis, apoptosis and severe failure of cell functions. DNA is protected from damage by detoxification enzymes belonging to two different classes or damage triggering phases: phase I enzymes metabolise the exogenous agent to a reactive state, whereas phase II
The aim of our study is to investigate the frequency of polymorphisms involved in detoxification and double strand break (DSB) repair via homologous recombination (HR) pathways and to correlate them with AML or therapy-related AML (t-AML) risk. Methods: We studied 160 patients with AML (132 de novo and 28 therapy-related) and 144 control subjects, matched for age and sex. RTPCR were used to analyze genotypes of DNA repair genes for RAD51 (RAD51-G135C) and XRCC3 (XRCC3-Th241Met) and detoxification genes for NQO1 (NQO1-Pro185Ser) and GSTA1 (GSTA1 promoter polymorphism, A*B). The polymorphism in the promoter region of detoxification gene CYP3A4 (CYP3A4-A290G) was examined by mismatch PCR. Results: When comparing AML patients to controls, a statistically higher prevalence of the g/c + c/c genotype of the DNA repair enzyme RAD-51 was found in AML patients (22% vs 12.7%, O.R. 1.9, 95% C.I. 1.36-3.6, p=0.04), in particular when associated to the CYP3A4-A290G detoxification enzyme polymorphism. This was confirmed by the multivariate analysis (p=0.047 for the association). Similarly the homozygous met/met mutant of XRCC3 was more frequent in AML patients (24% vs 12%, OR 2.3, 95% C.I. 1.2-4.3). No differences were found when looking at NQO1, GSTA1 and CYP3A4 polymorphisms, alone or in association to XRCC3. In the AML patient group, we found no associations between enzymatic polymorphisms and type of AML (de novo versus therapy-related). Summary/Conclusions: DNA repair and enzymatic polymorphism in RAD51 and XRCC3 genes may increase the risk of developing acute myeloid leukemia. This risk is particularly high when the RAD51-G135C DNA repair polymorphism is associated to the CYP3A4-A290G detoxification enzyme polymorphism.

0141
THE ANTI-PROLIFERATIVE AND APOPTOSIS-INDUCING EFFECTS OF THE PROTEASOME INHIBITORS BORTEZOMIB AND PR-171 IN ACUTE MYELOGENOUS LEUKAEMIA
C. Stanpe, A. Daszekand, K.J. Hatfield, E. Ersvær, B.T. Gjertsen, E. Ersvær, O. Bruserud
Institute of Medicine, BERGEN, Norway; Department of Biomedicine, BERGEN, Norway; Haukeland Univ. Hospital, BERGEN, Norway

Background. Proteasome inhibitors represent a new class of anti-neoplastic drugs with documented effects in multiple myeloma and mantle cell lymphoma. in vitro studies suggest that these drugs also have effects on other hematological malignancies. Aims. The aim of this study was to investigate the in vitro effects of proteasome inhibitors on human primary acute myelogenous leukaemia (AML) blast proliferation, viability/apoptosis induction and clonogenic potential. Furthermore, possible correlations with genetic features of AML cells, such as Fms mutations and cytogenetic abnormalities were also assessed. Methods. Native human AML blasts from peripheral blood of more than 50 consecutive patients were analysed. The impact of proteasome inhibition was examined in the following experimental models: - Cell proliferation: Leukaemia cells were cultured in vitro in the presence of exogenous growth factors (IL-3, SCF, GM-CSF) for 7 days and cell proliferation was measured by either [3H]-thymidine incorporation or a colony formation assay. - Cell viability: Leukaemia cells were cultured in the presence (7 days) or absence (2 days) of exogenous cytokines and apoptosis/viability was measured by flow cytometry analysis of Annexin-V expression/Propidium iodide exclusion. - Cytokine secretion: ELISA and Multiplex assays for CXCL10, CCL3, CXCL8, GM-CSF, IL-1β, IL-6 and TNFα. - Proteasome activity: release of the Fluorochrome 7-aminocoumarin (AMC) from N-Suc-Leu-Leu-Val-Tyr-AMC. We investigated the reversible proteasome inhibitor bortezomib (Velcade®) and a novel epoxomicin derivative, PR-171, which is an irreversible inhibitor (Demo 5D, et al. Biochemical and cellular characterization of the novel proteasome inhibitor PR-171 [abstract]. Blood 2005; 106:455a). Results. Basal proteasome specific activity in AML blasts varied 1-10 fold. Both drugs inhibited the proteasome complex with equivalent potency and suppressed cell viability as determined by annexin/PI staining after 48 hr compound treatment (% Annexin/PI negative cells; mean with standard deviation: control 33 ± 15; bortezomib 25 ± 9.5 and 50 ± 12 ± 11; PR-171 25 ± 9 ± 5 and 50 ± 8 ± 9). The drugs also inhibited AML cell proliferation as measured by incorporation of [3H]-thymidine after 7 days of compound treatment (IC50 95% confidence intervals: bortezomib 20.99-24.78 nM; PR-171 9.75-10.43 nM). Cytotoxic and antiproliferative responses of blasts to proteasome inhibition were heterogeneous, and marked effects on proliferation, viability and clonogenic inhibition even in CD34 negative AML (one third of the patients in our series). Conclusion. Our studies show that proteasome inhibitors have dose-dependent and marked effects on proliferation, viability and clonogenic properties of human native AML blasts at nanomolar levels in vitro.

0142
SINGLE CELL ANALYSIS OF PHOSPHOINOSITIDE 3-KINASE/AKT AND ERK ACTIVATION IN ACUTE MYELOID LEUKAEMIA BY FLOW CYTOMETRY
V. Bardet, T. Tamburini, N. Ifrah, P. Dreyfus, C. Lacombe, P. Mayeux, D. Boussac
Institut Cochin, PARIS, France; Service des Maladies du sang, CHU, ANGERS, France; Unité D’Hématologie, CHU Cochin, PARIS, France

Background. Acute myeloid leukaemia (AML) is an aggressive malignancy and new therapeutic agents are needed. Abnormal activation of several signal transduction pathways such as phosphoinositide 3-kinase (PI3K) and MAP kinase has been reported in AML. To test new targeted therapeutics, it is critical to develop sensitive analytical tools to detect aberrant activation of these pathways and monitor their inhibition in response to treatment. Aims. Our aim was to establish the feasibility of a flow cytometry analysis in patients samples even with a low blast infiltration and to analyze the correlation between flow cytometry and western blot analysis (WB). Methods. We analyzed AKT and ERK phosphorylation in blast cells of patients with hematologic anomalies. From 32 patients with high blast infiltration we compared WB and flow cytometry techniques. The flow cytometry protocol associated intra cellular staining for phospho proteins and membrane staining for several antigens including CD34 and CD38. Using CD34 allowed to identify the blast cell population even in CD34 negative AML (one third of the patients in our experience). For the CD34 positive AML, we set up a four color protocol using CD34, CD38 and CD123 as membrane antigens. We analyzed constitutive phosphorylation in blast cells of 72 patients with high blast infiltration by WB and flow cytometry. Results. AKT and ERK phosphorylation in blast cells of patients with AML were detected by WB and flow cytometry and their expression was correlated. In patients with high blast infiltration we compared WB and flow cytometry techniques. The flow cytometry protocol associated intra cellular staining for phospho proteins and membrane staining for several antigens including CD34 and CD38. Using CD34 allowed to identify the blast cell population even in CD34 negative AML (one third of the patients in our experience). For the CD34 positive AML, we set up a four color protocol using CD34, CD38 and CD123 as membrane antigens. This protocol allowed us to detect phosphorylated proteins in the most immaturle leukemic cells with high blast infiltration by WB and flow cytometry. In the positive samples we detected PI3K and ERK pathway activations in 45% and 70% of the samples. Flow cytometry allowed the analysis of samples that were not suitable for WB analysis (low material amount) and of samples with low blast infiltration that were not interpretable with WB. In the positive samples, we could identify an immature blast cell population among the whole leukemic bulk that already harbored PI3K and ERK activation. Conclusion. A flow cytometry assay is a fast and reliable method for the detection of constitutive phospho AKT and phospho ERK in leukemic samples. All samples can be analyzed with a protocol using CD34 and new therapeutic agents are needed. Abnormal activation of several signal transduction pathways such as phosphoinositide 3-kinase (PI3K) and MAP kinase has been reported in AML. To test new targeted therapeutics, it is critical to develop sensitive analytical tools to detect aberrant activation of these pathways and monitor their inhibition in response to treatment. Aims. Our aim was to establish the feasibility of a flow cytometry analysis in patients samples even with a low blast infiltration and to analyze the correlation between flow cytometry and western blot analysis (WB). Methods. We analyzed AKT and ERK phosphorylation in blast cells of patients with hematologic anomalies. From 32 patients with high blast infiltration we compared WB and flow cytometry techniques. The flow cytometry protocol associated intra cellular staining for phospho proteins and membrane staining for several antigens including CD34 and CD38. Using CD34 allowed to identify the blast cell population even in CD34 negative AML (one third of the patients in our experience). For the CD34 positive AML, we set up a four color protocol using CD34, CD38 and CD123 as membrane antigens. This protocol allowed us to detect phosphorylated proteins in the most immaturle leukemic cells with high blast infiltration by WB and flow cytometry. In the positive samples we detected PI3K and ERK pathway activations in 45% and 70% of the samples. Flow cytometry allowed the analysis of samples that were not suitable for WB analysis (low material amount) and of samples with low blast infiltration that were not interpretable with WB. In the positive samples, we could identify an immature blast cell population among the whole leukemic bulk that already harbored PI3K and ERK activation. Conclusion. A flow cytometry assay is a fast and reliable method for the detection of constitutive phospho AKT and phospho ERK in leukemic samples. All samples can be analyzed with a protocol using CD34. We analyzed the phosphorylation status of the PI3K and ERK pathways in the most immature blast cells with the CD34+ CD38-/low CD123+ phenotype. When we detected phosphorylated proteins in the whole blast cell population, this activation was already present in the most immature cells, that represent exquisite target cells for new therapeutics.
Chronic myeloid leukemia I

0143
ABERRANT EXPRESSION OF CELLULAR RETINOL-BINDING PROTEIN-1 (CRBP-1) IN MEGAKARYOCYTES AND MARROW STROMA CELLS OF CHRONIC MYELOPROLIFERATIVE AND MYELODYSPLASTIC/MYELOPROLIFERATIVE DISORDERS


1Institute of Pathology Universityhospital, FREIBURG, Germany; Division of Haematology-Oncology University, FREIBURG, Germany; 2Department of Pathology, University of, GENEVA, Switzerland

Background. The effects of retinol (ROL) are mediated by cytoplasmic binding proteins involved in retinoid transport and/or metabolism, as well as nuclear receptors which act as ligand-dependent transcriptional regulators. Cellular retinol-binding protein (CRBP-1) contributes to the esterification of ROL to retinyl esters, the oxidation of ROL to retinal, the hydrolysis of retinyl esters into ROL. It also has been implicated in cellular growth and differentiation. Lipid droplets of hepatic stellate cells of normal and fibrotic liver which are the main storage site for retinoids contain high amounts of CRBP-1. Moreover, CRBP-1 is widely expressed in many extrahepatic vitamin-A target tissues including normal prostate, breast, endometrial glands and stroma, and cervical epithelium. Aims. Heterogeneous patterns ranging from over-expression of CRBP-1 to down-regulation via epigenetic silencing through DNA hypermethylation has been reported in several malignancies. To investigate the involvement of this key protein of retinoid homeostasis and metabolism in myeloproliferative diseases (MPD) and in myelodysplastic/myeloproliferative overlap syndromes (MDS/MPD), we analysed the in situ expression patterns of CRBP-1. Methods. This study was performed on a cohort of healthy bone marrow donors (n=15), patients with essential thrombocythemia (ET; n=25), chronic idiopathic myelofibrosis (CIMF; n=25), polycythemia vera (PV; n=25) and in MDS/MPD with features of so-called essential thrombocythemia with ringed leukemia (ET/RS; n=79). The tissue localization of CRBP-1 in marrow trephines was visualized using a well characterized antibody. Imaging was performed by bright-field and confocal laser scanning microscopy (CLSM). Double-labeling experiments included a panel of antibodies such as CD6, CD34 or α-smooth muscle actin (SMA). Evaluation focused on CRBP-1 expression in megakaryocytes and bone marrow stromal cells/myofibroblasts (MSCs/MFs). CRBP-1+ MSCs/MFs were present in subsets of MPD patients, but not in normal controls and increased from prefibrotic CIMP to CIMP III. Co-localization of CRBP-1 and SMA was documented by CLSM. Results. The up-regulation of CRBP-1 in MSCs/MFs was associated with an increased fibre density in the various MPD entities including CML, CIMF and PV, but not in ET. Bone marrow stromal cells from a subset of patients presenting with high platelet counts and high megakaryocytes exhibited high CRBP-1 expression and were shown to be similar to classical CIMP. Megakaryocytes from healthy control persons showed a moderate to high cytoplasmic CRBP-1 immunoreactivity. In contrast to the stroma, heterogeneous levels were demonstrated in megakaryocytes of PV and subsets of ET. CRBP-1 loss or abnormal spotty localization was most prominent in the bizarre giant megakaryocytes of CIMP while smaller megakaryocytes of CIMF showed a stronger cytoplasmic immunolabeling. Conclusions. The modulation of CRBP-1 in MSCs/MFs of the marrow microenvironment may affect proliferation, migration, differentiation, matrix synthesis and turnover in MPD. Moreover, the retinoid-signaling cascade may be impaired in megakaryocytes. Abrupt regulation may occur as a consequence of point mutations or a disruption of the ordered pattern of DNA methylation. Until now, the molecular mechanisms affecting CRBP-1 in MPD and MDS/MPD overlap syndromes remain to be identified.

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CHRONIC MYELOID LEUKEMIA CELLS EXPRESS TUMOR ASSOCIATED ANTIGENS ELICITING SPECIFIC CD8+ T CELL RESPONSES AND ARE LACKING COSTIMULATORY MOLECULES


1University of Ulm, ULM, Germany; Medical School of Southeast University, NANJIING, China

Background/Aims. Specific immunotherapies for patients with chronic myeloid leukemia (CML) might eliminate residual CML cells after therapy with imatinib or chemotherapy, and might enhance a specific graft versus the leukemia effect after allogeneic stem cell transplantation. Methods. Here, we investigated the mRNA expression and T cell recognition of tumor/leukemia-associated antigens (TAA/LAAs) in 54 patients with CML. Results. Several LAAs are expressed in CML and therefore candidate structures for specific immunotherapies: bcr-abl (100%), G250 (24%), kTERT (52%), MMP11 (91%), NEWREX60 (94%), PRAME (52%), Proteinase3 (71%), RHAMM/CD168 (83%) and WT1 (53%), but not BAGE, MA GE-A, SX2 or NY-ESO-1. The frequency of mRNA expression of RHAMM/CD168, Proteinase3 and PRAME was higher in accelerated phase and blast crisis. In flow cytometry, CD34+ progenitor cells were sensitive to HLA-molecules but not to CD40, CD80, CD86 and CD86. However, RHAMM/CD168 R3-specific (ILSLELMKL) specific T cell responses in CML patients were demonstrated by ELISPOT analysis and specific lysis of RHAMM/CD168 R3-pulsed T cells in chromium-51 release assays. RHAMM-R3 specific T cells could be phenotyped as CD8+tetramer+CD45RA+CCR7-CD27- early effector CD8+ T cells. Therefore, vaccination strategies inducing such RHAMM-R3 directed effector T cells might be a promising approach to enhance specific immune responses against CML cells.

Molecular response at 6 months is a good early predictor of duration of treatment response in imatinib-treated chronic phase chronic myeloid leukemia

M. Ross, S. Branford, J. Reynolds, R. Koelmeyer, T.P. Hughes

1IMVS, ADELAIDE, Australia; 2ALLG, MELBOURNE, Australia

Background. Imatinib mesylate induces a complete cytogenetic response (CCR) in >75% of de novo chronic phase CML patients. Monitoring of disease response with highly sensitive real-time quantitative reverse-transcriptase PCR (RQ-PCR) provides prognostic information in addition to that obtained by cytogenetic monitoring. Achievement of a 3-log reduction in BCR-ABL/BCR from standardized baseline (major molecular response, MMR) on imatinib confers improved progression-free survival and occurs in 40% of patients treated with 400 mg daily for one year (IRIS study). Aims. The TIDEL study aimed to assess the effect of imatinib dose escalation on treatment outcome. The starting dose was 600 mg daily. Where possible, dose-escalation treatment was continued. Results. MMR was achieved in 66 patients (64%) after a median of 9 months (range 3-29). Defined events for loss of response occurred in 6 patients (2.2%) (1 treatment-related, 1 suicide, 1 transplant-related) and were excluded from EFS analysis. The starting dose was 600 mg daily. Where possible, dose-escalation treatment was continued. Results. MMR was achieved in 66 patients (64%) after a median of 9 months (range 3-29). Defined events for loss of response occurred in 6 patients (2.2%) (1 treatment-related, 1 suicide, 1 transplant-related) and were excluded from EFS analysis. The starting dose was 600 mg daily. Where possible, dose-escalation treatment was continued.
Only molecular response at 6 months was significantly associated with EFS at 80 months: <1-log reduction 40%; 1-<2-log reduction 66%; 2-<3-log reduction 96%; ≥3-log reduction 100%. The degree of log-reduction in BCR-ABL/BCR at 3 months, and at the time of CCR was not predictive of EFS. Summary. In this study where achievement of CCR is accelerated (in comparison with IRIS), molecular response at the time of detecting CCR lacks prognostic value with regard to EFS, highlighting the importance of molecular monitoring at regular intervals, rather than waiting until CCR is achieved. Despite excellent treatment responses overall, with early molecular monitoring it is possible to identify a group of patients whose chance of achieving MMR is low, and in whom closer monitoring or alternative treatments should be considered.

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P210 BCR-ABL TYROSINE KINASE INTERACTS WITH HISTONE DEACETYLASE 1 IN CHRONIC MYELOID LEUKAEMIA HAEMATOPOIETIC PROGENITORS: CONSEQUENCES ON HISTONE H4 ACETYLATION AND CHROMATIN STRUCTURE

G. Brusa,1 M. Mancini,1 E. Zuffa,1 P. Corrado,2 S. Luatti,2 E. Barbieri,1 M.A. Santucci1

1Università di Bologna, BOLOGNA, Italy; 2Istituto di Ematologia, BOLOGNA, Italy; 3Istituto di Radioterapia, BOLOGNA, Italy

The BCR-ABL fusion gene originated from balanced (9;22) translocation is the molecular hallmark and the causative event of Chronic Myeloid Leukaemia (CML). The interactions of its p210 protein constitutively activated and improperly confined to the cytoplasm with multiple regulatory signals of cell cycle progression, apoptosis and self-renewal induce the illegitimate enlargement of clonal hematopoiesis and generate an instability that drive the trans-formation phenotype of blast crisis. However, its effects on the basic transcription machinery and chromatin remodeling are unknown. Our study underscored histone H4 hyperacetylation associated with p210 tyrosine kinase (TK) in vitro and in vivo and its role in BCR-ABL transcription. Histone H4 acetylation status was assayed in 32D murine myeloid progenitors and the cell line expressing a ts BCR-ABL mutant by labelling immunoprecipitated (IP) chromatin (CHIPs) with an anti-Ac-H4 antibody. Under permissive culture condition for p210 TK (33°C), histone H4 acetylation was reduced between 4 and 24 h of imatinib mesylate (IM) exposure concomitantly with p210 dephosphorylation and enzymatic activity reduction. In vivo histone H4 acetylation signals on CHIPs of CD34+ progenitors from CML patients at diagnosis were more intense than those of normal controls and were significantly reduced at day 15 of IM therapy. To address the putative p210 TK role on histone H4 methylation in vitro and in vivo advanced by mass spectrometry analyses we proved that histone H4 trimethylation at Lys20 was significantly reduced in presence of p210 TK and restored after p210 TK inhibition by IM in vitro. Histone H4 hyperacetylation associated with p210 TK in vitro and in vivo proceed, at least in part, from Hdac1 loss of function arising from its cytoplasmatic compartmentalisation by p210 TK. Indeed p210 TK is associated with histone H4 hyperacetylation at a BCR promoter region (−40 to +285) critical for BCR-ABL transcription in LAMA cell line. BCR-ABL transcript levels were reduced by approximately 20% at 4 h of IM exposure and further declined to 40% of untreated control at 24 h. Amplification signals of DNA from anti-Ac-H4 CHIPs were significantly reduced at 2 h of IM exposure and remained lower compared with untreated control up to 24 h (Figure 1, part A and B). Complementary activities are probably implicated in the control of histone H4 acetylation status relative to p210 TK.
BCR/ABL ONCOGENIC KINASE DISRUPTS MISMATCH RECOGNITION AND REPAIR COMPLEX TO INDUCE GENOMIC INSTABILITY

T. Stoklosa, A. Slupianek, G. Basak, T. Skorski

‘The Medical University of Warsaw, WARSAW, Poland; ‘Temple University, PHILADELPHIA, USA; ‘The Medical University of Warsaw, Poland, WARSAW, Poland

Background. BCR/ABL oncogenic tyrosine kinase is present in most chronic myeloid leukemia (CML) and in a cohort of acute lymphocytic leukemia (ALL) patients. BCR/ABL is responsible for malignant transformation of hematopoietic cells rendering them independent of their environment. The other, less understood, role of BCR/ABL in haematological malignancies is deregulation of DNA damage response, which results in drug resistance and genomic instability. Mismatch repair proteins (MMR) are responsible for detecting and removing misincorporated nucleotides, which escaped proofreading activity of DNA polymerases. MMR proteins assembled on the mismatch can signal to repair or apoptosis. Defects in expression of MMR genes leads to drug resistance and mutator phenotype, observed in different solid tumors. Aims. Deciphering the role of mismatch repair in drug resistance of BCR/ABL-transformed cells. Methods. MNNG (N-methyl-N-nitro-N-nitrosoguanidine), methylating agent was used as a genotoxic treatment. 32Dcl3 cells myeloid cell line along with p510 BCR/ABL expressing counterparts and primary leukemia and normal bone marrow cells were employed. Cell viability was assessed by trypan blue exclusion and/or propidium iodide staining. Clonogenicity of parental and BCR/ABL cells after MNNG challenge was examined in semi-solid medium. Protein expression was analyzed by Western blotting. Immunofluorescence analysis of the nuclear localization of MMR proteins was performed with primary antibodies to MSH2, MSH6, MLH1 and PMS2. Mutation rate and phenotype was analyzed using TA cloning kit and sequencing. Results. Among different genotoxic agents, BCR/ABL cells were more resistant to MNNG than parental cells (as shown in viability and clonogenic tests). Parental cells and BCR/ABL expressing clones were incubated with MNNG for 4 weeks resulting in their MNNNG-resistant derivatives, which may accumulate mutations in their genomic DNA resulting from methylating activity of the drug. To investigate the mutation rate and phenotype, ouabain-resistance test was employed. The clonogenic assay revealed over 5 times more ouabain resistant colonies in MNNG-resistant BCR/ABL-positive cells than in parental counterparts. The dominating mutation in BCR/ABL MNNG-resistant cells was C to T, while A to G mutations was prevalent in parental cells (Figure 1). In order to check the status of MMR proteins, Western blotting and immunofluorescence studies were performed. Expression of MMR proteins in BCR/ABL-transformed cells was similar to parental, however immunofluorescence visualized dramatic changes after DNA damage in the nuclear co-localization of MMR proteins in BCR/ABL-transformed in comparison to normal cells. Co-localization of MSH2 and MSH6 proteins, forming a heterodimer homologous to bacterial MutS, remained similar in parental and leukemia cells upon MNNG treatment. However, co-localization of MLH1 (which form a heterodimer with PMS2 homologous to bacterial MutL) and MSH2 was detected in non-transformed cells and not in BCR/ABL leukemia cells. Interaction of MMR proteins in leukemia cells was restored after inhibition of BCR/ABL kinase by imatinib. Summary/Conclusions. BCR/ABL impairs assembly of MMR proteins on mismatched nucleotides and subsequent signaling to repair and/or apoptosis. This results suggest a novel mechanism how oncogenic tyrosine kinase can modulate mismatch recognition and repair leading to genomic instability and drug resistance of leukemic cells.

COMPARATIVE PROTEOMIC ANALYSIS OF CHRONIC MYELOGENOUS LEUKEMIA CELLS : INSIDE THE MECHANISM OF IMATINIB RESISTANCE

V. Santini, G. Ferrari, A. Gozzini, R. Pastorelli
University of Florence, FIRENZE, Italy

Background. Development of imatinib resistance represents a critical factor for the therapy of chronic myelogenous leukemia (CML). Resistance is mainly due to mutations in the abl kinase domain, and to overexpression of Bcr/abl protein, provoked by amplification of the genomic locus. Aims: We undertook a comparative proteomic approach of human chronic myeloid leukemia cells Imatinib sensitive and resistant, to dissect the molecular mechanism of resistance. In fact, the characterisation of biochemical pathways involved with and connected to Bcr/abl could be extremely useful in identifying new therapeutic targets to bypass resistance to the kinase inhibitor. Methods. Total cell protein LAMA 84-S and LAMA 84-R extracts were separated by two dimensional electrophoresis (2DE), and gel images were compared by adequate software in order to establish characteristic protein signatures typical of Imatinib sensitive and Imatinib resistant cells. Results. Matrix assisted Laser Desorption ionisation-Time of Flight Mass spectrometry (MALDI-TOF MS) analysis allowed the identification of 45 differentially expressed proteins We categorized these proteins into five main functional classes: i) Chaperones and Heat shock proteins ii) Nucleic acid interacting proteins (binding/synthesis/stability), iii) structural proteins, iv) cell signalling and v) metabolic enzymes. i) Heat shock proteins HS60 and HS70 interact with HSP70 respectively. HSP60 and HSP70 isoform 1 and 2, valosin containing protein (VCP) known to bind the HSP90-interacting Bcr-Abl complex, resulted to be significantly over expressed in LAMA 84-R cells, indicating a possible involvement of several of these chaperone proteins in the mechanism of Imatinib resistance, via a possible block of bcr/abl proteosome degradation. ii) A relevant number of proteins interacting with DNA and RNA (hnRNPF, hnRNPH1, hnRNPK and eIF3) were found to be more abundant or even expressed only in imatinib resistant cells. iii) Structural proteins: vimentin, α tubulin, γ actin were instead significantly more expressed in imatinib sensitive cells. The identified proteins involved in cell signalling and in metabolic pathways (classes iv and v) resulted differentially expressed in LAMA 84-S and LAMA 84-R, but without a clear signature. Summary/Conclusion. Bcr/abl and FLT3 are client of chaperon protein HSP90 and it has been shown that HSP90 inhibitors are active in blocking CML cell proliferation. HSP70 is involved in inhibition of apoptosis. However the overexpression of these class of proteins seems to be directly responsible for the stability, maintenance and function of Bcr/abl, akt and other tyrosine kinase substrate of bcr/abl. This is fundamental for CML cells, basing imatinib resistance on constitutionally overexpression of bcr/abl. A similar pivotal role in the maintenance of the resistant phenotype may be attributed to RNA stabilizing proteins, like hnRNPF, hnRNPH1, hnRNPK. The optimal characterization of the protein signature of CML imatinib resistant cells and the identification of critical actors in drug resistance may lead to new therapeutic approaches, synergistic with all tyrosine kinase inhibitors at present in clinical trials. To our knowledge, this is the first direct proteomic comparison of imatinib sensitive versus resistant CML cells.
DASATINIB IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN MYELOID BLAST CRISIS (MBC) THAT IS IMATINIB-RESISTANT OR IM-INTOLERANT: RESULTS OF THE CAL006006 START-B STUDY

M. Baccarani, 1 J. Cortes, 1 D.W. Kim, 2 T. Facon, 3 J.F. Apperley, 4 P. Rousselet, 5 E. Bleikardt, 6 R. Zink, 7 M. Muller, 8 C. Sawyer, 6
1 University of Bologna, BOLOGNA, Italy; 2 MD Anderson Cancer Center, HOUSTON, USA; 3 The Catholic University of Korea, KYUNGGI-DO, South-Korea; 4 Hôpital Haveluy, CHU de Lille, LILLE, France; 5 Hammersmith Hospital, LONDON, United Kingdom; 6 Institut St. Louis, PARIS, France; 7 Breast Centre, Squibb, WALLINGFORD, USA; 8 Universität Heidelberg, MANNHEIM, Germany; 9 UCLA School of Medicine, LOS ANGELES, USA

Background. Dasatinib (D) (BMS-354825) is an oral, multitargeted tyrosine kinase inhibitor of Bcr-Abl and SRC with activity against imatinib-resistant cell lines. A phase I study demonstrated preliminary evidence of activity of D in MBC-CML pts. Aims. To demonstrate the activity of D in pts who are resistant to or intolerant of imatinib. Methods. START-B is an open-label study of D in IM-R or IM-IMBC carried out in 46 sites worldwide. From December 2004 to July 2005, 109 MBC pts were treated. D was given orally at 70 mg twice a day (BID) with dose escalation to 100 mg BID for poor initial response or dose reductions to 50 mg and 40 mg BID for toxicity. Pts had weekly blood counts and monthly bone marrow evaluations, including cytogenetics. Molecular monitoring of BCR-ABL transcript levels by RT-PCR were obtained at baseline and in pts who achieved cytogenetic response. The primary endpoint was confirmed (minimum 4 weeks duration) major hematologic response (MaHR). Results. Among the 109 treated patients, 99 were IM-R and 10 IM-I; median age was 55 yrs (range 21’81), 58% were male. Prior therapy included interferon in 55 (49%) pts, stem cell transplant in 15 (14%) pts and prior chemotherapy 49 (66%) pts. Prior IM dose was >600 mg/day in 49% of pts and 41% of pts received IM for > 5 years. WBC count >20×10^9/mu in 46% of pts, Platelet count <100×10^9/mu in 64% and 32% had ≥20% bone marrow blasts. Preliminary safety and efficacy analyses are currently available on the first 74 pts (48 IM-R, 6 IM-I). Mutations in the BCR-ABL domain were found in 27/65 (43%) pts. Median duration of therapy was 35 months. Doses were reduced in 35% of pts, temporarily interrupted in 58% pts, and escalated in 41% pts. With a minimum of 6 months follow-up, hematologic responses were seen in 39 (53%) pts: confirmed MaHR in 24 (32%) pts, Complete in 18 (24%) and No Evidence of Leukemia in 6 (8%). Major cytogenetic responses were documented in 22 (30%) pts and were complete in 20 (27%). The median time to MaHR was 56 days. None of the 24 pts who achieved a MaHR have relapsed with a duration of MaHR ranging from 1.2 to 7.8+ months. The median PFS had not been reached. Severe myelosuppression was common, but manageable. Non-hematologic toxicities were usually mild to moderate. The most common Grade 3-4 toxicities included diarrhea in (7%), pleural effusion (9%), peripheral edema 25%, rash 21%, fatigue, headache 25% (n=6 each), anemia, extremity pain 21% (n=5 each), peripheral edema, pruritus 17% (n=4 each) and leukocytosis, arthralgia, pharyngolaryngeal pain, upper abdominal pain in 13% (n=3 each). Overall Grade 3/4 adverse events included thrombocytopenia 29% (n=7), neutropenia 25% (n=6), anemia 17% (n=4), pyrexia 8% (n=2), and leukocytosis, pruritus 4% (n=1 each). Summary/Conclusions. Nilotinib has clinical activity in patients with imatinib-resistant or intolerable BC and relapsed/refractory Ph+ ALL.

jej l J. Giles, 5 K. Bhalla, 6 H. Kantarjian, 3 A. Hochhaus, 4 M. Muller, 4 D. Jones, 3 K. Dawson, 3 K. Rose, 6 L. Alland, 4 M. Dugan, 6 S. Lilleburg, 6 O. Ottman, 6
5 MD Anderson Cancer Center, HOUSTON, USA; 6 H. Lee Moffitt Cancer Center, HOUSTON, USA; 7 Hi Med. Klinik Mannheim, HEIDELBERG, Germany; 8 Novartis Pharmaceuticals, FLOURHAM PARK, USA; 9 Transge nomics, Inc, NEW JERSEY, USA; 10 JW Goethe University, FRANKFURT, Germany

Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor which in vitro is 50-fold more potent than imatinib and active against 32/33 imatinib resistant Bcr-Abl mutations. Aim. To evaluate the spectrum of mutations in Bcr-Abl was evaluated by direct sequencing of the kinase domain and surrounding regions on samples from patients with imatinib-resistant Ph+ CML or ALL. Methods. This is a Phase I study of nilotinib (total oral doses ranging from 50 to 1200 mg administered daily or twice daily). The template was created by semi-nested PCR using primers in the BCR and ABL regions of the gene. Mutations correlated with clinical response. Results. There were 119 patients enrolled of whom 86 had both pre and post treatment analyses. Mutations were present at baseline for 39 (45%) patients of whom 27 (69%) responded. Of the 90 patients with data available 47 (55%) had no mutation at baseline of which 34 (72%) responded. The most common baseline mutations were G250E, E255K, E355G, F317L, H396R and M351T. New mutations were found during median follow-up of 112 (6-350) days in 37 patients (26 without baseline mutations). New most commonly included E595V, E595K, E595G, F2920, M244V and T315I. Of the 57 patients, 30 had evaluations after mutations emerged, and 15 continued to respond for median of 160 (41-351) days. Fourteen mutations not previously reported occurred, 6 at codons with known imatinib-resistance mutations resulting in novel amino acid substitutions. New mutations in several patients included E534C, F311L, E453K, and E549Q. The T315I mutation was present at baseline in 1 patient who failed to respond, and emerged in 4 patients, of whom follow-up was available for three. Two continued to respond ≥ 20 days after developing the mutation and one progressed when the mutation emerged. Summary/Conclusions. Nilotinib has clinical activity in patients with Ph+ ALL, and with new mutations. New mutations used in this Phase I study, new mutations often identified during follow-up, but were not a reliable predictor of clinical relapse. Future studies using more sensitive methods of mutation detection, such as D-HPLC, are needed to determine whether mutations detected during AMN107 therapy are present at low levels prior to therapy.
ROLE OF ENDOTHELIAL PROGENITOR CELLS IN MYELOPROLIFERATIVE DISEASES

C. Zwicky,1 E. Oppliger Leibundgut,2 M.P. Horn,3 C. Brunold,2 B. Pfanner-Meyer,2 D. Marti,2 H. Hiringer,2 A. Tobler,2 G. Baerlocher2
1University Berne, BERNE, Switzerland; 2Hematology Department, BERNE, Switzerland; 3Pathology Department, BERNE, Switzerland

Background. The presence of circulating hematopoietic progenitor cells has been described in patients with myeloproliferative diseases (MPD). However, the exact nature of such progenitor cells has not been specified until now. Aim: The aim of this work is to proof the hypothesis that the endothelial cell lineage is primarily involved in the pathophysiology of myeloproliferative diseases. Methods. Expression of the heman-gioblast markers (early common precursors to the hematopoietic and endothelial cell lineage) in the circulating cells of 53 patients with MPD was assessed. Peripheral blood was analysed for expression of CD34, promin (CD135), KDR (kinase insert domain receptor, or vascular endothelial growth factor receptor 2, VEGFR2) and vWF (von Willebrand factor) mRNA by quantitative PCR. Clonogenic stem cell assays were performed to assess differentiation towards the hematopoietic and endothelial cell lineage. Patient data (essential thrombocythemia (ET), n=17), polycythemia vera (PV, n=21) and chronic idiopathic myelofibro-sis (CIMF, n=15)) were compared with data from normal controls (n=16) and patients with secondary thrombo- or erythrocytosis (n=17). Results. Trafficking of CD34 positive cells was increased above the physiological level in 4/17 patients with ET, 5/21 patients with PV and 3/15 patients with CIMF. A subset of patients with CIMF co-expressed the heman-gioblast markers CD34, Prominin (CD135) and KDR, suggesting the presence of hemangioblasts among the circulating progenitor cells. Clonogenic stem cell assays confirmed differentiation towards both the hematopoietic and the endothelial cell lineage in 5/10 patients with CIMF. Furthermore, trisomy 8 was found in the grown endothelial cells of a patient in which trisomy 8 was diagnosed in the peripheral blood, confirming the common clonal origin of both cell lines. Conclusion. Hemangioblasts are present in the blood of a subset of patients with CIMF, suggesting a primary role of pathological endothelial cells in this disease.

COMPLIANCE AND PERSISTENCY WITH IMATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS

W. Feng,1 J. Henk,2 K. Thomas,3 J. Baladi,1 A. Hatfield,1 A. Goldberg,2 J. Cortes1
1Novartis Pharmaceuticals Corporation, FLORHEM PARK, USA; 2I3Magni-fitie, An Ingenx Company, EDEN PRAIRIE, USA; 3MD Anderson Cancer Center, HUSTON, USA

Background. Imatinib is an oral molecularly targeted therapy with unprecedented efficacy in chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST). Optimal dosing and adherence to treatment is critical to achieve the best clinical outcomes. Aim: This study examined compliance and persistency with imatinib in CML patients and identified the clinical and patient characteristics that are related to compliance and persistency. Methods. Claims data from a large US health plan were used to identify imatinib-treated patients from 6/1/01-3/31/04 who had continuous pharmacy and medical benefits in the 3 months prior and 12 months following initiation of imatinib ther-apy, and a diagnosis of CML (ICD-9-CM 205.1, 205.10, or 205.11). Compliance was defined by medication possession ratio (MPR=total days supply of imatinib in the first year divided by 365). Persistency was defined as failure to refill imatinib within 30 days from the run-out date of the prior prescription. Multivariate analyses were used to identify the key factors that are associated with compliance and persistency. Results. Total 878 imatinib-treated patients were identified of whom 413 had at least 15 months’ continuous eligibility. Sixty-nine percent (n=286) were diagnosed with CML and are the subjects of this analysis. The average age was 50 (range 5 to 86, median 50.5) and 59% were males. The average starting daily dose was 428 mg, with 61% (n=242) initiating on 400 mg daily. The mean MPR was 76%. Overall, 32% patients discon-tinued imatinib for at least 30 consecutive days during the 1-year follow up period. Multivariate analyses indicated MPR improved with age until age 52 and then deteriorated (p<0.001) but at a diminishing rate, decreased cell population by 6% for each 1-year increase (p=0.01), and was lower in women (p=0.004) and patients with more cancer complications (p=0.005). Other variables included in this analysis were starting daily dose and geographic region. In the multivari-
A PHASE II STUDY OF NILOTINIB (AMN107) A NOVEL INHIBITOR OF BCR-ABL ADMINISTERED TO IMATINIB-RESISTANT OR INTOLERANT PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN ACCELERATED PHASE

H. Kantarjian,1 O. Ottmann,2 N. Gattermann,3 R. Larson,4 T. Rafferty,5 L. Alland,6 F. Giles,7 S.G. O’Brien,8 P. Le Coutre9

1The University of Texas MD Anderson Canc, HOUSTON, USA; 2Universitätsklinik Frankfurt, FRANKFURT, Germany; 3Heinrich-Heine-Universitat, WESTHALLA, Germany; 4University of Chicago Hospital, CHICAGO, USA; 5Novantis, NEW JERSEY, USA; 6University of New Castle, NEWCASTLE UPON TYNE, United Kingdom; 7Virchow-Klinikum, CHARITE, Germany

Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor of Bcr-Abl which in vitro is 80-fold more potent than imatinib. It is active against imatinib-resistant and imatinib-sensitive B-Cr-Abl mutants. Aim. This study was designed to evaluate the safety and efficacy of nilotinib as defined by hematologic and cytogenetic response (HR/CyR) rates in imatinib-resistant or intolerant AP patients. Methods. This is a Phase II open-label, multicenter, study of nilotinib administered orally at a dose of 400 mg twice daily. Results. This study remains open to enrollment. Preliminary data are reported for 22 patients (77% resistant and 23% intolerant to imatinib). The median age was 62 (range 43-76) years and the median time from AP diagnosis was 6 months (range 0-2-56). Three of five patients with data available had a BCR-ABL mutation at baseline. The median duration of nilotinib exposure was 124 days (range 3-207). Treatment is ongoing for 16/23 (73%) patients. HR occurred in 14 (64%) patients of which 10 (45%) were complete. Three (14%) were marrow responses/no evidence of leukemia, and 1 had a return to chronic phase. CyR occurred in 6 patients (1 complete, 1 partial, 1 minor, and 3 minimal). The AE’s occurring in ≥ 10% patients were thrombocytopenia 36% (n=8), fatigue 32% (n=7), anemia, pruritus, muscle spasms 27% (n=6 each), bone pain, cough, rash 25% (n=5 each), diarrhea, headache, myalgia, pyrexia 18% (n=4 each), abdominal pain, chills, constipation, dyspnea, nausea, extremity pain, and peripheral edema 14% (n=3 each). The overall incidence of Grade 3/4 AE’s were thrombocytopenia 27% (n=6), anemia, neutropenia 13% (n=3 each) and rash 5% (n=1). Two deaths occurred: one patient with thrombocytopenia had a CNS bleed and one patient had disease progression. Summary/Conclusions. These data suggest that nilotinib is clinically active and has an acceptable safety profile when administered to patients with CML-AP.

IMATINIB 800 MG IN INTERMEDIATE SOKAL RISK PATIENTS IN EARLY CHRONIC PHASE: RESULTS OF A PHASE II TRIAL OF THE GIMEMA CML WORKING PARTY

G. Rosti,1 F. Castagnetti,1 M. Amabile,2 N. Testoni,3 G. Marzocchi,4 M. Breccia,5 F. Falandri,6 C. Bigazzi,7 A. Poerio,8 E. Stagno,9 F. Guliani,4 E. Abruzzese,6 F. Fane,1 G. Saglio,1 G. Martinelli,2 M. Baccarani3

1University of Bologna, BOLOGNA, Italy; 2Chair of Hematology, UNIVERSIT-‘T ‘LA SAPIENZA’ - ROMA, Italy; 3Division of Hematology, ASCOLI PICENO UNIVERSITY HOSPITAL, ASCOLI PICENO, Italy; 4Novapharm, ROME, Italy; 5University of New Castle, NEWCASTLE UPON TYNE, United Kingdom; 6Chair of Clinical and Biological Sciences, TORINO UNIVERSITY, Italy

Background. Imatinib standard dose (400 mg) gives impressive results in chronic myeloid leukemia (CML) in early chronic phase. Results, stratified by Sokal risk, are inferior in intermediate and high risk with respect to low risk. In intermediate Sokal risk patients, the IRIS trial (T Hughes et al., NEJM 349:15, 2005) reported within 12 months a complete cytogenetic response (CCG.R) rate of 67% and a major molecular response (MMR) rate of 45%. Phase I and II trials of imatinib have clearly shown a dose-dependent effect. Kantarjian et al. (Blood 103, 2004) reported increased results with imatinib high dose (800 mg) in 114 early chronic phase patients treated at the MD Anderson Hospital. The CCGR was 90% and MMR 60% within 12 months of therapy. Aim. The GIMEMA CML WP opened in January, 2004 a phase II, multicentric prospective study (Sokal 1992 and 2002 categories) devoted to investigate the efficacy of imatinib high dose (800 mg) in intermediate Sokal risk patients. Methods. Clinical and anagorical data were collected through a web-based system. Responses were evaluated at fixed time-points during treatment. Hemato-logic: continuously; cytogenetic at 6 and 12 months (local labs); molecular response at 3, 6 and 12 months. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR; Bcr-Abl/ABL x 100 - Taqman) were centralized in Bologna at 6, 3 and 12 months. Results. Patient between January 1, 2004 and April, 2005 23 Italian centers enrolled 81 patients (72 evaluable). Median age was 56 yrs (range 26-79), 43 males and 29 females. 72 patients are evaluable for response at 3 months, 65 at 6 months and 48 at 12 months. The median observation time is 6 months. Responses were: CCR 100% in 36 and 66% in 12 months. The cumulative incidence of CCGR was 94% at 12 months. CCGR rates were 100% and MMR in 53% of the patients at 12 months, the CCGR rate was 90% and the MMR rate in CCGR patients was 86%. The cumulative incidence of CCGR was 94%. The overall incidence of Grade 3/4 AE’s were thrombocytopenia 27% (n=6 each), anemia, neutropenia 18% (n=4 each), myalgia, pyrexia 18% (n=4 each), abdominal pain, chills, constipation, dyspnea, nausea, extremity pain, peripheral edema 14% (n=3 each). The overall incidence of Grade 3/4 AE’s were thrombocytopenia 27% (n=6), anemia, neutropenia 13% (n=4 each) and rash 5% (n=1). Two deaths occurred: one patient with thrombocytopenia had a CNS bleed and one patient had disease progression. Summary/Conclusions. The preliminary results of our trial suggest that imatinib 800 mg is highly effective for intermediate Sokal risk CML in early chronic phase, being superior to 400 mg (IRIS trial, same risk category) and in the range of the MD Anderson results.

0159
DASATINIB (D) IN PATIENTS (PTS) WITH ACCELERATED PHASE CHRONIC MYELOID LEUKEMIA (AP-CML) RESISTANT OR INTOLERANT TO IMATINIB: RESULTS OF THE CALB0055 START-A STUDY

F. Guihot, 1, J.F. Apperley, 1, A. Hochhaus, 1, R. Silver, 1, S. Amadori, 1, A. Gratwohl, 1, S. Cheng, 1, M. Iyer, 1, J. Radich, 1, M. Talpaz 1 1CHU La Milicière, POITIERS, France; 2Hammersmith Hospital, LONDON, United Kingdom; 3Universitaet Heidelberg, MANNHEIM, Germany; 4Cornell University, NEW YORK, USA; 5 Policlinico Toe Virgata, ROME, Italy; 6University Hospital Basel, BASEL, Switzerland; 7Bristol Myers Squibb, WALLING-FORD, USA; 8Fred Hutchinson Cancer Research Center, SEATTLE, USA; 9The University of Michigan, ANN ARBOR, USA

Background: D (BMS-354825) is an oral multi-targeted kinase inhibitor with preliminary evidence of efficacy in a previously reported phase I study. Aims. To demonstrate the activity of D in patients (pts) with AP-CML resistant to or intolerant of imatinib. Methods. START A is an open-label study of dasatinib in AP-CML who were imatinib resistant (IM-R) or imatinib intolerant (IM-I). Dasatinib was given orally at 70 mg twice daily (BID). Dose escalation to 100 mg BID was allowed for inadequate initial response and reduction to 50 or 40 mg BID for persistent toxicity. Evaluation included weekly blood counts and monthly bone marrow including cytogenetics. Molecular evaluation of Bcr-Abl transcript by real-time quantitative polymerase chain reaction was performed at baseline and after documentation of complete cytogenetic response. The primary endpoint was major confirmed (maintained at least 4 weeks) hematologic response (MaHR) in IM-R pts. Results. A total of 174 pts (161 IM-R and 13 IM-I) were enrolled between December 2004 and July 2005 in 59 centers worldwide. There were 96 (55%) males; median age 57 yrs (range 22-86); median time from diagnosis of CML 82 months. Prior therapy included IM-600 mg/day in 91 (52%) pts, IM for > 3 years in 103 (59%) pts, interferon in 126 (72%) pts, stem cell transplantation in 23 (13%) pts. Major Cytogenetic Response (MCyR) to prior IM was seen in 57 (33%) pts. Preliminary assessment of efficacy and safety was performed on the first 107 pts (99 IM-R, 8 IM-I) with 26 months of follow-up. The average daily dose was 119 mg/day, 52 (49%) pts required a dose reduction mostly due to hematologic toxicity. MaHR was documented in 63 (59%) pts (95% CI: 49-68) with Complete Hematologic Response in 53 (33%) and No Evidence of Leukemia in 28 (26%). MCyR was documented in 33 (32%) pts (95% CI: 22.9;41.6); complete in 23 (22%), partial in 10 (10%). In the 99 IM-R pts, the MaHR rate was 59%. In the 56 pts with Bcr-Abl mutations the MaHR was 66%. Molecular response analysis is ongoing. Fifteen pts had disease progression including one loss of MaHR. Myelosuppression was significant with grade 3/4 thrombocytopenia and neutropenia in 79% and 69% of pts, respectively. Non-hematologic toxicities were generally mild to moderate. The most frequent were diarrhea (46%), peripheral edema (27%), pleural effusion (16%), rash (8%), and GI hemorrhage (7%). Conclusions: Dasatinib is very effective in pts with IM-R AP-CML with high rates of durable MaHR and MCyR. Data on all 174 pts will be presented at the meeting including the molecular response analysis.

0160
INVolVEMENT OF RAS, JAK2 AND GM-CSF IN THE PATHOGENESIS OF PROLIFERATIVE VARIANT OF CHRONIC MYELOMONOCYTIC LEUKEMIA

D. Boskovic, 1 F. Servida, 2 C. Ricci, 2 E. Fermo, 2 M.C. Pasquini, 2 A. Vismara, 2 A. Cortezzoni, 2 S. Soligo, 2 G. Lambertenghi-Delliliers 2 2Institute of Hematology, BELGRADE, Serbia and Montenegro; 3Matterelli Foundation for Blood Diseases, MILANO, Italy; 4Fondazione Ospedaliero Maggiore Policlinico, MILANO, Italy; 5Azienda Ospedaliera G. Salvini, RHO (MILA-NO), Italy

Background. Chronic myelomonocytic leukemia (CMML) is a heterogeneous malignancy classified among MDS/MPD disorders. The paucity of known pathogenetic events contributes to the lack of effective treatment and to its dismal prognosis. On the basis of the peripheral leucocyte count, a dysplastic subtype (MD-CMML, WBC <12×10^9/L) can be distinguished from a proliferative subtype (MP-CMML, >12×10^9/L WBC/L) of the disease. Among factors that have been implicated in pathogenesis of CMML, GM-CSF produced by either autocrine or paracrine mechanisms has been shown to be a major growth determinant. Aims. To investigate cellular and molecular differences between MD- and MP-CMML which could contribute in clarifying pathogenesis of the disease and in identifying targets for possible therapeutic applications. Methods. Peripheral blood mononuclear cells (MNC) were isolated on Ficoll-Paque density gradient from 29 patients affected by CMML (17 with MD-CMML and 12 with MP-CMML). Samples were screened for the presence of N/K-RAS genes mutations by PCR and direct sequencing. Identification of the JAK2 V617F mutation was carried out by both allele-specific PCR and amplification of exon 12 followed by restriction analysis. Also, to evaluate the expression of intracytoplasmic GM-CSF and the expression of its receptor (GM-CSFR), MNC were stained with GM-CSF PE (Caltag) and CD116 (Pharmingen), respectively, in vitro growth of myeloid colonies was assessed in semisolid medium with or without the addiction of cytokines (SCF, GM-CSF, IL-3, Epo). Results. No RAS or JAK2 mutations were detected in the group of patients with MD-CMML. In the proliferative variant group, we identified two patients carrying the G12D substitution of N-RAS. Furthermore, a G60E point mutation of N-RAS was identified in 1 patient after progression from MD- to MP-CMML. The JAK2 V617F mutation was detected in 4 patients, all affected by the proliferative variant of CMML. Mean percentage of GM-CSF expression was 59.8% (range 14.5-90.7) in MP-CMML and 2.7% (range 0-9.3) in MD-CMML. The difference between MP and MD disease was statistically significant. In contrast, mean percentage of expression of GM-CSF was similar in MD- and MP-CMML samples (40.1 vs 42.2). However, when we considered median density of the GM-CSF expression, we observed significantly higher values in MP-CMML than in MD-CMML (123.2 and 51.4, respectively). The number of CFU-GM was higher in the MP-CMML than in MD-CMML (57 vs 17/5×10^9/L, cells plated) and a significant correlation with intracytoplasmic GM-CSF expression was observed (p<0.05). Conclusions. In summary, in our series of patients with proliferative variant of CMML, RAS and JAK2 mutations were quite frequent (25% and 35%, respectively). Because MP-CMML may evolve from MD-CMML, these findings support the hypothesis that molecular abnormalities could be acquired with disease progression. Moreover, since both JAK2 and RAS proteins are involved in the GM-CSF signalling pathway, the higher levels of intracytoplasmic cytokine and the increased density of its receptor in MP-CMML support the hypothesis that this pathway has a central role in malignant cell proliferation of CMML patients.
**Chronic myeloid leukemia II**

**0161**

**TRANSCRIPTIONAL PROFILING OF PRIMARY IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA**

O. Frank, B. Brohrs, A. Fabarius, L. Li, C. Zheng, M.C. Müller, R. Helmann, A. Hochhaus, W. Seifarth

Universität Heidelberg, MANNHEIM, Germany; Deutsches Krebsforschungszentrum, HEIDELBERG, Germany; III. Medizinische Klinik, Germany; Zentrum für Klinische Forschung, Germany

Background. Although the selective tyrosine kinase inhibitor imatinib is successfully used in the treatment of chronic myeloid leukemia (CML), inherent mechanisms confer primary resistance in a significant minority of patients. *Aims*. In order to search for potentially useful genes in predicting cytogenetic response, a retrospective microarray-based gene expression study was performed.

Methods. Quality-controlled RNA from leukocytes of bone marrow (BM) and/or peripheral blood (PB) of 34 interferon-α-pretreated chronic phase CML patients with or without major cytogenetic remission (i.e. <5% Ph+ metaphases) during the first year of imatinib treatment was comparatively analyzed using high-density Agilent U133A chips. Diagnostic groups (responders vs. non-responders, n = 11) were matched according to demographic and hematologic parameters and evaluated during their clinical course for hematologic, cytogenetic and molecular responses. For the assessment of differences in gene expression, BM and PB samples simultaneously taken from seven imatinib responders were statistically analyzed based on mixed loglinear models.

Results. Using support vector machines for gene classification, an outcome-specific gene expression signature consisting of 128 genes was identified. Expression comparative data of specific genes point to changes in apoptosis (e.g. casp9, trap1, bax), DNA repair (mhx3, dbb2), oxidative stress protection (gss, pon2, vnn1), and centrosomes (cdy1) within primary resistant patients. Independent statistical approaches (ANOVA, PAM) as well as quantitative real-time PCR studies on a selected subset of genes (vnn1, rph3a, tpsab1/b2, coch) verified the validity of the obtained data. Furthermore, the potential 128-gene predictor was tested on two independent patients with primary resistance that became accessible after having completed the study. Both test set patients were correctly assigned to their respective diagnostic group. Prospectively, our candidate predictor will be further explored on samples from CML patients currently treated in clinical studies.

Conclusions. This study establishes a candidate 128-gene predictor for early assessment of primary cytogenetic response of CML patients to imatinib. The data suggest that transcriptional regulation of genes related to apoptosis, disease progression, oxidative stress, DNA repair and centrosome fidelity are associated with imatinib resistance in chronic phase CML.

**0162**

**MONITORING SERUM LEVELS OF IMATINIB AND HAEMATOLOGICAL TOXICITY IN CML PATIENTS**


Ramón y Cajal Hospital, MADRID, Spain

Background. The development of imatinib mesylate (Glivec®) has been a major advance in the management of chronic myelogenous leukemia (CML). Although several clinical trials, revealed neutropenia, thrombocytopenia and/or anaemia in a proportion of patients on imatinib treatment, little is known about the potential association of these adverse effects and the serum levels of imatinib. *Aims*. We have developed a new method to analyze serum concentrations of imatinib mesylate and its active metabolite, N-demethyl-imatinib in Ph+CML patients. The aim of our study is to determine whether there is an association between serum levels of the drug and the development of either anaemia, thrombocytopenia or neutropenia, in order to establish an optimal therapeutic range.

Methods. We used a Beckmann Gold HPLC system in reverse phase under isocratic conditions, coupled to a visible ultraviolet detector fixed at 240 nm wavelength. We analyzed serum concentrations of imatinib and N-demethyl-imatinib in 114 samples from 36 patients with Ph+CML at different intervals. There were 19 males and 17 females, with a mean age of 57±15 years. Each patient was followed for at least six months. *Results*. Serum levels of imatinib showed a wide variation ranging from 0.43 ng/mL to 5.19 µg/mL. There was statistical correlation between therapeutical dose of Glivec® and serum concentration of the drug (r=0.47). Anaemia was present in 21 cases (58.3%), neutropenia in 3 cases (8.3%) and thrombocytopenia in 6 patients (16.5%). Patients with anaemia showed a mean concentration of imatinib of 2.10 µg/mL, compared to 1.41 µg/mL in non anaemic patients (P=0.015). Significantly higher levels of imatinib were also observed in patients with thrombocytopenia (2.60 µg/mL vs 1.70 µg/mL, p=0.047). No significant differences were observed with respect to neutropenia. Serum concentrations of imatinib rise above 3 µg/ml, the incidence of adverse events is high, and, therefore, the dose should probably be reduced. Taken together, our results demonstrate the feasibility and utility of monitoring serum levels of imatinib in CML patients. An optimal therapeutic range of between 0.57 µg/mL and 2 µg/mL is suggested.
0164
EARLY OR LATE COMPLETE MOLECULAR REMISSIONS IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS TREATED WITH IMATINIB RELY ON THE SPEED TO ACHIEVE A COMPLETE CYTOGENETIC REMISSION STATUS AND ON EARLY QUANTITATIVE BCR-ABL LEVELS

E.E. Nicollini, E. Bachy,1 S. Cormi,2 C. Plesa,3 O.H. Lê,4 S. Hayette,4 C. Preudhomme,1 M. Michallet1
1Édouard Herriot Hospital, LYON, France; 2Hôpital Huriez, LILLE, France; 3Centre Hospitalier Lyon Sud, PIERRE-BENITE, France

In chronic phase (CP) chronic myelogenous leukemia (CML) treated with imatinib (IM), the majority of patients that are in CCR still harbour, apparently indefinitely, a stable molecular disease, while IM is maintained. A small proportion of patients become RT-PCR negative (transcript levels <10-5) and there is still some controversy to know if these patients are cured. We retrospectively analyzed a cohort of 37 patients that became, at least once, BCR-ABL negative in the blood (Tag/man technology, sensitivity threshold 10-5-10-6, exchange of quality control samples between the 2 laboratories involved), to try to identify predictive factors for early [<12 Months, Group 1 (G1), 19 patients] versus late [>12 Months, Group 2 (G2), 18 patients] complete molecular remissions (CMR), in an univariate and multivariate analysis. Allogeneic transplantated patients were excluded from this study. All patients were in CP at diagnosis with 2 (1 in accelerated phase diagnosed on cytological criteria (G1), and 1 in myeloid blast phase (G2)). All patients had a ‘M-BCR transcript, and were 90-100% Ph+ at diagnosis, except 1 (61% Ph+ G1)). One patient had a masked Ph1 and had a variant [t(9;22), (G1)]. There were 19 males and 18 females with a median age of 52 (G1) and 50 (G2). Some patients have been treated with IFN prior to IM (12/19 in G1 and 13/18 in G2). All patients received IM 300-600 mg/day and some of them in association with Cytarabine (2 in G1; 2 in G2), PegIFN (1 in G1; 1 in G2), or daunorubicine + cytarabine (Patient in blastic phase). Two patients were in CMR, after IFN, when IM was started for IFN intolerance. None of the patients was in CMR at 3 Mo except 2 in G1. CMR appeared after a median interval of 6 Mo in G1 (0-11.2) and 25 Mo in G2 (12-53.7). Univariate analysis did not find any difference for pt or treatment by IFN, Sokal score and associations with Cytarabine or PegIFN. Analysis of variance indicated that a low RT-PCR value at 6 Mo and at 12 Mo was a significant factor for early CMR (p=0.03 for both). Multivariate analysis by logistic regression for Sokal score, prior treatment with IFN, initial IM dose had no influence on the early or late CMR status, whereas the interval between diagnosis and IM onset (p=0.04, HR=1.45 [1.01-2.08]), probably because there was a longer exposure to IFN; and a shorter time to reach a CCR status were significantly associated with early CMR (p=0.05, HR=0.67 0.46-0.97). However, the duration of IFN treatment variable was not significant by itself. With a median follow-up since IM onset of 24 (G1) and 43 (G2) Mo, all patients are alive, 16 out of 19 in G1 and 15 out of 18 in G2, in a stable CMR. In conclusion, when IM induces early CCR, and a quick reduction of BCR-ABL transcripts initially, CMR can be obtained within a year, but CMR are still present after a longer period. However gain in progression free survival remains to be demonstrated.

0165
THE IMPACT OF NON-COMPLIANCE WITH IMATINIB THERAPY ON HEALTH CARE COSTS IN CHRONIC MYELOID LEUKEMIA PATIENTS

W. Feng,1 J. Henk,1 K. Thomas,1 J. Baladi,1 A. Hatfield,1 A. Goldberg,2 J. Cortes1
1Novartis Pharmaceuticals Corporation, FLOREHM PARK, USA; 2I3Magni
tie, An Ingenix Company, EDEN PRAIRIE, USA; 3MD Anderson Cancer Center, HUSTON, USA

Background. While compliance to drug therapy is vital to receive optimal patient benefits, the costs of delivering adequate medical care for cancer patients remain an important consideration for society and payers. Aims. This study examined the relationship between compliance with imatinib therapy and health care costs for patients with chronic myeloid leukemia (CML). Methods. Claims data from a large US health plan were used to identify imatinib-treated patients from 6/1/01-3/31/04 who had continuous pharmacy and medical benefits in the 3 months prior and 12 months following initiation of imatinib therapy, and a diagnosis of CML (ICD-9-CM 205.1, 205.10, or 205.11). Compliance was defined by medication possession ratio (MPR−total days imatinib supplied in the first year divided by 365) and patients were stratified into three segments by MPR (<50%, 50-90%, 90-100%). Total health care costs include hospital, office, laboratory testing, emergency room, and pharmacy. Disease-related health care costs to treat CML were also analyzed. Multivariate analyses were used to examine the association between first-year MPR and first-year health care costs, controlling for age, sex, number of other medications used by the patient, initial starting dose, year of initial imatinib fill, and complications due to underlying disease. Results. Total 878 imatinib-treated patients were identified of whom 413 had at least 15 months of continuous eligibility. Of these, 277 were non-Medicare CML patients. Total health care costs per patient in the first year of therapy in patients with MPR <50%, 50-90%, and 90-100% were $17,277, $54,497, and $41,319 respectively (p<0.001). Inpatient care was the leading driver of health care costs followed by medication use and ambulatory care. The corresponding numbers for disease-related health care costs were $11,926, $35,856, and $34,964 (p<0.001). Controlling for the variables listed above, the multivariate analyses demonstrated that a 10% increase in MPR was associated with a 4.7% decrease in total health care costs (p=0.032) and 2.4% decrease in disease-related health care costs to treat CML (p=0.057). For example, when MPR was improved from 75% to 85% for a patient, the annual total health care costs for the patient were reduced by $3,100 and the annual CML related health care costs went down by $1,200. Conclusions. Improved compliance with imatinib therapy is associated with decreased total health care costs and disease-related health care costs. Improving compliance to imatinib therapy may not only optimize clinical outcomes but also reduce the overall societal burden of health care costs associated with cancer.

0166
MOLECULAR RESPONSE TO IMATINIB IN EARLY CHRONIC PHASE VERSUS LATE CHRONIC PHASE CML PATIENTS IN COMPLETE CYTOGENETIC RESPONSE: A COMPARISON AT 24 MONTHS OF 2 CLINICAL TRIALS OF THE GIMELA-CML WP

A. Poerio,1 G. Rosti,1 M. Amabile,1 I. Iacobucci,1 S. Soveni,1 S. Colarossi,1 A. Gnani,1 E. Ottaviani,1 C. Terragna,1 T. Grafone,1 S. Luotti,1 F. Castagnetti,1 F. Pane,1 G. Saglio,1 M. Baccarani,1 G. Martinelli1
1Inst.of Hematology Sergnoli, BOLOGNA, Italy; 2Inst of Hematology Sergnoli, BOLOGNA, Italy; 3Cengea Advanced Biotechnologies, NAPLES, Italy; 4Div of Hematology and Internal Medicine, TURIN, Italy

Background. The introduction of imatinib (IM) has changed the current approach to the management of chronic myeloid leukemia (CML). It is currently unclear whether patients (pts) treated with IM first-line treatment have a greater reduction of BCR-ABL transcript with respect to pts treated with IM after IFN-α failure, giving the same complete cytogenetic response (CCR). Aims. We sought to determine the differences in molecular response (MR) between early and late chronic phase (CP) pts with CML who achieved a CCR after treatment with IM at the standard dose of 400 mg/d. We studied 2 different cohorts of pts in CCR: - 67/191 (35%) pts after α-Interferon (α-IFN) failure enrolled on the CML/002/STI571 protocol - 53/76 (70%) pts treated front line with a combination of IM and peglated IFN-α (PEG-IFN) enrolled on the CML/011/STI571 protocol. Methods. Cytogenetic response was monitored on bone marrow (BM) metaphases and MR was assessed by real time RT-PCR (TaqMan) BM and peripheral blood (PB) samples, collected at baseline, 3, 6, 9 and 12 months during the first year, and every 6 months thereafter. MR was expressed as the ratio between BCR/ABL and β2-microglobulin (β2-M) x100. The lowest level of detectability of the method was 10-5. Negative results (i.e. undetectable transcript) were confirmed by nested PCR performed 4 times (sensitivity 10^-6). For the purpose of this analysis, a major molecular response (MMR) was defined as a BCR-ABL/β2M value <0.0001%, which turned out to be roughly equivalent to a 3-log reduction and a complete molecular response (CMR) was defined as negative (undetectable) BCR/ABL levels confirmed by nested PCR.
Results. We observed a progressive decrease of the amount of BCR/ABL transcript in pts who achieved CR. At 24 months the median reduction in BCR/ABL transcript level was: a 3-log reduction in late CP pts; a 4-log reduction in early CP pts. In the latter group of pts MR was assessed also at 36 months. So we observed that 36 months after the first dose of IM and PEG-IFN pts who were still in CCR had the median value of BCR/ABL transcript of 0.00001% both in BM and PB. Therefore all these pts achieved a CR (0). However only 52% (44/84) pts were in CMR (undetectable BCR/ABL at least once as assessed by nested PCR). Conclusions. Although after 24 months of therapy front line treatment of CML pts with IM determines a major percentage of CCR in comparison of pts treated with IM after IFN failure (in our experience, 70% versus 35%, respectively) the difference in MR (reduction in BCR/ABL transcript level) observed in the 2 groups of pts were not significant. Nevertheless excellent results were obtained in both groups, with a median reduction in BCR/ABL transcript level of at least 5 log. In the pts treated with a combination of IM and PEG-IFN a further reduction of BCR/ABL transcript (about another log) was observed at 36 months of treatment.


0167
HOMOHARRINGTONINE IS ASSOCIATED WITH A HIGH PROPORTION OF HEMATOLOGIC RESPONSE IN CML PATIENTS WITH HEMATOLOGIC FAILURE TO IMATINIB
P.R. Rousselet,^1 A.S. De Sarrazin,^1 J.M.C. Cayuela,^2 O.M. Maarek,^2 M.P.G. Gaub,^2 E.M. Maloise^1
^1Hôpital Mignot, LE CHESNAY, France; ^2Hôpital Saint-Louis, PARIS, France; ^3Hôpital Hautepiere, STRASBOURG, France

Background and Aims. Imatinib mesylate (IM) is now the gold standard for first line therapy in patients with chronic myelogenous leukemia (CML). Homoharringtonine (HHT) is an alkaloid obtained by an original hemi-synthetic process from Cephalotaxus. HHT having hematologic toxicity reported. grade 4 hematotogic toxicity , 6 of them presenting anemia requiring therapy may still be of interest for treating CML patients in the tyrosine kinase inhibitors era in case of resistant disease. Indeed, hematologic response, aiming so to determine its role in the current context of the disease management. Results. In total, 15 CML (CP, n=10 and AP, n=5) patients started HHT between 09-2000 and 10-2004 for lack or loss of hematologic response in the two institutions. Main previous therapy was interferon α (IFN, n=6, including 3 patients with IFN + Ara-C), or IM (n=9, including IM 400 mg per day for 8 patients, and IM 600 mg per day for 1 patient). Median age at the first HHT treatment was 57 years (range: 38-70). Median time from diagnosis was 49 months (range: 18-121). HHT was administered at 1.25 to 2.5 mg/m^2 daily dose, as a continuous 24h infusion or as 2 divided doses via the subcutaneous route. The course durations were maximum 6 days when combined to IM (n=5) or Ara-C (n=7), and up to 14 days when used as a single therapy (n=5). Of these 15 patients, 11 (73.3%) achieved a complete hematologic response (CHR) after 2 courses in median (range: 1-6), including 1 patient in accelerated phase and 5 patients with BCR-ABL kinase domain (KD) mutations (E255K, M244V, F317L + K247R, V244Q; Y255H). Responding patients received HHT as a single therapy (n=5), or combined with IM (n=3) or Ara-C (n=5). One additional patient who was in AP returned to chronic phase after 2 HHT + Ara-C courses. There was no hematologic response in the 3 remaining patients. Two of them received HHT as a single therapy and 1 received HHT + Ara-C. Among the patients detected with a BCR-ABL KD point mutation, all CP patients obtained CHR and the mutated patient in accelerated phase (Q252H) did not respond to HHT. Nine patients (60%) experienced grade 4 hematologic toxicity, 6 of them presenting anemia requiring blood transfusion (9 occurrences). There was no significant extra-hematologic toxicity reported. Conclusion. HHT as a single or a combination therapy may still be of interest for treating CML patients in the tyrosine kinase inhibitors era in case of resistant disease. Indeed, hematologic responses to HHT have been obtained in case of BCR-ABL KD mutations. The role of HHT alone or in combination will be refined with well-designed prospective studies.

0168
ACETYLEMONE AND PHOSPHOPROTEOMIC MODIFICATIONS OF IMATINIB SENSITIVE AND RESISTANT CML CELLS AFTER SHORT CHAIN FATTY ACID HISTONE DEACETYLASE INHIBITOR TREATMENT.
V. Santini, R. Pastorelli, G. Ferrari, A. Gozzini, D. Tombaccini
University of Florence, FIRENZE, Italy

Background. CML patients may become irresponsive to Imatinib because of resistance developed by amplification of the BCR-ABL genomic locus or by point mutations within the kinase domain of BCR-ABL. Innovative dual src/ABL kinase inhibitors with higher power additive native and imatinib-resistant mutants of BCR-ABL give remarkable therapeutic benefits, but at least one mutation remains resistant to any kinase inhibitor (T315I). Given these evidences, the investigation of alternative therapeutic agents effective in CML still remains a subject of primary interest. Aims. We analysed whether HDACIs short chain fatty acids (SCFAs) valproic acid and butyrate were synergistic with imatinib. SCFAs acetylation of non-histone proteins is not well characterized; we thus compared the acetylome and phosphoproteome of CML cells treated and not treated with HDACIs, alone and in combination with imatinib, by immunoproteomic techniques. Methods. The human CML cell lines K562, LAMA-84 S (Imatinib sensitive) and LAMA-84 R (HDACsb resistant), KBA-R and -S were in the presence of valproic acid at the escalating doses 0.2 mM to 2 mM or in the presence of butyric acid derivative D1 (0.2-1 mM) for 24 and 48 hrs. Apoptosis was monitored by annexin V test and propidium iodide uptake. Bcr-abl mRNA was measured by real time PCR. Bcr-Ab1 protein expression was determined by western blot with specific antibodies. Total cell proteins were separated by 2D electrophoresis (pH 3-11). We used a pan-pan-acetylated and anti- phospho-tyrosine antibody for 2D WB, followed by matching with 2D gel and MALDI-TOF mass spectrometry for protein identification. Results. Apoptosis was induced time and dose dependent by VPA and D1. Imatinib was synergistic with both HDACIs in inducing apoptosis and cell proliferation arrest (WST-1-assay). VPA and D1 were able to induce a significant decrease in the number of copies of Bcr- abl mRNA both in sensitive and in resistant cells. A significant decrease in BCR-ABL protein expression was observed by WB of total cell lysates from CML cells. Twenty two proteins differentially acetylated were identified. At least two chaperone proteins were identified as target of acetylation after VPA and D1 treatment of CML cells, other targets were proteins involved in the synthesis and stability of RNA. Sixteen proteins differentially phosphorylated were identified. For 13 of these proteins the phosphorylation level was not significantly affected by HDACIs in resistant cells, while the combination of both Imatinib and HDACIs produced a considerable decrease of phosphorylation in both sensitive and resistant cell lines. This category includes: HSP90, HSP70, HOPI and nucleophosmin. Summary/Conclusion. Short chain fatty acids are not the most powerful HDACIs, but have been used successfully in clinical trials. Our analysis show significant evidences of their effects on CML cells in terms of induction of apoptosis and arrest of CML cell proliferation arrest. Further effects were observed on Imatinib expression and modifications on both acetylation and phosphoproteome. This study characterises proteome modifications provoked by SCFAs and may help to understand the molecular effects of different HDACIs in order to improve their use in combination with imatinib or new SRC/ABL inhibitors.

0169
IMATINIB THERAPY FOR CHRONIC MYELOID LEUKEMIA PATIENTS WHO RELAPSE AFTER ALLOGENIC STEM CELL TRANSPLANTATION: A MOLECULAR ANALYSIS
E. Falardi,^1 M. Amabile,^1 G. Bandini,^1 E. Benedetti,^2 M. Usala,^4 E. Angelucci,^1 M. Tiribelli,^2 F. Ranin,^G. Rosti,^4 G. Martinelli,^2 M. Baccarani^2
^1University of Bologna, BOLOGNA, Italy; ^2Istituto of Hematology L. e A. Seràgnoli, BOLOGNA, Italy; ^3BMIT Unit, Azienda Ospedaliera di Verona, VERONA, Italy; ^4Divisione di Ematologia, CAGLIARI, Italy; ^5Istituto di Ematologia, UDINE, Italy

Background. Allogeneic stem cell transplantation (SCT) is date to the only curative therapy for CML patients, but relapse is still one of the major causes of failure. Discontinuation of the immunosuppressive therapy and donor lymphocyte infusion (DLI) is the treatment of first choice in a substantial number of patients. Nonetheless, it requires the availability of the donor and may be associated with severe graft-versus-host-

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In order to better define the role of imatinib in CML recurrence after allografting. Aims. In order to better define the role of imatinib in this setting, we report an extended molecular follow-up of 16 CML patients treated with imatinib while in relapse after allogeneic SCT. Methods. Patients underwent allogeneic, non T-cell depleted, standard conditioning regimen SCT. At evidence of relapse, 5 patients were in immunosuppressive therapy, which was discontinued. At start of imatinib, five patients were in hematological relapse, Nine had a cytogenetic relapse and two a molecular relapse. Results. Median follow-up is 33 months (range 12-45). All patients achieved a complete cytogenetic response (CCgR) within 12 months. Molecular response was evaluated in bone marrow and/or peripheral blood samples at start of imatinib therapy and every 3 months during treatment by a standardized real-time quantitative PCR (RTQ-PCR) method and/or by qualitative nested PCR. Complete molecular response (CMR) was defined as reduction of BCR-ABL/B2 Microglobulin below 0.00001 or negativity of qualitative nested PCR in bone marrow samples. Eight patients achieved and maintained a stable CMR. In seven patients, CMR has been achieved but lost at least once during follow-up. In these patients, median duration of longer CMR was 12 months (range 3-24). All patients are in ongoing treatment with imatinib except for one patient who discontinued the therapy 6 months ago and maintain a CMR. No further treatment was administered in all but two patients, who received DLI after the achievement of CMR. Conclusions. In our experience, response rate to imatinib is comparable with that expected from treatment with DLI alone. All patients achieved cytogenetic and molecular responses, which were associated with reconstitution of full donor chimerism without increasing of GVHD. Moreover, no major side effects were observed. Although no direct comparison may be made, the data suggest that in our patients percentage of and time to molecular response to imatinib appeared to be better than in newly diagnosed CP CML patients, possibly due to residual GVL effect. An important observation is that even patients relapsed in advanced phase of disease obtained durable molecular responses (median duration of CMR: 20 months, range 6-24). Compared to other therapeutic approaches, our experience confirms that imatinib is effective and feasible, with a very high overall response and a manageable side-effects profile, at least in the short-term.

**0170**

**INFLUENCE OF CYP3A4 ACTIVITY ON IMATINIB RESPONSE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA**

H. Gréen,1 K. Skoglund,1 F. Rommel,1 L. Bertillon,2 K. Lotfi1

1Linköping University, LINKÖPING, Sweden; 2Karolinska Institute, STOCKHOLM, Sweden

Background. Imatinib, also known as Gleevec/Glivec, is a potent BCR-ABL tyrosine kinase inhibitor currently used for the treatment of chronic myeloid leukemia (CML) patients. Imatinib induces complete cytogenetic responses (CCR) in the majority of patients with CML in chronic phase (CP). However, a subgroup of patients is refractory at the cytoge

**Aims.** The aim of this study was to investigate the role of the drug metabolising enzyme i.e. the CYP3A4 activity in vivo, for the response to imatinib treatment. Methods. 16 chronic myeloid leukemia patients were included in the study. To assess the in vivo CYP3A4 activity the patients were given 250 mg of quinine, 16 h later a blood sample was collected and the ratio of quinine/3-hydroxyquinine was measured using HPLC. The response of imatinib treatment was measured by cytogenetic analysis and the patients were divided into CCR within nine months and partial cytogenetic responders (PCR) who had failed to achieve a CCR. Results. Patients with PCR showed significantly (Mann-Whitney U-test, p=0.013) higher CYP3A4 activity (low Quinine ratio, mean = 9.4, SD = 2.7) compared to patients that were PCR (high Quinine ratio, mean = 8.1, SD = 0.5), see figure. Conclusions. CML patients with high CYP3A4 activity respond better to imatinib treatment than patients with low activity. Clinically, it would be advantageous to identify such patients a priori, since they may benefit from more aggressive therapy. This also indicates that the effect and potency of the metabolites might be of clinical importance.

**0171**

**IMATINIB 400 MG IN LOW SOKAL RISK CML PATIENTS: EARLY RESULTS OF AN OBSERVATIONAL, MULTICENTRIC TRIAL OF THE GIMEMA CML WP**

G. Rosti,1 F. Castagnetti,1 I. Iacobucci,1 N. Testoni,1 M. Breccia,1 F. Palandini,1 G. Specchia,1 B. Martino,1 S. Luatti,1 A. Capucci,1 A. Poerio,1 R. Bassan,1 F. Pane,1 G. Saglio,1 G. Martinelli,1 M. Bacciarni1

1University of Bologna, BOLOGNA, Italy; ‘Chair of Hematology, UNIVERSITÀ LA SAPIENZA’ - ROMA, Italy; Division of Hematology, REGGIO CALABRIA, Italy; ‘Cenege, NAPOLI UNIVERSITY FEDERICO II, Italy; Dpt of Clinical and Biological Sciences, TORINO UNIVERSITY, Italy

Background. Imatinib 400 mg is the established first line treatment of chronic myeloid leukemia (CML) in chronic phase. The efficacy of imatinib in early chronic phase has been demonstrated by multicentric randomized controlled trials like the IRIS trial (O’ Brien et al NEJM 348:11, 2004). Large multicentric studies aimed to evaluate the impact of imatinib 400 mg outside strictly monitored trials are not yet available. Aims. The GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Adulto) CML Working Party opened in January, 2004, an observational study (serial n. CML 023) to investigate the efficacy of imatinib 400 mg in newly diagnosed CML patients. Methods. Clinical and anagraphical data were collected through a web-based system. Responses were evaluated at fixed time-points during treatment. Hematologic: continuously; cytogenetic at 6 and 12 months (local labs); molecular response at 3, 6 and 12 months. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR, Bcr-Abl/Abl × 100 - Taqman) were centralized in Bologna at 3, 6 and 12 months. Patients. Overall, 54 italian centers enrolled 209 (188 evaluable) low Sokal risk patients between January 1, 2004 and November, 2005. Median age was 44 yrs (range 20-69), 117 male and 71 females. 58 patients were evaluable for response at 3 months, 151 at 6 months and 84 at 12 months. The median observation time is 6 months. Results. At 3 months, 95% of the patients reached a stable complete hematologic response. At 6 months, 81% of the evaluable cases obtained a complete cytogenetic response (100% Ph-neg, CCGR). A major molecular response (MMR) defined as a Bcr-Abl/Abl × 100 ratio < 0.1%, was shown in 51% of CCGR patients. At 12 months, the CCGR rate was 88% and the MMR rate in CCGR patients was 57%. At 12 months, 4% of CCGR cases showed a undetectable level of transcript (ratio Bcr-Abl/Abl × 100 < 0.00001). With this short observation period, only 1 pt progressed to accelerated/blastic phase, while 2 patients were censored at the time of allogenic stem cells transplantation. SUMMARY AND Conclusions. The preliminary evidences of our observational trial confirm that imatinib 400 mg is a highly effective treatment for CML in early chronic phase, as far the CCGR and MMR response rates. 201 low Sokal risk patients were enrolled in the IRIS trial and received imatinib as first line treatment. The CCGR rate within 12 months was 76% with 66% of patients reaching a MMR (defined as reduction of Bcr-Abl transcript level > 3 logs; control gene Bcr) (T Hughes et al., NEJM 349:15, 2003). Our results (81% and 57% MMR rate at 6 and 12 months, 51% and 57% MMR at 6 and 12 months) compare favourably with the IRIS trial results.


Figure 1. CYP3A4 activity and Imatinib response.
O172 IMATINIB AND AGING: PRELIMINARY RESULTS OF A SUB-ANALYSIS WITHIN 3 TRIALS OF THE GIEME MA CML WP

F. Castagnetti,1 G. Rosti,1 I. Iacobucci,1 N. Testoni,1 M. Amabile,1 E. Usala,1 D. Russo,1 E. Orlandi,1 S. Soverini,1 E. Montefusco,1 G. Rege Cambrin,1 S. Rupoli,1 G. Martellini,1 F. Pane,1 G. Saglio,1 M. Baccarani1

1Division of Hematology, OSPEDALE BUSINO - CAGLIARI, Italy; 2Department of Clinical and Biological Sciences, TORINO, Italy; 3Institute of Hematology 'Sergio', BOLOGNA, Italy; 4Geige, NAPOLI, Italy

Background. Older age constitutes a poor prognostic variable in Ph+ CML. This study assessed whether high BCR-ABL expression and the functional consequence of expressing CCN3 in BCR-ABL+ cells were statistically different between younger and older patients. Methods. A sub-analysis within 3 simultaneously running trials of the GIEMEA (Gruppo Italiano Malattie Ematologiche del L’Adulti) CML WP (n.CML/02, phase II - ima 800 in intermediate Sokal risk; CML/022, phase III- ima 400 vs 800 mg in high Sokal risk, n. CML/023, observational - ima 400 mg) have been performed. Overall, 454 patients have been enrolled (January 2004 - November 2005). At enrollment 85/404 (21%) were ≥65 yrs (median age 71, range 65-85) and 319/404 (79%) <65 yrs (median age 46, range 18-64). Sokal risk distribution was different between the 2 groups: low Sokal risk cases were 15% in older cohort vs 54% in younger cohort (p=0.01); 21% of older and 22% of younger pts received high dose (800 mg) of imatinib front-line. Timing of response evaluation: hematologic, continuously; cytogenetic, at 6 and 12 months; molecular, at 3, 6 and 12 months. Results. The numbers (%) of evaluable cases (older/younger) at 3, 6 and 12 months were: 85/319 (27%), 27/141 (21%) and 22/66 (36%). At 3 months, both groups achieved a 93% complete hematologic response (CHR) rate. At 6 months, the complete cytogenetic response (CCGr) rate was 66%/80% (p=0.39). The major molecular response (MMR, defined as a Bcr-Abl/AbI × 100 ratio <0.1%) rates (CCGr only) were 67%/49% (p=0.08). At 12 months, CCGr rates were 81%/82% (p=0.67) and MMR 50%/60% (p=0.04). With a median observation time of 6 months, 1 pt (1%) of older cohort and 4 (1%) of younger cohort progressed to accelerated/blast phase. Summary and Conclusions. This sub-analysis was generated from 3 trials with different aims and dosages of imatinib. The observation period is still short. However, it is noteworthy that notwithstanding a worsen risk distribution of older cases (15% low risk vs 54% for younger), results at 6 and 12 months are comparable. The only significant difference was demonstrated for MMR at 12 months. Consequently, we may foresee that the long-term survival and progression free survival will not differ between the 2 groups. Acknowledgments. Supported by: COFIN 2003, FIRB 2004, A.I.R.C., C.N.R., Fondazione Monte di Bologna e Ravenna, LeukemiaNet, A.I.I.

O173 BCR-ABL REDUCES CCN3 EXPRESSION THEREBY EVADING NEGATIVE GROWTH REGULATION

L. McCallum,1 S. Price,1 W. Lu,3 N. Planke1; B. Perbal,5 A.D. Whetten,3 A.E. Irvine1

1Queen’s University Belfast, BELFAST, United Kingdom; 2Univiersité Paris, PARIS, France; 3University of Manchester, MANCHESTER, United Kingdom; 4Dpt of Clinical and Biological Sciences, TORINO, Italy; 5Chair of Hematology, BRESCIA, Italy

Background. Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active BCR-ABL tyrosine kinase. Consequently, we have identified downregulation of the negative growth regulator, CCN3, as a result of BCR-ABL kinase activity and detected reduced CCN3 expression in human CML cells and primary human CML cells. Aims. To identify the relationship between BCR-ABL and CCN3 expression and the functional consequence of expressing CCN3 in BCR-ABL+ cells. Methods. Real-time PCR was used to examine the relationship between BCR-ABL and CCN3 expression in human K562 cells using siRNA directed against BCR-ABL or the BCR-ABL tyrosine kinase inhibitor, Imatinib. CCN3 function was investigated in K562 cells transfected with vector or vector containing CCN3 by assessing cell growth using flow cytometry and colony formation on methyl cellulose. Results. Parental K562 cells showed high expression of BCR-ABL whilst CCN3 expression was present at low levels. Treatment with siRNA directed against BCR-ABL resulted in a 3.7 fold decrease in BCR-ABL and 6.1 fold increase in CCN3 expression (mean Ct change 1.9±0.2 and 2.6±0.5 for BCR-ABL and CCN3 respectively; n=3, p=0.001). Similarly, BCR-ABL was associated with increased promoter ABL activity which confirmed a 5.9 fold decrease in BCR-ABL expression and a 4.2 fold increase in CCN3 expression (mean Ct change 2.5±0.1 and 2.1±0.2 for BCR-ABL and CCN3 respectively; n=3, p=0.001). To investigate CCN3 function, we expressed CCN3 in BCR-ABL expressing cells. K562 cells were transfect- ed with the pC86+ vector or pCase vector containing the CCN3 expression construct. Cell cycle analysis was performed: CCN3 expression in BCR-ABL+ cells resulted in an accumulation of cells in the subG0 phase of cell cycle, indicative of cell death (mean for subG0 9.9±4.6 and 21.8±0.7 for the pC86+ vector alone and pC86+ vector containing CCN3 construct respectively). In addition, CCN3 expression reduced the clono- genetic capacity of BCR-ABL+ cells. K562 cells transfected with vector containing CCN3 construct formed significantly fewer colonies on methyl cellulose in comparison to cells that had been transfected with the pC86+ vector alone (n=3, p=0.027). Conclusions. This study demonstrates a reciprocal relationship between CCN3 and BCR-ABL expression. CCN3 is known as a negative growth regulator and increased expression of CCN3 in BCR-ABL+ cells inhibits proliferation and decreases clonogenic potential. Thus CCN3 down-regulation mediated by BCR-ABL offers growth advantage to hematopoietic cells.

O174 THE PERSISTENCE OF P190 BCR-ABL TRANSCRIPTS IS ASSOCIATED WITH LOWER PROBABILITY OF MOLECULAR RESPONSE TO IMATINIB IN EARLY AND LATE CHRONIC PHASE CML PATIENTS

I. Abarci,1 A. Garuti,2 S. Pozzi,1 G. Cimerna,1 E. Bertolotti,1 M. Miglino,1 N. Sessarego,2 R. Vittido,1 N. Colombo,1 M. Podestà,1 M. Gobbi,1 M. Sessarego,1 F. Patrone,1 F. Frassoni,1

1Ospedale San Martino, GENOVA, Italy; 2Clinica Medica, GENOVA, Italy; 3Clinica Ematologica, GENOVA, Italy; 4Seminario Medica, GENOVA, Italy

Background. It has been demonstrated that the about 70% of p210CML patients in chronic phase (CP) at diagnosis co-expressed p190 BCR-ABL transcripts and 2) during the treatment with imatinib in 2 different groups, those with previously untreated disease (early CP-CML) and those who previously failed IFN therapy (late CP-CML). The aim of the study was to monitor in CML pts <65 yrs old, who were treated with either the pCb6+ vector or pCb6+ vector containing the CCN3 construct. Cell cycle analysis was performed: CCN3 expression in BCR-ABL+ cells resulted in an accumulation of cells in the subG0 phase of cell cycle, indicative of cell death (mean for subG0 9.9±4.6 and 21.8±0.7 for the pC86+ vector alone and pC86+ vector containing CCN3 construct respectively). In addition, CCN3 expression reduced the clono- genetic capacity of BCR-ABL+ cells. K562 cells transfected with vector containing CCN3 construct formed significantly fewer colonies on methyl cellulose in comparison to cells that had been transfected with the pC86+ vector alone (n=3, p=0.027). Conclusions. This study demonstrates a reciprocal relationship between CCN3 and BCR-ABL expression. CCN3 is known as a negative growth regulator and increased expression of CCN3 in BCR-ABL+ cells inhibits proliferation and decreases clonogenic potential. Thus CCN3 down-regulation mediated by BCR-ABL offers growth advantage to hematopoietic cells.

O173 BCR-ABL REDUCES CCN3 EXPRESSION THEREBY EVADING NEGATIVE GROWTH REGULATION

L. McCallum,1 S. Price,1 W. Lu,3 N. Planke1; B. Perbal,5 A.D. Whetten,3 A.E. Irvine1

1Queen’s University Belfast, BELFAST, United Kingdom; 2Univiersité Paris, PARIS, France; 3University of Manchester, MANCHESTER, United Kingdom

Background. Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active BCR-ABL tyrosine kinase. Consequently, we have identified downregulation of the negative growth regulator, CCN3, as a result of BCR-ABL kinase activity and detected reduced CCN3 expression in human CML cell lines and primary human CML cells. Aims. To identify the relationship between BCR-ABL and CCN3 expression and the functional consequence of expressing CCN3 in BCR-ABL+ cells. Methods. Real-time PCR was used to examine the relationship between BCR-ABL and CCN3 expression in human K562 cells using siRNA directed against BCR-ABL or the BCR-ABL tyrosine kinase inhibitor, Imatinib. CCN3 function was investigated in K562 cells trans-
expression of p190 and p210 transcripts by CP-CML patients at diagnosis. However, some patients with low levels of p210 transcripts continue to display p190 expression. The persistence of p190 signals despite the 2-3-log fall in p210 BCR-ABL levels may be of prognostic value. The significance of the lack of correlation between p190 and p210 transcript levels warrants further investigations and may disclose unfolded biological relevance.

**0175**

**IMPACT OF BCR/ABL GENE EXPRESSION ON THE PROLIFERATIVE RATE OF DIFFERENT SUBPOPULATIONS OF HEMATOPOIETIC CELLS IN CHRONIC MYELOID LEUKEMIA**

D. Primo, J. Flores,1 S. Guijano,1 M.L. Sanchez,1 M.E. Sarasquete,1 J. Del Pino-Montes,1 P.L. Gaarder,1 M. Gonzalez,1 A. Orfao1

1Centro de Investigación del Cáncer, SALAMANCA, Spain; 2Servicio de Hematología, SALAMANCA, Spain; 3Servicios de Traumatología y Ortopedia, SALAMANCA, Spain; 4Department of Immunology, OSLO, Norway

**Background and Aims.** Despite the effects of BCR/ABL on cell proliferation, no study has been reported so far in which the proliferative rate of different hematopoietic cell compartments from chronic myeloid leukemia (CML) has been compared to normal bone marrow (NBM). In order to gain further insight into the potential impact of BCR/ABL gene expression on leukemic CML cells, we compared the proliferative rate of different BM cell subpopulations from CML patients and normal subjects and explored the correlation between the proliferation of each cell population from CML with BCR/ABL gene expression in highly-purified fractions of BM cells. Methods. A total of 26 BM samples corresponding to 15 patients diagnosed of CML and 11 NBM were studied. The proportion of S+G2/M cells was analyzed on CD45/CD19/DRAQ5, CD34/CD117/DRAQ5, CD11b/CD13/DRAQ5, CD56/CD14/DRAQ5 and HLA-DR/CD123/DRAQ5 stained BM cells. Fluorescence activated cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD).

**Results.** A total of 26 BM samples corresponding to 15 patients diagnosed of CML and 11 NBM were studied. The proportion of S+G2/M cells was analyzed on CD45/CD19/DRAQ5, CD34/CD117/DRAQ5, CD11b/CD13/DRAQ5, CD56/CD14/DRAQ5 and HLA-DR/CD123/DRAQ5 stained BM cells. Fluorescence activated cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAnia flow cytometer (BD) and the FACSDiVa software (BD).

**Discussion.** Our results suggest that each IAP homologue has a different mechanism of action and because more than one member of this family may be overexpressed in CML, successful treatment strategies for this disease will be defined by the ability to block all of the IAP expressed or to associate its inhibitor with other therapies.

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**0176**

**SURVIVIN AND CIAP-1 GENE EXPRESSION IS LINKED TO CHRONIC MYELOID LEUKEMIA PROGRESSION AND POOR RESPONSE TO IMATINIB**

F.A. Castro,1 J.F. Jacsyn,1 A.G. Ulbrich,1 F.R. Tobo,1 L.R. Lopes,2 D.L.C. Alves,3 M.A. Zanichelli,2 M.D. Colassanti,2 D. Camanho,2 C.A. Moreira-Filho,2 N. Hamerschlik,1 G.P. Amarante-Mendes1

1FCERP-USP, RIBEIRO PRETO, Brazil; 2Departamento de Hematologia, ICB-USP, SO PAULO, Brazil; 3IEP-S.B.I.B. Hospital Albert Einstein, SO PAULO, Brazil; 4Servicio Hematología-Hospital Brigadier, SO PAULO, Brazil

**Background.** Chronic myeloid leukemia (CML) is a myeloproliferative disease in which bcr-abl oncogene enhances survival of leukemic cells through modulation of proapoptotic and antiapoptotic molecules. The IAPs (inhibitor of apoptosis proteins) are a family of caspase inhibitors that block the execution phase of apoptosis. Overexpression of IAPs confers chemoresistance and, in some groups of cancer patients, is associated with a poor prognosis. Fully understanding the basic apoptotic pathway and its regulation in Bcr-Ab1-positive cells will unveil more targets for manipulation, which can be translated into novel therapies. Aim. The objective of this work was to determine the IAPs gene expression (ciap-1, ciap-2 and survivin) in 15 healthy individuals and on 71 CML patients (20 chronic phase, 15 accelerated phase, nine blastic phase, 20 in cyrogenetic remission and seven refractory patients post-imatinib). Methods. The iaps gene expression was performed in CML patient’s peripheral blood mononuclear blood by quantitative real-time RT-PCR. Results. The results are expressed by relative expression e.g. ratio of investigated gene to the reference GAPDH gene. survivin and ciap-1 gene overexpression was observed in 61 (86%, p<0.001) and 55 (77%, p<0.001) patients, respectively. The survivin levels (mean/SD) were: 0.11/0.10 in controls; 0.55/0.14 in chronic (CP), 2.13/0.55 in accelerated (AP), 9.63/3.3 in blastic (BP) phases, 0.04/0.02 in CML remission and 18.09/3.8 Gleevec-refractory patients. The ciap-1 expression was 17.6/3.53 in controls; 31.02/5.9 in CP, 15.75/2.6 in AP, 57.25/15.49 in BP, 32.8/5.1 in CML remission and 59.04/12.05 in Gleevec-refractory patients. Conclusion. There was an association between survivin (p<0.001) and ciap-1 (p<0.001) mRNA level with CML stage and response to imatinib. The ciap-2 gene expression observed in healthy controls and CML patients are similar (p>0.05). Taken together our results suggest that each IAP homologue has a different mechanism of action and because more than one member of this family may be overexpressed in CML, successful treatment strategies for this disease will be defined by the ability to block all of the IAP expressed or to associate its inhibitor with other therapies.

**Supported by:** CNPq, FAPESP, Instituto de Investigação em Imunologia-Instituto do Milênio/CNPq and IIEP-HIAE

**0177**

**ABL KINASE DOMAIN MUTATIONS ARE IMPORTANT MECHANISMS OF RESISTANCE IN ASIAN PATIENTS WITH IMATINIB-RESISTANT CHRONIC MYELOID LEUKAEMIA**


Singapore General Hospital, SINGAPORE, Singapore

**Introduction.** While the efficacy of imatinib in chronic myeloid leukemia (CML) is without doubt, resistance remains a problem, especially in the advanced phases. The most common mechanism of resistance is point mutations (leading to amino acid substitutions in the Abl kinase domain) and has been described mainly in the Western population. Little information is currently available if this mechanism is also common among Asian patients. Methods. Samples from 32 patients with suboptimal responses or progression on imatinib have been analysed so far. DNA was extracted from peripheral blood and complementary DNA synthesised using random hexamers. The Abl kinase domain was amplified using a two-step semi-nested reverse transcriptase/polymerase chain reaction (RT/PCR) and the PCR products subjected to direct sequencing. Results. The median age was 47 (19-77) years with a equal sex distribution. There were 15 Chinese, 9 Indian and 8 Malay patients. Thirteen patients were in the chronic phase (CP), 14 in accelerated phase (AP) and 5 in blast phase (BP). The median duration of imatinib treatment was 27 (7-65) months. A total of 20 mutations were detected in 14 patients, with 8 located in the ATP-binding loop, 4 in the imatinib-binding site, 2 in the catalytic domain and 2 in the activation loop. The remaining 4 were in other sites within Abl kinase, of which 3 were deletions (Tyr 492-Val 494) and 1 a new mutation will be verified by amplifying the ABL alleles to exclude polymorphisms. Four patients had more than one mutation. Of these 14 patients, 12 were enrolled into clinical trials with second generation Abl
kinase inhibitors (dasatinib, Bristol-Myers Squibb or AMN107, Novartis). Mutation testing was also performed in 6 patients after 3 months of dasatinib (see Table).

Table 1. Amount of shading = rel size of mutant clon.

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<th>Patient</th>
<th>Phase</th>
<th>Mutation pre-dasatinib</th>
<th>Mutation post-dasatinib</th>
<th>Response to dasatinib at 3 months</th>
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<td>1</td>
<td>AP</td>
<td>F350V</td>
<td>T315I</td>
<td>Haemat progression, no cytogenetic response</td>
</tr>
<tr>
<td>2</td>
<td>AP</td>
<td>T240A</td>
<td>G256E, G250E</td>
<td>Complete haem response, no cytogenetic response</td>
</tr>
<tr>
<td>3</td>
<td>AP</td>
<td>H369R</td>
<td>H369R</td>
<td>Complete haem response, no cytogenetic response</td>
</tr>
<tr>
<td>4</td>
<td>CP</td>
<td>E555K</td>
<td>E555K</td>
<td>Complete haem response, minor cytogenetic response</td>
</tr>
<tr>
<td>5</td>
<td>CP</td>
<td>E355G</td>
<td>E355G</td>
<td>Complete haem response, minor cytogenetic response</td>
</tr>
<tr>
<td>6</td>
<td>CP</td>
<td>E255V</td>
<td>E255V</td>
<td>Complete haem response, minor cytogenetic response</td>
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</table>

Haematologic progression was observed with the development of the T315I mutant in 2 patients and in 1 patient, a minor cytogenetic response was associated with a relative reduction in size of the mutant clone. Conclusions. Our study shows that Abl kinase mutations are common in Asian CML patients resistant to imatinib. Currently, mutation testing is only available in a few laboratories across the continent. It is therefore important that screening for mutations be performed routinely in imatinib-resistant Asian CML patients as this will have an impact on therapeutic decision making.

The degree of intratumoral neo-vascularization as well as the expression of some proangiogenic factors is of prognostic significance in some solid tumors. It is still to be elucidated the possible prognostic role of increased angiogenesis in patients with haematological diseases. The aims of the present study are: (1) to analyze the neo-vascularization of bone marrow in patients with chronic myeloid leukemia (CML); (2) an assessment of bone marrow cellular expression of Vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (KDR) as well as the plasma levels of VEGF in CML; (3) to analyze the correlation dependency of angiogenic factors with the prognostic and biologic markers of the disease. A totally of 37 patients with CML as well as 50 healthy individuals were analyzed for VEGF plasma levels by using ELISA technique. Immunohistochemical methods were applied to visualize the vascular structures as well as the VEGF/KDR cellular expression in 17 trephine biopsies from newly diagnosed patients with CML and in 15 normal bone marrows. We observed that the mean vessel count per field was 3.13 per 0.0625 mm² in normal bone marrows vs. 24.6 per 0.0625 mm² in CML (p<0.001).

Figure 1. Increased MVD in CML bone marrow (A) comparing to the normal (B) case. (Von WF immunostaining)

The VEGF/KDR cellular expression levels were nearly 5-fold that in normal control samples and the VEGF plasma levels were significantly higher in CML group (Mean 429 vs. 36.8 pg/ml; p<0.001). A good correlation was found between plasma VEGF and platelets as well as leucocytes but not with the blast per cent and Hasford prognostic score. Likewise, plasma VEGF levels could not predict the acceleration of the disease, moreover, their levels decline with the progression of CML. We found a good correlation between MVD and cellular VEGF/KDR expression but only cellular KDR is in a significant correlation with Hasford prognostic score. According to our results the high MVD, VEGF and KDR expressions indicate that angiogenesis is an inevitable event in the pathophysiology of CML. The complex angiogenic assessment of bone marrow provides more reliable information about the occurrence and the significance of this process in CML than using VEGF plasma concentration alone. That is to say, the precisely defined patients with CML, which have a high rate of angiogenic activity in bone marrow, could benefit by angio-suppressive therapy.
Non-Hodgkin’s Lymphoma - Clinical I

0179
A PHASE II, MULTICENTER, SINGLE-AGENT STUDY OF BENDAMUSTINE HCl IN PATIENTS WITH RITUXIMAB-REFRACTORY INDOLENT B-CELL NON-HODGKIN’S LYMPHOMA
A. Foroza-Torres,1 J. Foran,1 P. Cohen,2 B.D. Cheson,3 K.S. Robinson,3 L. Fayad,7 A.S. La Casce,4 A. Bessudo,5 E.S. Camacho,5 M.E. Williams,6 R.H. van der Jagt,7 J.W. Oliver,7 J.W. Friedberg1

1University of Alabama at Birmingham, BIRMINGHAM, AL, USA; 2George-town University Hospital, WASHINGTON, DC, USA; 3QEI Health Sciences Centre, HALIFAX, NS, Canada; 4MD Anderson Cancer Center, HOUSTON, TX, USA; 5Dana-Farber Cancer Institute, BOSTON, MA, USA; 6San Diego Cancer Center, ENCINITAS, CA, USA; 7Desert Regional Medical Center, PALM SPRINGS, CA, USA; 8University of Virginia, CHARLOTTESVILLE, VA, USA; 9Ottawa General Hospital, OTTAWA, ON, Canada; 10Cephalon, Inc., FRAZER, PA, USA; 11Wilmot Cancer Center, Uni of Rochester, ROCHESTER, NY, USA

Background. Bendamustine HCl (TREANDA™) is a novel, multifunctional, hybrid, cytotoxic agent with novel mechanisms of action. Unlike other commonly used chemotherapeutic agents, bendamustine induces durable DNA damage resulting in rapid cell death in apoptosis-resistant cancer cell lines through the apoptosis-independent pathway of mitotic catastrophe. Studies have reported single-agent activity in patients with relapsed/refractory non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia, multiple myeloma, and breast cancer. Aim. This study evaluated the efficacy and safety of bendamustine in patients with NHL who had relapsed and were refractory to prior rituximab treatment. Methods. This Phase II, multicenter study enrolled patients with relapsed, indolent, or transformed, rituximab-refractory B-cell NHL. Rituximab-refractory disease was defined as no response or progression within 6 months of completing rituximab treatment. Patients received bendamustine 120 mg/m² IV over 30 to 60 minutes on days 1 and 2 every 21 days for 6 cycles. Response was measured using the International Working Group criteria. Results. The intent-to-treat (ITT) population consisted of 77 heavily pretreated patients (53% male) with a median of 4 prior systemic therapies (range: 1-9), enrolled in 14 sites in the US and Canada. Median age of patients was 63 years (range: 38-84), and 87% had stage III/IV disease. Indolent histologic phenotype was seen in 82% of patients, while 18% had transformed disease. The overall objective response rate (ORR) in the ITT population was 74%; 38% had a complete response (CR) or unconfirmed complete response (CRu), 36% had a partial response (PR), 6% had stable disease (SD), and 16% had progressive disease (PD) (4% unknown). In the 14 patients with transformed disease, the ORR was 64%, with 14% CR + CRu, 50% PR, 7% SD, and 29% PD. By comparison, in the 62 patients with indolent lymphoma, the ORR was 78%, with 44% CR + CRu, 34% PR, 6% SD, and 15% PD (3% unknown). The median duration of response was 7.7 months for all patients, 2.5 months for transformed patients, and 8.4 months for indolent patients. The most common nonhematologic adverse events were neutropaenia (72%), fatigue (47%), and vomiting (41%). Most of these events were grade 1 or 2; mild alopecia rarely observed (5%). The primary hematologic toxicity was reversible myelosuppression, with grade 3 or 4 adverse events including neutropaenia (53%), thrombocytopenia (25%), and anemia (12%). MDS reported with similar frequency to the published incidence in this population. Conclusions. Single-agent bendamustine HCl (TREANDA™) was well tolerated and produced a high rate of durable objective responses, despite unfavorable prognostic features, in heavily pretreated rituximab-refractory, indolent and transformed NHL patients. The findings suggest that bendamustine may be an effective treatment for this patient population, which currently has very few treatment options. A Phase III trial with bendamustine as a single agent in patients with rituximab-refractory indolent NHL is ongoing.

0180
CLINICAL FEATURES AND PROGNOSTIC ASSESSMENT OF NODAL MARGINAL ZONE B-CELL LYMMPHOMA, A RARE DISEASE WITH FOLLICULAR-LIKE BEHAVIOUR
L. Arcaini,1 M. Pauli,2 E. Boveri,2 A. Rossi,2 M. Spina,2 F. Passamonti,3 S. Burchen,1 M. Montanari,2 A. Gallamini,1 L. Uziel,1 C. Cugnola,1 C. Pascutto,1 E. Morra,1 M. Lazzarino1

1IRCCS Policlinico San Matteo, PAVIA, Italy; 2Division of Hematology, BERG-AMO, Italy; 3Division of Oncology, AVIANO, Italy; 4Ospedale S.Paolo, MILANO, Italy; 5Division of Hematology. Osp. Niguarda, MILANO, Italy

Background. Primary nodal marginal zone B-cell lymphoma (MZL) is a rare entity recognized by the WHO classification. Diagnosis requires a lymph node localization in the absence of prior or concurrent involvement of extranodal sites. Most studies reported so far focus mainly on histopathology, while the clinical features and outcome of this uncommon lymphoma remains less defined. Aim. To define the clinical features and to assess prognosis of primary nodal marginal zone B-cell lymphoma. Methods. We studied a series of 47 newly diagnosed patients with primary nodal marginal zone B-cell lymphoma. Diagnosis was made on histologic examination of lesional tissues integrated with immunohistochemical data. No patient showed MALT or splenic localization of lymphoma at diagnosis. Results. Patients: 17 males and 30 females, median age 68 years (25-79) with 64% aged more than 60 years. 15% of patients had stage I disease, 10% stage II, 52% stage III, 48% stage IV (bone marrow involvement). 11% had peripheral blood involvement, 11% had bulky disease, 15% B symptoms, 6% ECOG score 2. 23% had hemoglobin <12 g/dL. LDH was above normal in 15% and b2-microglobulin in 45%. 11% had an autoimmune Background. HCV serology was positive in 24% (9/38). With the IPI score 37% ranked in the low risk, 22% in the low-intermediate, 35% in the intermediate-high, and 7% in the high risk category. Using the FLIPI score, 33% were classified as low risk, 34% as intermediate risk, and 33% as high risk. After treatment, 57% achieved a complete response and 24% a partial response, for an overall response rate of 81%. At a median follow-up of 2.6 years, no patient developed splenic or MALT involvement. 5-years and 10-years OS is 69% (95% CI 52-86%).

Figure 1. OS of primary nodal MZL according to FLIPI.

Death occurred in 10 pts (related to NHL in 9, to another neoplasm in one). In univariate analysis the following factors were associated with shorter event-free survival (EFS): B symptoms (p=0.001), high vs intermediate vs low risk FLIPI score (p=0.009). The following factors were associated with worse overall survival: high vs intermediate vs low risk FLIPI score (p=0.02) (Figure 1), age > 60 years (p=0.05), LDH above normal (p=0.05). HCV positivity was of borderline significance (p=0.06). In multivariate analysis hemoglobin < 12 g/dL (p=0.02, HR 14.3) was predictive of shorter EFS. Concerning overall survival, only the FLIPI retained statistical significance in predicting a worse outcome (p=0.02, HR 3.5). Positive HCV serology was of borderline significance (p=0.06, HR 4.4). Conclusions. Among marginal zone neoplasms, primary nodal marginal zone lymphoma appears a distinct disorder with an indolent behaviour. The association with HCV infection (28%) is particularly high in comparison with non-marginal zone lymphomas. Considering the prognostic assessment of this rare disease, the FLIPI score is effective in detecting patients at worse prognosis with the same power as in
folicular lymphoma. Thus, the application of the FLIPI may be of clinical value for treatment decision also in primary nodal marginal zone lymphoma.

0181
PRELIMINARY EVALUATION OF EFFICACY AND TOXICITY OF TWO DOSES SCHEDULES OF BORTEZOMIB PLUS R-CHOP REGIMEN IN FRONT-LINE B LYMPHOMA PATIENTS

F. Remy,1 V. Ribrag,2 N. Mounier,1 C. Haioun,3 J. B. Gollier,1 C. Ferme,1 J. Briere,1 M. Ertault,1 M. T. Ertault,1 N. Chagny,1 P. Eeftens,1 P. Debie,2 P. Koltzenburg,3 B. Delabesse,4 H. Maertens,5 S. V. Kneba,6 E. K. Cho1

Cancers were randomized between two doses schedules of administration of bortezomib: A (bi-weekly: day 1, 4, 8 and 11) and B (weekly: day 1 and 8). For the first 24 patients (step 1), Velcade was administered at 1mg/m² in group A and 1.3 mg/m² in group B. For the next 24 patients (step 2), in absence of severe toxicity in step 1, it was planned to increase velcade to 1.3 mg/m² in group A and 1.6 mg/m² in group B. Results. We report here the safety results on 29 patients, there were 11 females, 18 males, with a median age of 59 years old (32-76). Histology: 2 Lymphoplasmocytic Lymphoma, 5 Marginal Zone Lymphoma, 8 Follicular lymphoma, 3 Follicular Lymphoma with histological Transformation, 3 Mantle Cell Lymphoma and 8 Diffuse Large B Cell Lymphoma without adverse factor (IPSI=0). Performance status > 2: 0; LDH > N : 9; number of extra-nodal sites > 1: 11. 27 patients received 6 cycles (1 patient was in progression after 5 cycles and 1 patient did not receive the 6th cycle). In step 1, group A, 90 to 100% of scheduled dose of bortezomib was administered. For group B it was 99 to100%. In step 2, group A, 78 to 100% of scheduled dose of bortezomib was administered. It was 100% for group B. Dose reduction were made after cycle 4. G-CSF and EPO support was used when necessary. Grade 3-4 hemototoxicity (per cycle) occurred in 13% for platelets, 45% for leukocytes. There was no red blood cells transfu-

There was no difference with regard to genetic aberrations: trisomy 3, trisomy 18, t(11;18), t(14;18) involving IGH/MALT1) and clinical data (site and extent of disease, relapse rate, time to relapse, monoclonal gammopathy) of these patients with autoimmune diseases were compared to those with-out AD. Results. We have investigated the clinical characteristics of MALT lymphomas have a different clinical course or a different responsiveness to treatment compared to their counterparts in patients without autoimmune diseases. Aims. We have investigated the clinical characteristics of MALT lymphoma patients and the influence of AD on the clinical course as compared to controls without an underlying autoimmune condition.

Patients and Methods. We evaluated retrospectively 219 patients with histologically verified MALT lymphoma within a case-control study. In 134 cases, clinical and serologic data to judge the presence of AD were available. The epidemiologic (age), genetic (trisomy 3, trisomy 18, t(11;18), t(14;18) involving IGH/MALT1) and clinical data (site and extent of disease, relapse rate, time to relapse, monolocal gammapathy) of these patients with autoimmune diseases were compared to those without AD. Results. In total, 68/134 patients (47%) suffered from a concurre-

0182
CLINICAL FEATURES AND TREATMENT OUTCOMES OF ANGIOMYOPLASMOCTIC T-CELL LYMPHOMA


1 Samsung Medical Center, SEOUL, South-Korea; 2 Korea Cancer Center Hospital, SEOUL, South-Korea; 3 Asan Medical Center, SEOUL, South-Korea; 4 Gachun Medical School Gil Medical Center, INCHEON, South-Korea; 5 Dong-A Cancer Center, BUSAN, South-Korea; 6 Dankook University Hospital, CHEONAN, South-Korea; 7 Hallym University Kangnam Hospital, SEOUL, South-Korea; 8 Gyeongsang National University Hospital, JINJU, South-Korea

Angiomyoplasmocitic T-cell lymphoma (AITL) is one of the most serious complications in patients with autoimmune diseases (AD). Most of these lymphomas originate from B-cells and the extranodal mucosa associated lymphoid tissue (MALT) lymphomas is the most common subtype. Currently, it is unclear whether these lymphomas have a different clinical course or a different responsiveness to therapy compared to their counterparts in patients without autoimmune diseases. Aims. We have investigated the clinical characteristics of MALT lymphoma patients and the influence of AD on the clinical course as compared to controls without an underlying autoimmune condition.

0183
AUTOIMMUNE DISEASES IN PATIENTS WITH MAL-T-LYMPHOMA: CHARACTERISTICS AND CLINICAL COURSE

S. Wöhrer, M. Troch, B. Streubel, J. Zwerina, C.C. Zielinski, A. Chott, M. Raderer

Medical University of Vienna, VIENNA, Austria

Background. The development of a Non-Hodgkin’s lymphoma (NHL) is one of the most serious complications in patients with autoimmune diseases (AD). Most of these lymphomas originate from B-cells and the extranodal mucosa associated lymphoid tissue (MALT) lymphomas is the most common subtype. Currently, it is unclear whether these lymphomas have a different clinical course or a different responsiveness to therapy compared to their counterparts in patients without autoimmune diseases. Aims. We have investigated the clinical characteristics of MALT lymphoma patients and the influence of AD on the clinical course as compared to controls without an underlying autoimmune condition.

Patients and Methods. We evaluated retrospectively 219 patients with histologically verified MALT lymphoma within a case-control study. In 134 cases, clinical and serologic data to judge the presence of AD were available. The epidemiologic (age), genetic (trisomy 3, trisomy 18, t(11;18), t(14;18) involving IGH/MALT1) and clinical data (site and extent of disease, relapse rate, time to relapse, monoclonal gammapathy) of these patients with autoimmune diseases were compared to those without AD. Results. In total, 68/134 patients (47%) suffered from a concurrent AD, with patients being significantly younger (59 vs 67 years, p=0.002). Elevated autoimmune parameters without clinical significance and symptoms were found in 15.7% of patients. Patients with AD had significantly more extragastrointestinal lymphoma (p=0.011), but showed a comparable number of multifocal disease (p=0.7). Surprisingly, equal relapse rates (p=0.3) and a similar time to relapse were found in both groups (p=0.3, Figure 1).

Figure 1. Time to relapse.

There was no difference with regard to genetic aberrations: trisomy

CLINICAL COURSE

Group Step Stable RC Stable RCU Partial Remission RP Progression

Bi-weekly

(n=15)

1 (n=12)

2 (n=3)

5

9

1

0

Weekly

(n=14)

1 (n=12)

2 (n=2)

8

4

1

1

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11th Congress of the European Hematology Association
3 (p=0.057), trisomy 18 (p=0.8), t(11;18) (p=0.1) and t(14;18) (p=0.6). The presence of a monoclonal gammapathy/paraprotein production was evenly distributed (p=0.1) between both groups. Summary/Conclusions. This is the first study to suggest that the clinical course and the genetic aberrations of MALT lymphoma patients are not related to the presence or absence of autoimmune diseases. However, patients with autoimmune diseases develop MALT lymphomas at a significantly younger age. It was also shown that elevated autoimmune parameters were in fact associated with underlying AD in 85% of cases and were not merely a paraneoplastic phenomenon. Since a significant number of patients with MALT lymphoma suffer from an underlying AD, it is reasonable to determine autoimmune parameters on a routine basis in such patients and to search for the presence of an underlying AD.

0184
RITUXIMAB-M/VACOP-B COMBINED WITH RADIOTHERAPY IN PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA: A PROSPECTIVE ITALIAN INTERGROUP PHASE II STUDY

M. Martelli,1 V. Stefoni,2 P. Cabras,3 S. Cortellazzo,1 E. Brusamolinol,2 A. Levis,2 G. Gallo,1 A. De Vivo,2 F. Natalino,2 E. Fineolley,1 A. Di Rocco,1 R. Feà,1 P.L. Zinzanil
1University La Sapienza, ROMA, Italy; 2Hematology Institute, BOLOGNA, Italy; 3Hematology Division, CAGLIARI, Italy; 4University La Sapienza, ROMA, Italy

Background. Weekly 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 (Zinzani 2005). The superiority of R-CHOP over CHOP or CHOP-like regimens has been recently demonstrated in younger low risk NHL (Phreundschuh 2005). 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. A total of 40 patients with PMLBCL were treated in six participating centers between February 2002 and July 2005. The median age was 38 years (range 17-54); 21/19 (53%) were females; 30 patients had stage II and 10 stage IV, 38 (95%) presented a bulky disease; LDH was increased in 26 (65%) and 21 (53%) had a superior vena cava syndrome. According to the age-adjusted IPI score, 24 patients had an IPI = 0-1 and 16 an IPI = 2-3. All patients were treated with standard MACOP-B (30 patients) or VACOP-B (10 patients) regimens plus six cycles of Rituximab (375 mg/m²) given at weeks 3, 5, 7, 9, 11, 13. Twenty-six patients (65%) received mediastinal IFRT at a median dose of 36 Gy. The response was evaluated in all patients after six cycles of chemo-immunotherapy, at the end of planned chemotherapy and after IFRT. Results. The response rate after six cycles of the planned R-M/VACOP-B regimen was CR/CRu = 20 (50%), PR = 19 (47%) and NR = 1 (3%). Eight/40 patients received a second line therapy followed by HDT-ASCT (6/8 patients) because considered low responders (PR= 7 and NR=1). At the end of the chemo-immunotherapy program 28 patients witnessed a CR/CRu (70%) and 12 a PR (30%). Seven/12 PR patients obtained a CR/CRu following IFRT for an overall CR/CRu rate of 67% (35/50). After a median follow up of 13 months, the 2-year OS and PFS were 75% and 78%, respectively. No additional toxicities other than those related to the chemotherapy were observed during and after Rituximab infusion. Conclusions. R-M/VACOP-B are an active therapeutic regimens devoid of severe toxicity for the management of patients with PMLBCL. Further studies are required to demonstrate if the addition of Rituximab to front-line third generation regimens might overcome the need of more aggressive strategies, such as consolidation with IFRT or HDT-ASCT.

0185
ASSOCIATION OF REDUCED RELATIVE DOSE INTENSITY AND SURVIVAL IN LYMPHOMA PATIENTS RECEIVING CHOP-21 CHEMOTHERAPY

P. Bettengell,1 A. Bosily,2 D. Bron,1 M. Schwenkglenks,3 P. Johnson,2 A. Van Hoof,1 B. Hancock,1 R. De Bock,1 A. Pagliuca,4 Z. Bereman,5 R. Thomas,1 A. Ferrant,1 M. Joyner,1 M. Daouve,1 G. Verhoef,1 P. Hoskin6
1St. George’s Hospital, LONDON, United Kingdom; 2UCI, Mont-Godinne, YVOIR, Belgium; 3Institut Jules Bordet, BRUXELLES, Belgium; 4University of Basel, BASEL, Switzerland; 5Southampton General Hospital, SOUTHAMPTON, United Kingdom; 6A.Z. St-Jan, BRUGGE, Belgium; 7Wessex Park Hospital, SHEFFIELD, United Kingdom; 8A.Z. Middleheim, ANTWERPEN, Belgium; 9King’s College Hospital, LONDON, United Kingdom; 10U.Z. Antwerpen, ANTWERPEN, Belgium; 11Addenbrooke’s Hospital, CAMBRIDGE, United Kingdom; 12UCL, St-Luc, BRUXELLES, Belgium; 13Royal Devon and Exeter Hospital, EXETER, United Kingdom; 14Amgen NV, BRUXELLES, Belgium; 15KUL Leuven, LEUVEN, Belgium; 16Mount Vernon Cancer Centre, NORTHWOOD, United Kingdom

Background. Chemotherapy delivery in patients with non-Hodgkin lymphoma (NHL) is sometimes impaired by treatment side-effects. It remains unclear whether moderate reductions in relative (i.e., administered compared to planned) chemotherapy dose intensity (RDI) affect overall survival. Aims. To assess the relationship of reduced RDI and overall survival in NHL patients receiving CHOP chemotherapy with a cycle length of 21 days (CHOP-21). Methods. Retrospective audits of NHL patients were conducted in Belgium (lymphodose ‘02) and the UK (Audit of Lymphoma Patients). Variables available from both datasets were merged into a dataset of individual observations, and definitions and exclusions were harmonised, to allow for comparisons and combined analyses. RDI was averaged across anti-malignant drugs. Potential predictors of survival were assessed using extended Cox proportional hazards regression.

Results. The Belgian study included 211 NHL patients receiving CHOP-21 and the UK study included 78. Of these, 59% (Belgium) vs. 46% (UK) were female. Mean age (SD) at chemotherapy initiation was 63 (14) years (Belgium) vs. 65 (18) years (UK). Mean RDI (SD) was 90% (17%) in Belgium vs. 94% (9%) in the UK. Mean RDI of survivors at 60 months was estimated to be 61% in Belgium vs. 67% in the UK. This analysis confirms earlier reports that reduced RDI is associated with decreased survival. The strength of the association and stage of disease decreased over time. Summary/Conclusions. This analysis confirms earlier reports that reduced
RII may have a negative impact on survival in NHL patients receiving CHOP chemotherapy. While further investigation is needed, delivering full chemotherapy dose intensity remains an important goal in this group of patients.

0186

THE EMERGING ROLE OF FDG PET/CT IN THE PRIMARY STAGING OF LYMPHOMA

J. Trotman,1 M. Fulham,1 M. Gibson,1 A. Mohamed,1 I. Cunningham,1 G. Young2

1Concord Hospital, SYDNEY, Australia; 2Royal Prince Alfred Hospital, CAMBERDON, Australia

Background. The rapid adoption of FDG PET/CT scanning for imaging lymphoma has occurred in the absence of a large body of published experience of this imaging modality. Aim. To evaluate the role of PET/CT in primary staging of previously untreated Hodgkin (HL) or Non-Hodgkin lymphoma (NHL) and assess its impact on clinical management. Method. Over the period June 2003 to December 2005, we performed 1600 PET/CT scans for lymphoma. The primary staging cohort comprised 563 (16%) patients, (130 female, 133 male; mean age 52 years) from 64 referring specialists. All patients received 350 MBq FDG i.v. with an uptake period of one hour; the study extended from the vertex to upper thighs; diabetic patients were not excluded. All scans were read without access to the histopathology or results of anatomical imaging. Histological diagnosis was HL (n=52) and NHL (n=211). DLBCL accounted for 41% and follicular lymphoma 34% of the NHL cohort. Referring doctors provided details of the clinical stage, results of other investigations and the management plan prior to the PET/CT. After the PET/CT they were asked for a revised clinical stage and management plan and the pre- and post-PET/CT staging and management plans were compared. Results were collated for 175 patients retrospectively and prospectively for 88. Results. There were 26 negative PET/CT scans: 28 were stage I patients where the sole primary site of disease had been resected, 2 had lesions beyond the resolution of the scanner (conjunctival MAIT, cutaneous anaplastic large cell) and 1 scan was negative in a patient (HL) undergoing TPN with an elevated blood sugar level where the technical quality of the study was suboptimal. All were included in the analysis. Pre- and post-PET/CT staging was obtained in all patients. PET/CT altered staging in 105 (40%), 22 (42%) in HL and 83 (39%) in NHL. Staging was unchanged in 159. Up-staging was seen in 82 (76%) of those with change of stage (18 HL, 64 NHL). Pre- and post-PET/CT management plans were obtained in 91%. The 25 patients for whom we did not receive post-PET/CT management plans were excluded from further analysis. Management was changed in 80 patients (84%) overall; 12 (27%) with HL and 68 (85%) with NHL. 64 (44%) patients were pre-PET/CT Stage 1, 14 Stage 2, 10 Stage 3 and 12 Stage 4. Management changes were made in 50% (56) of patients upstaged; 27% (59) with staging unchanged; and 26% (5) of those downstaged. The greatest impact on management occurred in upstaging patients with Stage I follicular lymphoma, of whom 71% had a change in management plan. Conclusions. Our data suggest that PET/CT detects a greater volume and extent of disease, and Karnofsky Performance Status ≥50% (ECOG 0-2). No prior bortezomib was allowed and patients who had received a prior regimen that included rituximab were not included. A total of 242 patients were enrolled in 8 PLRG centers: 11 pts had HL at diagnosis, 6 pts had a PR (Arm A). Response rates were similar in patients who previously received rituximab compared with the total study population. Median progression-free survival has not yet been reached (median follow-up 3.9 months). Treatment was well tolerated in both arms; grade ≥3 adverse events (AEs) were seen in 14 (54%) patients in Arm A and 6 (18%) patients in Arm B. The most common grade ≥3 AEs were gastrointestinal toxicities, neutropenia, thrombocytopenia and peripheral neuropathy. Serious AEs were seen in 11 (27%) patients in Arm A versus 6 (15%) patients in Arm B. Conclusions. Weekly and twice weekly bortezomib plus rituximab are active and well tolerated treatments for relapsed/refractory CD20+ indolent NHL. The more convenient weekly regimen appears to offer a similar response rate with less toxicity. The weekly regimen allowed delivery of a higher fraction of the intended dose, and a similar total cumulative dose, compared with the twice weekly regimen.

0187

BORTEZOMIB PLUS RITUXIMAB IN PATIENTS WITH INDOLENT NON-HODGKIN’S LYMPHOMA: A PHASE 2 STUDY

A. Goy,1 S. De Vos,1 S. Dakhil,2 S. Dakhil,2 S. Dakhil,2 S. Dakhil,2 A. Goy,1 A. Goy,1 R. Belt,1 T. Zhang2

1Hackensack University Medical Center, HACKENSACK, USA; 2UCLA School of Medicine, LOS ANGELES, USA; 3Cancer Center of Kansas, WICHITA, USA; 4MD Anderson Cancer Center, HOUSTON, USA; 5Georgia Cancer Specialists, ATLANTA, USA; 6Kanas City Cancer Care, KANSAS CITY, USA; 7Winship Cancer Institute, ATLANTA, USA; 8Mid Ohio Oncology/Hematology, COLUMBUS, USA; 9Florida Cancer Specialists, FORT MYERS, USA; 10Northern Virginia Hematology/Oncology, FAIRFAX, USA; 11Huntsman Cancer Institute, SALT LAKE CITY, USA; 12Rush Medical Center, CHICAGO, USA; 13Charleston Cancer Center, CHARLESTON, USA; 14Millennium Pharmaceuticals Inc, CAMBRIDGE, USA

Background. Bortezomib (VELCADE®), a first-in-class proteasome inhibitor, has demonstrated single-agent activity in non-Hodgkin’s lymphoma (NHL) with response rates of 14-56%. Preclinical data with combined bortezomib and rituximab suggest increased activity with no overlapping toxicity. Weekly bortezomib is active in animal models for myeloma and is expected to be more convenient than the approved twice-weekly regimen. Weekly dosing was therefore studied in NHL. Aims. This randomized, phase 2 study investigated the response rate to bortezomib plus rituximab, weekly or twice-weekly, in patients with relapsed follicular lymphoma (FL) or marginal zone lymphoma (MZL). Methods. Eligibility criteria included CD20+ FL or MZL with measurable disease, and Karnofsky Performance Status ≥25% (ECOG 0-2). No prior bortezomib was allowed and patients who had received a prior regimen that included rituximab were not included. A total of 242 patients were enrolled in 8 PLRG centers: 11 pts had HL at diagnosis, 6 pts had a PR (Arm A). Response rates were similar in patients who previously received rituximab compared with the total study population. Median progression-free survival has not yet been reached (median follow-up 3.9 months). Treatment was well tolerated in both arms; grade ≥3 adverse events (AEs) were seen in 14 (54%) patients in Arm A and 6 (18%) patients in Arm B. The most common grade ≥3 AEs were gastrointestinal toxicities, neutropenia, thrombocytopenia and peripheral neuropathy. Serious AEs were seen in 11 (27%) patients in Arm A versus 6 (15%) patients in Arm B. Conclusions. Weekly and twice weekly bortezomib plus rituximab are active and well tolerated treatments for relapsed/refractory CD20+ indolent NHL. The more convenient weekly regimen appears to offer a similar response rate with less toxicity. The weekly regimen allowed delivery of a higher fraction of the intended dose, and a similar total cumulative dose, compared with the twice weekly regimen.

0188

CONSOLIDATION OF CHEMOTHERAPY RESPONSE IN MANTLE CELL LYMPHOMA PATIENTS WITH 90Y-BRITUNOMAB TIUXTEN RADIOIMMUNOTHERAPY


1CMUJ, KOPERNIKA, Poland; 2AM, GDANSK, Poland; 3SAM, KATOW- ICE, Poland; 4MCS, WARSAW, Poland

Background. MCL is an aggressive and prognostically unfavorable subtype of B-cell NHL, with a 5-yr survival rate <20%. Standard chemotherapy for MCL includes fludarabine, cyclophosphamide, and mitoxantrone (FCM). Addition of rituximab (R) to FCM increases the overall response rate (ORR) from 46% to 58%. Conventional non-myeloblastic RT has been unsuccessful in MCL patients, with a large tumor burden and no initial cytoreduction. Aims: The Polish Lymphoma Research Group Trial (PLRG MCL1) assessed whether 90Y-Zevalin would consolidate the response achieved from FCM-R and provide better ORRs and longer survival in MCL patients. Methods. Eligibility criteria included measurable disease, and Karnofsky Performance Status ≥50% (ECOG 0-2). No prior bortezomib was allowed and patients who had received a prior regimen that included rituximab were not included. A total of 242 patients were enrolled in 8 PLRG centers: 11 pts had HL at diagnosis, 6 pts had a PR (Arm A). Response rates were similar in patients who previously received rituximab compared with the total study population. Median progression-free survival has not yet been reached (median follow-up 3.9 months). Treatment was well tolerated in both arms; grade ≥3 adverse events (AEs) were seen in 14 (54%) patients in Arm A and 6 (18%) patients in Arm B. The most common grade ≥3 AEs were gastrointestinal toxicities, neutropenia, thrombocytopenia and peripheral neuropathy. Serious AEs were seen in 11 (27%) patients in Arm A versus 6 (15%) patients in Arm B. Conclusions. Weekly and twice weekly bortezomib plus rituximab are active and well tolerated treatments for relapsed/refractory CD20+ indolent NHL. The more convenient weekly regimen appears to offer a similar response rate with less toxicity. The weekly regimen allowed delivery of a higher fraction of the intended dose, and a similar total cumulative dose, compared with the twice weekly regimen.
Recent studies of gene expression in diffuse large B-cell lymphoma (DLBCL) have identified gene signatures associated with germinal center or post-germinal center lymphocytes. These signatures have been shown to have prognostic significance in DLBCL, and to correlate with similar classifications based on immunohistochemical biomarkers. Protein kinase C β II (PKC-β II) was considered as one such gene, and recent studies have suggested that its expression is associated with a poor prognosis. Aim: To determine the prognostic significance of the expression of PKC-β II in patients with nodal DLBCL.

Methods: Patients with de novo nodal DLBCL treated in two hospitals were enrolled retrospectively. Inclusion criteria were treatment with frontline chemotherapy regimens, HIV-negative status, and tissue availability. Diagnosis was confirmed by a pathologic review board. Clinical data were obtained from the charts. The IPI was grouped in low-risk (0-2 factors) and high-risk (3-5 factors). Formalin-fixed, paraffin-embedded tissues were stained with a monoclonal antibody against PKC-β II protein (Sigma, P-2201). Cases with more than 10% stained large cells were considered positive. Results: A total of 125 patients were enrolled. The median follow-up was 5.3 years for the surviving patients. Data were available for IPI classification in 118 patients, and 83 patients were in the low-risk group. Forty-eight patients (38%) were positive for PKC-β II.

1019
PKC-BETA II EXPRESSION HAS PROGNOSTIC IMPACT IN NODAL DIFFUSE LARGE B-CELL LYMPHOMA

R.S. Schaffer

Federal University of Rio de Janeiro, RIO DE JANEIRO, Brazil

Background. Recent studies of gene expression in diffuse large B-cell lymphomas (DLBCL) have identified gene signatures associated with germinal center or post-germinal center lymphocytes. These signatures have been shown to have prognostic significance in DLBCL, and to correlate with similar classifications based on immunohistochemical biomarkers. Protein kinase C β II (PKC-β II) was considered as one such gene, and recent studies have suggested that its expression is associated with a poor prognosis. Aim: To determine the prognostic significance of the expression of PKC-β II in patients with nodal DLBCL.

Methods: Patients with de novo nodal DLBCL treated in two hospitals were enrolled retrospectively. Inclusion criteria were treatment with frontline chemotherapy regimens, HIV-negative status, and tissue availability. Diagnosis was confirmed by a pathologic review board. Clinical data were obtained from the charts. The IPI was grouped in low-risk (0-2 factors) and high-risk (3-5 factors). Formalin-fixed, paraffin-embedded tissues were stained with a monoclonal antibody against PKC-β II protein (Sigma, P-2201). Cases with more than 10% stained large cells were considered positive. Results: A total of 125 patients were enrolled. The median follow-up was 5.3 years for the surviving patients. Data were available for IPI classification in 118 patients, and 83 patients were in the low-risk group. Forty-eight patients (38%) were positive for PKC-β II.

Among these patients, there were 90 patients with IPI group according to the expression of PKC-β II. There were no differences in LDH, age, B symptoms, Ann Arbor stage or IPI group according to the expression of PKC-β II. More females than males were PKC-β II positive (54% vs 27%, p=0.005). Complete remission was obtained in 70%, and was not influenced by PKC-β II status (67% vs 71%). The 5-year event-free survival (EFS) was shorter in high-risk patients (14% vs 59%, p<0.001) and in those with PKC-β II positivity (56% vs 49%, p=0.054). Only the IPI influenced the 5-year overall survival (18% vs 70%, p<0.001). However, in low-risk patients, PKC-β II expression was related to a shorter 5-year OS (60% vs 76%, p=0.03) and a shorter 5-year EFS (48% vs 66%, p=0.014). In a Cox regression analysis for EFS, PKC-β II expression (hazard ratio = 1.6, p=0.04) and the IPI (HR=3.06, p=0.01) were independent poor prognostic factors for the OS. Conclusion: PKC-β II expression, along with the IPI, was associated with a shorter EFS and OS in patients with nodal DLBCL. PKC-β II identified a subgroup of patients, within the IPI low-risk group, who had a shorter OS.

0191
CIGARETTE SMOKING AND ALCOHOL CONSUMPTION AS DETERMINANTS OF SURVIVAL IN NON-HODGKIN'S LYMPHOMA: A POPULATION-BASED STUDY

T. Battaglioli,1 G. Gorini,1 A. Seniori Costantini,1 P. Crosignani,1 L. Miligi,2 O. Nanni,2 S. Stagnaro,3 R. Tumino,3 P. Vines1

1BrCCA Maggiore Hospital, MILAN, Italy; 2Center for Study & Prevention of Cancer, FLORENCE, Italy; 3National Cancer Institute, MILAN, Italy; 4Istituto Ortopedico Rizzoli, Bologna, Italy; 5National Cancer Research Institute, GENOA, Italy; 6Regional Hospital, RAGUSA, Italy; 7University of Turin, TURIN, Italy

Background. The risk of non-Hodgkin’s Lymphoma (NHL) seems to be enhanced by cigarette smoking and lowered by alcohol drinking. No study has ever focused on the role of these factors on survival from NHL. Aims: To assess whether cigarette smoking and alcohol drinking affect NHL survival. Methods: A population-based prospective study on 1,138 NHL patients, diagnosed between 1991 and 1993, followed-up until 2002, was carried out. At diagnosis, clinical and socio-demographic data were recorded and lifestyle habits were assessed through a validated questionnaire. Survival analysis was performed with Kaplan-Meier analysis.
Methods. Hazard ratios (HR) were estimated by Cox regression. Results. The mean follow-up was 6.6 years (st.dev. 4.3). The mean survival time was 7.56 years (st.dev. 0.155). At both univariate and multivariate analysis, heavy cigarette smoking and alcohol drinking were associated with poor survival. Compared with those with a lower cumulative exposure to tobacco smoking, those who had smoked >51 pack-years had a worse survival (HR=1.69, 95%CI=1.18-2.38). Drinkers had a higher risk of death than non-drinkers (at diagnosis HR=1.10-1.81). Considering only those who had NHL as cause of death, the HR for the higher category of pack-years smoked, compared with the lowest, was 1.69 (95%CI=1.15-2.33) and for drinkers, compared with non-drinkers, it was 1.38 (95%CI=1.01-1.80). Conclusions. Cigarette smoking and alcohol drinking may influence NHL survival.

**0192**

IBRITUMOMAB TIUXETAN COMBINED WITH HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM-CELL TRANSPLANTATION IN PATIENTS WITH CHEMO-REFRACTORY AGGRESSIVE NON-HODGKIN’S LYMPHOMA

A. Shimoni, T. Zwass, Y. Oksman, I. Hardan, N. Shem-Tov, R. Yerushalmi, A. Avigdor, I. Ben-Bassat, A. Nagler

Chaim Sheba Medical Center, TEL-HASHEM, Israel

**Background.** High-dose chemotherapy and autologous stem-cell transplantation (SCT) have an established role in the treatment of patients with first chemo-sensitive relapse of aggressive lymphoma. However, autologous SCT has limited success when performed in refractory or progressive stage of the disease and the expected 1-year progression-free survival (PFS) in this setting is less than 20%. The major cause of treatment failure is disease relapse. Aims. This study was designed to explore the safety and outcome following inclusion of Zevalin in the conditioning regimen given prior to autologous SCT. The primary endpoint was 1-year PFS. Methods. Patients were eligible for this study only if they had refractory lymphoma and a positive PET-CT prior to SCT. Rituximab 250 mg/m² followed by Zevalin 0.4 mCi/kg were given on day -14. Chemotherapy according to standard BEAM regimen was started on day -6. Results. The study included 22 patients, 14 men and 8 women, median age 55 years (range, 35-66). Histology was diffuse large cell (n=15), transformed follicular (n=6) and mantle cell lymphoma (n=1). Patients had active lymphoma at SCT, either primary refractory (n=12) or refractory relapse (n=10) and 12 patients had bulky disease at presentation. The median number of prior lines of therapy was 3 (range, 1-6). There were no early infusion reactions associated with Zevalin. Fifteen patients achieved CR (of them additional radiotherapy given after SCT), 5 achieved PR and 2 died early after SCT from organ toxicities. With a median follow-up of 9 months (range, 1-21), 15 patients are alive and 7 have died. The estimated 1-year survival is 54% (28-81). Only 4 patients relapsed so far with a 1-year cumulative incidence of only 22% (9-53%). As expected in this group of patients with refractory disease all relapses occurred within 4 months of SCT, such that the relative short follow-up relapse rate seems lower than expected. Two pts died of multi-organ toxicities and 2 of late occurring infections. The day 100 treatment related mortality was 9%. These rates of non-relapse mortality are expected in heavily pretreated patients with refractory lymphoma and there was no additional toxicity related to Zevalin. Conclusions. The inclusion of Zevalin in the conditioning regimens given prior to autologous SCT may reduce the risk of post SCT relapse and improve the poor outcome of patients with refractory lymphoma given SCT with standard regimens. This observation merits further study in larger comparative studies.

**0193**

CENTRAL NERVOUS SYSTEM INFECTION IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA. INCIDENCE AND CLINICAL CHARACTERISTICS


Institut Catala d’Oncologia, L’HOSPITALET DE LLOBREGAT, Cancer Purpose. The aim of the study was to evaluate the incidence of central nervous system infiltration in patients with Non-Hodgkin’s lymphoma diagnosed according to the REAL/WHO classification and to describe the clinical features and treatment outcome. Patients and Methods. All patients diagnosed of a lymphoid neoplasm in our centre between May 1994 and May 2004 (n=2544) were included in our analysis. We identified all the cases with CNS infiltration at diagnosis or during the clinical course and excluded those who received intrathecal prophylaxis. We evaluated the incidence, clinical characteristics and response to treatment. Results. Forty (3.8%) patients with CNS infiltration were identified. Twenty one (52.5%) males, with a median age of 56 years (range 31-82 years). Ten (25%) patients presented CNS infiltration at diagnosis. Thirty patients (75%) developed CNS involvement during the course of the disease at a median time of 12 months (range 3.4-20.5m) from initial diagnosis. Four (10%) were HIV+. CNS infiltration by REAL/WHO subtypes is shown in the table below. Clinical and laboratory features at diagnosis were as follows: IVL (n=5), extranodal involvement: 36 (90%), bone marrow infiltration: 15 (40.5%), bulky disease: 20 (50%), B symptoms: 14 (35%) and ECOG ≥ 2: 20 (50%). Response to treatment: CR 10 patients (27%), PR (13.5%) and failure 22 (59.5%). Median overall survival was 2.26 months (range 0.72-3.2 months). Conclusions. The high incidence of CNS involvement in lymphoma patients’ although previous CNS prophylaxis, makes us hypothesise that unknown factors could be associated to this phenomenon. Treatment response and survival of these patients is poor.

**0194**

CLINICAL FEATURES OF THE WESTERN AND ASIAN FORMS OF INTRAVASCULAR LYMPHOMA (IVL) VARIES ACCORDING TO THE PRESENCE OF HEMOPHAGOCYTIC SYNDROME (HPS) AND NOT TO THE GEOGRAPHICAL AREA

A.J.M. Ferreri,1 G.P. Donnini,2 E. Campo,3 E. Zucca,4 M. Martelli,5 A. De Renzo,6 C. Doglioni,1 M. Ferrari,7 C. Montalban,8 A. Tedeschi,9 A. Pavlovsky,10 A. Morgan,11 L. Uziel12 S. Ascani13 C. Patriarca14 F. Facchetti15 L. Mazzucchelli16 F. Cavalli17 M. Ponzo18 1San Raffaele Scientific Institute, MILAN, Italy; 2Hospital Clinic, BARCELONA, Spain; 3Istituto Oncologico Svizzera italiana, BELLINZONA, Switzerland; 4Università La Sapienza, ROME, Italy; 5Università Federico II, NAPOLI, Italy; 6Ospedale di Belluno, BELLUNO, Italy; 7Hospital Ramon y Cajal, MADRID, Spain; 8Ospedale Maggiore, MILAN, Italy; 9Fundaleu, BUENOS AIRES, Argentina; 10Hospital of Melbourne, MELBOURNE, Australia; 11Ospedale San Paolo, MILAN, Italy; 12Ospedale di Terni, TERNI, Italy; 13Ospedale Vizzolo Pedrotti, MELENGANO (MI), Italy; 14Spedali Civili, BRESÍA, Italy; 15Ospedale di Locarno, LOCARNO, Switzerland

Background. Published data suggest the existence of some clinical differences between IVL patients diagnosed in Asian and Western countries. Aim. To explore potentially different clinical forms of IVL by comparing the clinical features of the largest cumulative series of IVL patients diagnosed in Western countries and three subgroups of IVL patients diagnosed in different Asian countries and published in the English literature. Methods. Clinical records and pathological material of 45 HIV-negative patients with IVL diagnosed in 8 Western Countries (Western-IVL) were reviewed. Clinical features of this series were compared with 282 previously reported cases of IVL diagnosed in Western countries (Western-IVL) and with 120 previously reported cases of IVL diagnosed in Japan (n=86) and other Asian Countries (n=34). Analysis was performed according to the presence of HPS. Results. HPS was absent in our patients; it was diagnosed in 5 (2%) previously reported cases of Western-IVL (p= 0.57), in 36 (44%) Japanese patients (p = 0.0001) and in 4 cases (12%) diagnosed in other Asian countries (p = 0.03). Analysis of differences in clinical presentation and laboratory findings included four subgroups: our 45 Western-IVL patients, 38 Japanese patients with IVL and HPS (J-HPS), 48 Japanese patients with IVL without HPS (J-IVL) and 30 patients with IVL without HPS diagnosed in Asian countries other than Japan (Eastern-IVL). Median age was very similar among studied subgroups, oscillating between 62 and 69 years, with a constant slight prevalence among males. As reported in the table, there were no significant differences in presenting symptoms, sites of disease or laboratory findings among Western-IVL, J-IVL and Eastern-IVL patients. Conversely, stage-IV disease, fever of unknown origin, involvement of liver, spleen, bone marrow or lung, fatigue, jaundice, thrombocytopenia, increased serum levels of hepatic enzymes as well as a concomitant extravascular lymphoma were significantly more common among the
38) J-HPS patients in comparison with the other groups. Conversely, skin and central nervous system involvement was significantly more rare in J-HPS patients. No significant differences were observed in terms of anemia, leucopenia, monoclonal component, and peripheral blood involvement. In patients treated with anthracycline-based chemotherapy (21 from our Western-IVL series and 27 from J-HPS series), complete remission rate was 52% and 58% (p = 0.93), with a 2-year overall survival of 45±11% and 22±5% (p = 0.04), respectively. Conclusions. The association between IVL and HPS is anecdotally diagnosed outside of Japan. IVL significantly varies in clinical features and laboratory findings according to the presence of HPS and not to the geographical area. Patients with IVL but without HPS diagnosed in Western countries, Japan and other Asian countries display similar characteristics and should be considered as forming part of a classical form of IVL. J-HPS patients display numerous clinical differences with respect to the classical form and could be considered as a HPS-related variant of IVL. When treated with anthracycline-based chemotherapy, both variants exhibit a worse prognosis, specially in HPS-related cases; thus, rendering advisable treatment intensification. An extensive phenotypic and molecular characterization is needed to confirm whether these clinical differences might reflect discordant biological entities within IVL.

### Western (n=45) J-HPS (n=38) p J-IVL (n=48) p Eastern (n=30) p

<table>
<thead>
<tr>
<th>Category</th>
<th>Western</th>
<th>J-HPS</th>
<th>p</th>
<th>J-IVL</th>
<th>p</th>
<th>Eastern</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>19 (42%)</td>
<td>34 (89%)</td>
<td>0.00001</td>
<td>20 (42%)</td>
<td>0.96</td>
<td>18 (60%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Stage IV</td>
<td>34 (76%)</td>
<td>37 (97%)</td>
<td>0.01</td>
<td>45 (94%)</td>
<td>0.19</td>
<td>25 (83%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Skin</td>
<td>17 (38%)</td>
<td>3 (3%)</td>
<td>0.00011</td>
<td>12 (25%)</td>
<td>0.18</td>
<td>7 (23%)</td>
<td>0.19</td>
</tr>
<tr>
<td>CNS</td>
<td>18 (40%)</td>
<td>8 (21%)</td>
<td>0.014</td>
<td>24 (51%)</td>
<td>0.24</td>
<td>11 (37%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Liver</td>
<td>12 (27%)</td>
<td>25 (60%)</td>
<td>0.004</td>
<td>15 (31%)</td>
<td>0.63</td>
<td>13 (45%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Spleen</td>
<td>11 (24%)</td>
<td>7 (18%)</td>
<td>0.001</td>
<td>10 (22%)</td>
<td>0.68</td>
<td>10 (33%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Lymph n.</td>
<td>3 (4%)</td>
<td>9 (5%)</td>
<td>0.68</td>
<td>2 (4%)</td>
<td>0.69</td>
<td>4 (30%)</td>
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</tr>
<tr>
<td>Lung</td>
<td>8 (18%)</td>
<td>11 (37%)</td>
<td>0.05</td>
<td>5 (10%)</td>
<td>0.28</td>
<td>15 (50%)</td>
<td>0.003</td>
</tr>
<tr>
<td>B. marrow</td>
<td>14 (31%)</td>
<td>18 (37%)</td>
<td>0.014</td>
<td>16 (33%)</td>
<td>0.82</td>
<td>4 (13%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Thrombocyto 16 (38%)</td>
<td>15 (36%)</td>
<td>0.005</td>
<td>6 (13%)</td>
<td>0.07</td>
<td>25 (36%)</td>
<td>0.07</td>
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</tr>
<tr>
<td>Hight LDH 29/35 (85%)</td>
<td>36/50 (72%)</td>
<td>0.17</td>
<td>31/33 (94%)</td>
<td>0.03</td>
<td>31/33 (100%)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>High ALT</td>
<td>9 (20%)</td>
<td>10 (25%)</td>
<td>0.04</td>
<td>3 (10%)</td>
<td>0.73</td>
<td>8 (27%)</td>
<td>0.06</td>
</tr>
<tr>
<td>High bilirubin 2 (4%)</td>
<td>11 (29%)</td>
<td>0.004</td>
<td>0 (0%)</td>
<td>0.23</td>
<td>1 (3%)</td>
<td>1.00</td>
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</table>

### 0195

**A PHASE II STUDY OF CYCLOPHOSPHAMIDE, VINCRIESTINE, NON-PEGYLATED LIPOSOAMAL DOxorubicin, and PREDNISONE PLUS RITUXIMAB (R-COMP) IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)**

M. Federico, 1 M.D. Caballero, 2 E. Thiel, 3 S. Bologna, 4 M.J.S. Dyer, 5 S. Luminari, 6 1Università di Modena e Reggio Emilia, MODENA, Italy; 2Hospital Clinico Universitario, SALAMANCA, Spain; 3Charité Campus Benjamin Franklin, BERLIN, Germany; 4Hospital Bariatric Adulte, VANDEOUVRE LES NANCY, France; 5University of Leicester, LEICESTER, United Kingdom

**Background.** R-CHOP regimen has become the standard treatment for CD20+ diffuse large B-cell lymphoma (DLBCL). However, the majority of cases occur in elderly patients whose tolerance to immunochemotherapy is limited. Liposomal doxorubicin citrate (Myocet™) has an improved therapeutic index in comparison to standard doxorubicin, and may increase the tolerability of effective therapy in vulnerable populations.

**Methods.** Up to eight cycles of the R-COMP regimen containing Myocet(m) 50 mg/m², cyclophosphamide 750 mg/m², and vincristine 1.4 mg/m² (max. 2 mg) were administered on d1 every 3 weeks, plus rituximab 375 mg/m² (d3 C1, d1 thereafter) and prednisone 100 mg/d d1-5. Restaging was performed after 3 cycles and patients (pts) with an objective response received 5 additional cycles. A two stage Simon design was adopted. Complete response rates of 40% and 60% were defined in advance as the level of no interest (P0) and the level of interest (P1), respectively. Previously untreated elderly (>60 years) pts with CD20+ and stage I/II bulky IV DLBCL were included. Pts with CNS involvement or VH+ were excluded. Results. Between Oct 2002 and Apr 2005, 75 pts were registered. Fifty-nine pts were evaluable for efficacy. Reasons for exclusion from the evaluable population included patient ineligibility, patient not treated, early termination and failure to undergo disease re-evaluation according to the protocol total 16 patients. The median age was 71 years (range 60-83). At diagnosis, 56% of pts had an international prognostic index (IP) score > 2, 2% had B symptoms, 3% had Bulky disease > 10 cm in diameter, 70% had stage III or IV disease, 52% had increased LDH and 64% had extranodal disease. Median IVEF at baseline was 61% (range 50-89%). The mean number of cycles admini-
Background: the outcome of patients (pts) with PTCL is dismal. Because of this, there is an increasing interest to investigate intensive treatments in these pts. Aims: to analyze the results in terms of toxicity, response and outcome, of a phase II trial that includes high-dose chemotherapy (CT) plus ASCT as first line treatment for pts with PTCL. Methods: forty pts (29M/11F; median age: 47 yrs) diagnosed with PTCL (excluding cutaneous and anaplastic ALK+), in stages II-IV and <65 yrs, who have finished the planned therapy, are the subject of this analysis. Pts received intensive CT (3 courses of high-dose CHOP [cyclophosphamide 2000 mg/m² day 1, adriamycin 90 mg/m² day 1, vincristine 2 mg day 1, prednisone 60 mg/m² day 1 to 5, mesna 150% of cyclophosphamide dose, G-CSF 300 µg/day days 7 to 14], alternating with 3 courses of standard ESHAP). Responders (CR or PR) were submitted to ASCT. Results: twenty-three patients had a PTCL unspecified, nine angioimmunoblastic, two panniculitic and six other subtypes. Eleven pts (28%) presented with primary extranodal disease, 28 (70%) were in stage IV, and 14 (35%) had bone marrow involvement. Forty five percent of the pts had high/intermediate or high-risk IPI, whereas 49% were in the groups 3 or 4 according to the Italian Index for PTCL. Twenty seven pts (68%) received the planned 3 courses of CT. Response rate after CT was: CR, 19 cases (47.5%); PR, 4 (10%); failure, 17 (42.5%), including one pt who died because of sepsis. Hematological toxicity of CT mainly consisted of neutropenia (grades 3-4 in 87 and 62%, respectively) and thrombocytopenia (grades 3-4 in 63 and 68%, respectively). Severe infection requiring hospitalization was observed in 38 and 15% of courses of high-dose CHOP and ESHAP, respectively. Only 16 of the 23 candidates (70% of all candidates and 40% of all pts) received ASCT due to the lack of stem-cell mobilization (3 cases), severe previous toxicity (2), early relapse (1) and pt decision (1). No differences in the outcome were seen among these 23 pts according to whether or not they eventually received ASCT. No major toxicity was observed after ASCT. Response after the whole treatment was: CR, 20 cases (50%); PR, 3 (8%); failure, 17 (42%). Two of 14 pts in CR and 2 pts in PR eventually progressed. Four-year failure-free survival (FFS) was 58% (95%CI: 14-64%), whereas 4-year event-free survival for pts achieving CR was 63% (95%CI: 43-89%). Twenty-one pts have died during follow-up, with a 4-year overall survival (OS) of 40% (95%CI: 21-55%). Most patients died because of PTCL progression, but 2 died in CR due to secondary leukemia and lung cancer, respectively. Both the IPI and the Italian Index were able to predict FFS and OS. Conclusion: in this series of patients with PTCL, a relatively high CR rate was obtained with high-dose CHOP/ESHAP followed by ASCT. Toxicity was manageable. However, the prognosis of patients with PTCL, particularly of those not achieving CR, is still very unfavorable.
Non-Hodgkin’s Lymphoma - Clinical II

0199

CLINICAL OUTCOME OF LOW GRADE NON HODGKIN’S LYMPHOMA PATIENTS WITH BONE MARROW INVOLVEMENT DETECTED BY FLOW CYTOMETRY ALONE

M. Lisherer, N. Gronich, J. Radnay, H. Shapiro, Y. Manor, M. Lahav
'Mic Medical Center, KFAR SABA, Israel; 'Rabin Medical Center, PETAHC-TIKVA, Israel

Background. BM involvement in low grade NHL patients results in stage IV clinical classification and has a negative impact on survival. The standard practice is morphologic examination of BM biopsy conducted at diagnosis. In many institutions flow cytometry (FC) is also routinely performed on BM aspirate samples accompanying respective biopsies. FC is believed to increase the sensitivity of the morphologic analysis by detecting occult lymphoma cells evading the pathologist’s eyes. However, the prevalence of such finding and especially its clinical significance are largely unknown. In our institute bone marrow biopsies of NHL patients conducted after 1993 were routinely accompanied by bone marrow aspirates with FC analysis. Aims. We aimed to analyze the prevalence and clinical significance of BM FACS findings in patients with low grade NHL. Methods. We retrospectively reviewed the charts of all low grade NHL patients (small lymphocytic lymphoma, follicular small cleaved cell NHL, follicular mixed small and large cell, marginal zone B-cell lymphoma, mantle cell lymphoma and Waldenstrom macroglobulinaemia) diagnosed or followed in the Hematology Unit between 1994 and 2004, who had undergone bone marrow biopsies and aspirates as a part of their diagnostic workup or before treatment. Flow cytometric results were considered positive if they showed either a ratio of immunoglobulin light chain expression of kappa/lambda >3.2 and lambda/kappa >2.1 in at least 2% of the gated population. Selected cells were analyzed by two or three color combinations: CD5 versus CD19, CD20 versus CD10, and kappa light chain versus lambda light chain occasionally with the addition of CD19 or CD20. Results. Lymphoma involving BM by morphology was found in the biopsies of 48 patients (61.4%) (BM+ group). Of the remaining 1 patient had inconclusive results and 26 patients had normal BM biopsies. Of these 27 patients the FC analysis was positive in 9 patients (BM-FC+ group) and negative in 18 (BM-FC-) group. We could not compare the groups using FLIPI or IPI scores as a whole since both include the stage as one of the five summed parameters while BM involvement was different by definition between the groups. However, the groups had similar parameters that are prognostically important and are part of the FLIPI scoring system including age, hemoglobin and LDH levels and also the number of involved extranodal sites. Splenic involvement and number of involved nodal sites were higher in BM+ and BM-FC+ groups than in BM-FC- group. Significant differences in disease progression as indicated by time-to-treatment were observed. The median treatment-free period was shorter in the BM+ and BM-FC+ groups (1 month and 4 months, respectively) as compared with the BM-FC- group (31 months) (log rank test p=0.0195). BM-FC-patients had significantly longer survival time than BM+ and BM-FC+ groups. Median survival time was not reached for the BM-FC- patients while in the BM+ and BM-FC+ groups median survival times were 129 and 89 months respectively with no significant difference between them. (Log rank test=0.029 for the difference between BM-FC- and the two other groups). Conclusions. We conclude that the outcome of low grade NHL patients found to have malignant cells by FC analysis while their BM morphology is normal is the same as that of patients with histological involvement. This may imply that patients with localized disease who have bone marrow involvement by FC should be regarded as advanced stage disease.

0200

CHOP (DOXORUBICIN) CHEMOTHERAPY IS SUPERIOR TO CNOP (MITOXANTRONE) IN THE TREATMENT OF PATIENTS WITH AGGRESSIVE NON-HODGKIN’S LYMPHOMA (REVIEW)

M. Björkholm, T. Andersson, A. Ahlbom, E. Ösby
Karolinska University Hospital, STOCKHOLM, Sweden, Karolinska Institute, STOCKHOLM, Sweden

Introduction. Mitoxantrone (M), an anthracenedione, was introduced in the early/mid 1980s as a more tolerable alternative to anthracyclines. This agent has a broad anti-tumour activity including lymphoma with potentially less cardiotoxicity than doxorubicin (D), which may be of particular importance in the elderly patient population. However, an important issue is whether M is as efficacious as D in the treatment of NHL patients. Methods. Through search of several relevant databases and direct contacts with lymphoma investigators worldwide, we identified seven randomised studies of previously untreated patients comparing CHOP and CNOP chemotherapy in aggressive NHL. In this analysis we included five trials where (D; 50 mg/m²) was compared with (M;10-12 mg/m²); table) and the interval between chemotherapy courses was 3-4 weeks. Patients reported in the Pavlovsky article were included in the Bezwooda report, why analyses were performed with and without patients reported by Bezwooda et al. Odds ratios of complete remission (CR) and overall survival (OS) were pooled using a fixed effects model. Results. CHOP was significantly superior to CNOP with regard to both CR rate and OS (Figures 1-2).

Table. Randomised trials comparing doxorubicin with mitoxantrone in untreated patients with aggressive NHL.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Regimen</th>
<th>Dose of doxorubicin/mitoxantrone (mg/m²)</th>
<th>Treatment interval (weeks)</th>
<th>Median patients age (years)</th>
<th>Number of patients &gt;60 years (%)</th>
<th>CR rate (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osby et al. 2003 CHOP</td>
<td>50</td>
<td>3</td>
<td>205</td>
<td>71</td>
<td>205</td>
<td>60***</td>
<td>56***</td>
</tr>
<tr>
<td>Pavlovsky et al. 1995 CHOP</td>
<td>50</td>
<td>3</td>
<td>164</td>
<td>55</td>
<td>69</td>
<td>51*</td>
<td>40 n.s.</td>
</tr>
<tr>
<td>Sonneveld et al. 1995 CHOP</td>
<td>50</td>
<td>4</td>
<td>72</td>
<td>70</td>
<td>72</td>
<td>48*</td>
<td>42*</td>
</tr>
<tr>
<td>Sonneveld et al. 2001 CHOP</td>
<td>50</td>
<td>3-4</td>
<td>20</td>
<td>57*</td>
<td>57*</td>
<td>40 n.s.</td>
<td>53 n.s.</td>
</tr>
<tr>
<td>Sonneveld et al. 1999 CHOP</td>
<td>50</td>
<td>3-4</td>
<td>20</td>
<td>57*</td>
<td>57*</td>
<td>70 n.s.</td>
<td>58 n.s.</td>
</tr>
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<td>Sonneveld et al. 1990 CHOP</td>
<td>50</td>
<td>3-4</td>
<td>20</td>
<td>57*</td>
<td>57*</td>
<td>70 n.s.</td>
<td>58 n.s.</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001; n.s. = Not significant; NI = No information; Approximately (visual reading); *Mean; p<0.05

Myelosuppression was not more severe using CHOP, rather the opposite. However, the incidence of gastrointestinal toxicity and alopecia was significantly lower in patients treated with CNOP. Conclusion. CHOP chemotherapy is more efficacious than CNOP at equitoxic (myelosuppression) doses leading to higher CR rates and improved survival.
**Early-Mid Treatment CR-Active Protein Levels Predict Time to Disease Progression or Relapse as Well as Overall Survival in Aggressive Non-Hodgkin’s Lymphoma**

Y. Herishanu,1 C. Perry,2 U. Metzer,3 L. Gipstein,1 S. Trestman,1 O. Goor,1 A. Polliaic,1 E. Napartek1

1Tel-Aviv Sourasky Medical Center, TEL-AVIV, Israel; 2Hadassah Medical Center, JERUSALEM, Israel

**Background.** Higher pretreatment serum CRP levels in patients with aggressive non-Hodgkin’s lymphoma (NHL) are associated with a more aggressive histology, B-symptoms and a shorter overall survival (OS). In most patients who achieve complete remission (CR) at the end of therapy, serum CRP levels appear to return to normal range. In the light of an emerging role for early-mid treatment FDG-PET as an important prognostic indicator for progression free survival (PFS) and OS in NHL, we considered whether a simple parameter, such as early-mid treatment CRP, could also be a significant prognostic factor in this respect. Aims. To evaluate the possibility that wide ranged CRP could predict early response to treatment, time to progression or relapse and overall survival in aggressive NHL.

**Patients and Methods.** Serum CRP levels were monitored in fifty five patients with aggressive NHL (newly diagnosed and relapsed) at baseline and before receiving each of the next 5 chemotherapy cycles. The lowest value of the early mid-term CRP levels recorded was compared to the interim FDG-PET results, as well as to the clinical course and outcome. Results. At baseline, patients with aggressive NHL presenting with B-symptoms or bulky disease had higher pretreatment CRP levels compared to those recorded in asymptomatic patients and those without bulky disease (mean 90±71.9 mg/L Vs 37.7±41.9, p=0.0013 and mean 76.8±54.2 Vs 40.2±55.9 mg/L, p=0.04, respectively). Pretreatment CRP levels ≥20 mg/L were also associated with a shorter overall survival (p=0.029). During chemotherapy, the lowest value of early-mid treatment CRP levels significantly predicted the results of the interim FDG-PET (p=0.004 with a hazard ratio of 1.28). This implies that any increase of 1 mg/L in the serum CRP level enhances the risk of a positive FDG-PET scan by 12.8%. Moreover, patients who did not achieve an early-mid treatment CRP level of <5 mg/L, appear to have a shorter time to disease progression or relapse (p=0.001) and a reduced overall survival (p=0.016) (Figure 1). In multivariate analysis, both early-mid treatment CRP levels and interim FDG-PET findings significantly predicted PFS (p=0.02 and p=0.004, respectively), while OS was significantly predicted by the early-mid treatment CRP levels (p=0.016) and by the International Prognostic Index-IPI (p=0.08). Conclusions. The early-mid treatment serum CRP level is an important prognostic factor in aggressive NHL. Patients who do not achieve an early-mid treatment level of <5 mg/L have faster disease progression or earlier relapse and also appear to have an inferior overall survival.

![Figure 1. Kaplan-Meier curves of PFS and OS.](image-url)

**BEAC or BEAM Chemotherapy Followed by Autologous Stem Cell Transplantation in Non-Hodgkin’s Lymphoma Patients: Comparative Analysis on Efficacy and Toxicity**


Asan Medical Center, SEOUL, South-Korea

**Background.** Non-Hodgkin’s lymphoma (NHL) is the major indication of high dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT). However, little is known on the comparative efficacy and toxicity of various HDC regimens. Aims. This study aimed to compare the efficacy and toxicity of BEAC and BEAM regimen. Methods. Between April 1994 and February 2005, 97 NHL patients were received HDC with BEAC (N=69) or BEAM (N=28) followed by ASCT at Asan Medical center. We matched one patient received BEAM with two patients received BEAC who has same International Prognostic Index (IPI). Thus total 84 patients (56 in BEAC group and 28 in BEAM group) were analyzed. Results. Of 84 patients, 52 (62.5%) were male, 29 (34.5%) were female and median age was 40.5 (15-65) years. Baseline characteristics such as age, sex, disease status at ASCT, Histology, stage at ASCT, IPI were not different between two groups. Time to neutrophil engraftment (WBC >0.5×10^9/mm³) was significantly longer in BEAC group (14±5 days) than in BEAM group (11±2 days, p=0.002). Total amount of RBC transfusion was more in BEAC group than in BEAM group (6.5 units vs. 3.7 units, p=0.037). Time to platelet engraftment (platelet >20×10^9/mm³) was faster and total amount of platelet transfusion was less in BEAM group. Patients received BEAM had more frequent WHO grade ≥2 diarreha than those received BEAC (46.4% vs. 19.6%, p=0.010). But, other clinically important toxicity such as mucositis, nausea/vomiting, bleeding were not different between two groups. In addition, neutropenic fever and documented infection were not different between two groups. Two year overall survival (OS) rate was 30% in BEAC group and 66% in BEAM group. Two year event free survival (EFS) rate was 34% in BEAC group and 61% in BEAM group. Both OS and EFS was significantly superior in BEAM group than in BEAC group (p=0.049, p=0.032, respectively). Summary/Conclusions. BEAM appears to be a superior HDC regimen in the aspect of OS and EFS than BEAC while regimen related toxicity is similar except more frequent diarreha in BEAM.

**Rapid Infusion of Rituximab With Or Without Steroid Containing Chemotherapy. One Year Experience in a Single Centre**

A. Salar, D. Casao, M. Cervera, C. Pedro, E. Gimeno, E. Abella, A. Alvarez-Larrán, M. Calafell, C. Besses

Hospital del Mar, BARCELONA, Spain

**Background.** Infusion-related toxicity is frequent after the administration of rituximab despite the fact that strict guidelines have been recommended. Recently, a rapid rituximab infusion schedule in combination with a steroid containing chemotherapy regimen was well tolerated and safe. Aim. To assess the feasibility of a fast infusion of rituximab with or without steroid containing chemotherapy. Methods. Inclusion criteria: disease susceptible of treatment with rituximab and having been treated with a first infusion of rituximab according to the product monograph. Exclusion criteria: lymphocytosis > 5×10^3/L, toxicity grade 5/5 the previous infusion of rituximab or dose ≥ 375 mg/m². Schedule: First infusion of rituximab according to the product monograph; Further infusions over a total time of 90 minutes (20% in the first 30 minutes and the remaining 80% over 60 minutes). Premedication: acetaminophen and diphenhidramine, plus methylprednisolone in only those patients receiving steroid containing chemotherapy. Results. A total of 70 patients were treated for a total of 314 infusions. Patient characteristics: median age 64 yr (range 28-87), 47% males, DLBCL 36%, follicular 40%, mantle 6%, MALT 11%, other 7%. Number of rituximab infusions: 199 as treatment (combined or not with chemotherapy) and 115 as maintenance. Number of rituximab administrations with and without steroids: 123 and 191 infusions, respectively. Median time from previous rituximab infusion was 28 days (range 7-272). Sixteen rapid infusions were administered with an interval greater than 90 days from the previous standard infusions. This rapid rituximab administration schedule was very well tolerated. No grade 3/4 adverse events were seen. Three patients referred symptoms during rituximab infusion (grade 1) and all these reactions occurred in patients who did not receive premedication with steroids. Conclusions. Rituximab administration in a 90-minute infusion schedule is well tolerated and safe in this group of patients. This approach is beneficial, both in patients who are administered steroids and in patients who are not.
RITUXIMAB PLUS CLADRIBINE OR CLAD-RPHOSPHAMIDE IN HEAVILY PRETREATED PATIENTS WITH INDOLENT LYMPHOPROLIFERATIVE DISORDERS

T.R. Robak,1 P. Smolewski,1 A. Szmigielska-Kaplon,1 B. Cebula,1 J.Z. Błonski,1 K. Chojnowski1
1Medical University of Lodz, LODZ, Poland; 2Med Uniłodz, LODZ, Poland

Background. Preclinical studies have shown synergistic or additive effects of rituximab combined with purine nucleoside analogs, fludarabine or cladribine (2-CdA). Aim. In this report we present the results of our study evaluating the feasibility, efficacy and toxicity of the combined regimens consisting of rituximab plus 2-CdA (RC regimen) or rituximab, 2-CdA and cyclophosphamide (RCC) in the treatment of patients with heavily pretreated indolent lymphoid malignancies. Methods. Between March 2001 and November 2005 54 adult patients with relapsed or refractory low grade non-Hodgkin lymphoma (LG-NHL) and B-cell chronic lymphocytic leukemia (CLL) were treated according to RC/RCC regimens. The RC protocol consisted of rituximab at a dose of 375 mg/m² i.v. on day 1 and 2-CdA given at a dose of 0.12 mg/kg/d on days 2 to 6. In RCC protocol rituximab was administered at a dose of 375 mg/m² on day 1, 2-CdA 0.12 mg/days 2 to 4 and 2 cyclophosphamide at a dose 650 mg/m² i.v. on days 2 to 4. The cycles were repeated every 28 days or longer if severe myelosuppression occurred. Guidelines for response were those developed by the NCI-sponsored Working Group. Results. Fifty four patients, 52 with B-CLL and 22 with LG-NHL entered the study and all of them were eligible. Thirtysix patients (61.1%) were recurrent after prior therapy and 21 (38.9%) had refractory disease. All patients received 3 or more cycles of chemotherapy before RC/RCC treatment. Thirty-one patients were treated with RC regimen and 23 with RCC regimen. The RC/RCC courses were repeated at 4 week intervals or longer if severe myelosuppression occurred. One hundred fifty six cycles of RC/RCC with median of 3 cycles per patient were administered (range 1-5 cycles). Six patients (11.1%), 2 with B-CLL and 4 with LG-NHL, achieved a complete response (CR). Thirty two patients (59.25%), including 25 with B-CLL and 9 with LG-NHL, had a partial response (PR). Overall response rate (OR) was 70.4% in the whole group, from 59.1% in LG-NHL to 78.1% in B-CLL patients. The median failure-free survival (FFS) of responders was 10.5 months. Hypersensitivity to RIT was the major toxicity of RC/RCC regimens, and occurred in 14 patients (25.9%), mostly during the first infusion of RIT. Severe neutropenia (grade III-IV) was seen in 5 patients (9.25%). Eight (14.8%) episodes of grade III-IV infections were observed. One patients died from severe pneumonia complicated with septic shock after second cycle of RCC regimen. Severe thrombocytopenia (grade III-IV) occurred in 4 patients (7.4%). Conclusion. RC and RCC regimens are highly effective and well tolerated modalities of treatment in heavily pre-treated patients with indolent lymphoproliferative disorders.

A RETROSPECTIVE STUDY TO ASSESS RELATIVE DOSE INTENSITIES IN PATIENTS WITH LYMPHOMA IN CENTRAL EUROPEAN COUNTRIES

M. Trneny,1 M. Waldeck,1 M. Ladicka,1 G. Kreuzbauer,1 T. Skacel1
1Charles University General Hospital, PRAGUE, Czech Republic; 2Szent László Kórház, I. Belgyógyászat Ha, BUDAPEST, Hungary; 3MSC Cancer Centre, WARSAW, Poland; 4National Cancer Institute, BRATISLAVA, Slovak Republic; 5Azienda Ospedaliera Universitaria, FLORENCE, Italy; 6Integroup Italiano Linfomi, REGGIO EMILIA, Italy

Background. Maintenance chemotherapy drug dose intensity is important for the success of treatment of cancer patients. However, myelosupenia and its complications are major dose-limiting factors. Data on chemotherapy-related reductions in dose intensities for lymphoma patients from Central European (CE) countries remain sparse. Aim. To assess the relative dose intensities in patients with Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) in CE countries. Methods. Chemotherapy treatment data from 1995 to 2004 were retrospectively collected from 484 patients undergoing chemotherapy treatment for lymphoma from 24 centres in 4 CE countries: Czech Republic (26%), Hungary (13%), Poland (44%) and Slovakia (17%). For this sub-analysis, 510 patients who received either doxorubicin, vinblastine, bleomycin and dacarbazine (AVBD) treatment for HL (117 patients) or cyclophosphamide, doxorubicin, vincristine and prednisone + rituximab every 21 days (CHOP21±R) for NHL (193 patients) were considered. Results. Of 116 HL patients with full data records (median age 29 years), 112 (96%) had classical disease and 4 (3%) had lymphocyte-predominant HL; for 189 NHL patients with full records (median age 56 years), 179 (95%) were B-cell and 10 (5%) were T-cell. AVBD was administered to 100% of the 117 HL patients selected for this study and CHOP21±R was administered to the 193 NHL patients, of which 59 patients (30%) also received rituximab. Dose delays >7 days were observed in 271 out of 1583 cycles (17%; HL: 110 of 648-17%; NHL: 161 of 958 - 17%). Overall, 221 patients (72% of 306 considered for this analysis) experienced at least one dose delay during their treatment. This corresponds to 95 of 117 HL-AVBD patients and 126 of 189 NHL-CHOP21±R patients (61% and 66% respectively). One hundred and forty-three patients (47% of 305 patients) experienced a dose reduction ≥15% in at least one cycle, of which 90 patients (30% of 305) received ≥15% reduction in their overall dose. Dose reduction of ≥15% in any cycle occurred in 61 HL-AVBD patients (52% of 117) and in 82 NHL-CHOP21±R patients (44% of 188), with 51 HL-AVBD and 59 NHL-CHOP21±R patients receiving ≥15% reduction in their overall dose (44% and 21% respectively). The relative total dose intensity (RTDI, see Table 1) at the end of treatment was as follows: 55.6% of HL-AVBD patients received ≥85% RTDI and 59.3% received ≥90% RTDI; 73.4% of NHL-CHOP21±R patients received ≥85% RTDI and 59% received ≥90% RTDI. G-CSF was used in 71 of 765 cycles (9.3%) of chemotherapy and in 73 of 1124 cycles of NHL-CHOP21±R (6.5%). There were 48 unplanned hospitalisations in 30 patients (5 HD-AVBD and 25 NHL-CHOP21±R); 21 hospitalisations were neutropenia-related. Summary/Conclusions. The reduction of RTDI, and its associated problems, upstream of the chemotherapy patients receiving chemotherapy is a major concern. The data observed in CE countries are similar to US centres (Lyman et al. JCO 2004; 22: 4302-4311). Further analysis of these data will enable a better understanding of the implications of reduced RTDI in lymphoma and help to identify those patients who require preventative treatment.

Table 1. RTD at end of treatment.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
<th>total patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-AVBD</td>
<td>32 (27.35)</td>
<td>20 (17.03)</td>
<td>38 (32.48)</td>
<td>27 (23.08)</td>
<td>46 (39.3)</td>
<td>117</td>
</tr>
<tr>
<td>NHL-CHOP21</td>
<td>33 (27.55)</td>
<td>17 (14.90)</td>
<td>69 (59.70)</td>
<td>69 (59.70)</td>
<td>111 (95.0)</td>
<td>180</td>
</tr>
</tbody>
</table>

*Total number of patients with data available for this analysis.

A COMPARISON BETWEEN 2-DEXY-2-[18F]FLUORO-3-GLUCOSYL POSTIMISSION AND COMPUTER-TOMORAPHY FOR STAGING OF PATIENTS WITH HODGKIN LYMPHOMA

L. Rigacci,1 E. Merli,1 A. Castagnoli,1 A. Versari,1 L. Nassi,1 T. Chiesi,1 A. Gallamini,1 A. Biggi,1 U. Vitolo,1 R. Sacchetta,1 P. Pregno,1 F. Salvi,1 A.M. Scattolin,1 A. Levis,1 A. Bosi1
1Azienda Ospedaliera Universitaria, FLORENCE, Italy; 2Integroup Italiano Linfomi, REGGIO EMILIA, Italy

Background. Accurate staging in lymphoma patients (pts) has an important role in the treatment and allows minimization of toxic therapies, such as extended field radiation or chemotherapy. Particularly in HL a tailored therapy decrease the risk of secondary malignancies which exceeds 10% in several historical series in patients with early stage disease. Anatomic imaging modalities lack sensitivity and specificity because the definition of lymph node involvement is based on size criteria. During the last decade FDG-PET has been introduced for noninvasive staging of lymphoma. Methods. Herein we propose a prospective multicentric study with the aim to assess the impact of FDG-PET on the staging of pts with diagnosis of HL. A total of 186 consecutive pts coming from six Italian hematological Institutions underwent a FDG-PET scan in addition to conventional staging procedures, which include physical examination, laboratory data, bone marrow biopsy and imaging of the neck, thorax, abdomen and pelvis using CT scan. In general the adjunctive informations from PET did not influence the therapeutic options in use at a given centre at a particular time. Results. Pts characteristics were the following: 98 male and 88 female, 140 (75%) with diagnosis of nodular sclerosis classical HL, 28 (15%) mixed cellularity classical HL, 11 (6%) lymphocyte-rich classical HL, 2 (1%) lympho-
cyte-depleted classical HL and 5 (3%) non specified HL. At clinical and infrared standard staging (11 (6%) pts were stage I, 112 (60%) stage II, 42 (22%) stage III and 21 (12%) stage IV. FDG-PET and CT were concordant in 156 out 186 pts (84%). FDG-PET allowed to identify in 38 out 156 concordant stage more nodal (52 pts) or extranodal (6 pts: two bone, two spleen, two liver and spleen) involvement in comparison with CT imaging. In eight out 156 (5%) concordant stage CT showed one more involvement in comparison with FDG-PET. FDG-PET in 64%, relative to FDG in 100%, stage IV in 66% (bene marrow involvement in 86% and CNS involvement in 36%), Interna-

tional Prognostic Index (IPI) ≥ 3 in 71.4%, of the cases, respectively. Hos-
tological analysis and immunophenotyping performed on nodule tissue biopsy and/or bone marrow tumor cells showed a DLBCL in 9 and a ‘Burkitt-like’ in 4 patients, respectively. Cytogenetic analysis (conven-
tional cytogenetics and FISH analysis) showed in all cases the combina-
tion of (14:18) translocation and c-myc rearrangement. All patients were treated with chemotherapy regimens (R-CHOP (n=8) or High-dose CHOP (n=6)), and could receive subsequent high-dose front-line ther-

apy with autologous (n=5) or allogenic stem cell transplantation (n=2). Most patients (12/18=66%) initially responded to induction chemother-

apy but disease response was dramatically short, precluding a planned stem cell transplantation in most cases. Despite salvage chemotherapy, all patients, even those who could receive early stem cell transplantation, progressed and the median overall survival from diagnosis is 4 months (1-10). In conclusion, DLBCL with a tandem (14;18) translocation and c-myc rearrangement is a very aggressive entity with rapid progressive disease. Innovative strategies are warranted in this subgroup of patients.

**0207**

MARKED ACTIVITY OF BORTEZOMIB, RITUXIMAB, AND DEXAMETHASON (BORID) IN HEAVILY PRETREATED PATIENTS WITH MANTLE CELL LYMPHOMA

J. Drach, H. Kaufmann, O. Pichelmayer, V. Sagaster, S. Seidl, A. Chott, C. Zielinski, M. Raderer

*Medical University Vienna, VIENNA, Austria*

**Background.** Bortezomib (B) belongs to a new class of anti-cancer agents, the proteasome inhibitors, and has documented activity in multiple myeloma and mantle cell lymphoma (MCL). Preclinical studies suggest that B has synergistic activity with rituximab (R), which provides a rationale for the exploration of treatment combinations. Aims. We have initiated a phase II study in relapsed/chemotherapy refractory MCL to evaluate the activity and safety of B in combination with R and dexamethasone (BORID). Methods. A treatment cycle consists of B at 1.3 mg/m^2^ administered on days 1, 4, 8, and 11, R at 3.75 mg/m^2^ admin-
istered on day 1, and dexamethasone 40 mg orally on days 1 to 4. Cycles are repeated every 3 weeks for a total of 6 treatment cycles. Patients (pts) with progressive MCL after at least one prior line of therapy (including CHOP or a CHOP-like regimen) are eligible. Results. Up to now, we have enrolled 11 pts (median age, 67 years; range, 40 to 75 years) after a medi-
an of 3 lines of prior therapies (range, 1 to 6) including R in 9 pts, high-
dose therapy in 4 pts, and thalidomide in 5 pts. Median time between start of frontline therapy and study inclusion was 42 months (range, 11 to 96 months). Severe adverse events (> grade II) included infections (herpes zoster in 2 pts, bacterial pneumonia, mucosal candidiasis), peripheral neuropathy (3 pts), fatigue (2 pts) and vasculitic skin infil-
trates in 3 pts. Thrombopenia (< 50 g/L) occurred in 2 pts. All adverse events were manageable by standard means of supportive care and pro-
longation of the treatment interval between cycles. Of 9 pts evaluable for efficacy, 8 have achieved a response (3 CR, 4 PR), and 1 pt experi-
cenced stable disease. Pts in CR were also negative for disease activity by PET scanning. Skin infiltrates (histologically proven T-cell infiltrates) preceded achievement of CR in 2 pts. Among 7 pts with follow-up beyond 6 months, 2 pts have relapsed (progression-free survival 9 and 11 months, respectively), and 5 pts are still progression-free at 12, 11, 7, and 6 months, respectively, after treatment initiation. Recruitment of patients is ongoing, and updated results will be presented. Conclusions. Data obtained thus far indicate that BORID has promising activity and manageable toxicity in patients with heavily pretreated MCL, and develop-
ment of a vasculitic rash may be an early indicator of a favorable response.

**0208**

CLINICAL AND CYTOGENETIC CHARACTERISTICS OF HIGH-GRADENON-HODGKIN LYMPHOMA (RHNL) WITH A COMBINATION OF (14;18) TRANSLATION AND C-MYC REARRANGEMENT: A VERY AGGRESSIVE ENTITY WITH A DISMAL PROGNOSIS


*CHU Nantes, NANTES, France; Centre Cathedre de Sienne, NANTES, France*

Diffuse Large B cell lymphoma (DLBCL) is an heterogeneous entity with various clinical, cytological, cytogenetic and molecular features. In this single center, retrospective analysis, we report the results of a cohort of 14 patients treated from 1997 to 2005 with a diagnostic of DLBCL or ‘Burkitt-like’ lymphoma characterized by a tandem (14;18) translocation and c-myc rearrangement. Patients were 9 males, 5 females with a medi-
an age of 52 years (36-75). At the time of diagnosis, all patients present-
ed with poor clinical and biological features: B symptoms in 78.5%, ECG in 64%, elevated LDH in 100%, stage IV in 66% (bone marrow involvement in 86% and CNS involvement in 36%), Interna-
tional Prognostic Index (IPI) ≥ 3 in 71.4%, of the cases, respectively. Hos-
tological analysis and immunophenotyping performed on nodule tissue biopsy and/or bone marrow tumor cells showed a DLBCL in 9 and a ‘Burkitt-like’ in 4 patients, respectively. Cytogenetic analysis (conven-
tional cytogenetics and FISH analysis) showed in all cases the combina-
tion of (14:18) translocation and c-myc rearrangement. All patients were treated with chemotherapy regimens (R-CHOP (n=8) or High-dose CHOP (n=6)), and could receive subsequent high-dose front-line ther-

apy with autologous (n=5) or allogenic stem cell transplantation (n=2). Most patients (12/18=66%) initially responded to induction chemother-

apy but disease response was dramatically short, precluding a planned stem cell transplantation in most cases. Despite salvage chemotherapy, all patients, even those who could receive early stem cell transplantation, progressed and the median overall survival from diagnosis is 4 months (1-10). In conclusion, DLBCL with a tandem (14;18) translocation and c-myc rearrangement is a very aggressive entity with rapid progressive disease. Innovative strategies are warranted in this subgroup of patients.
90Y ibritumomab tiuxetan did not correlate with the risk of relapse as determined by univariate analysis. Summary/Conclusions. 90Y ibritumomab tiuxetan may be safely incorporated into conditioning regimens prior to ASCT, even in patients >60 years. Late relapses were uncommon, which suggests this approach may lead to durable remissions in MCL.

**0210**

GASTRECTOMY PLUS CHEMOTHERAPY VS. CHEMOTHERAPY ALONE IN GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA OF EARLY STAGE

D. Mihou,1 P. Konstantinidou,2 D. Markala,3 Fr. Patakiouta,7 N. Constantinou1

1 ‘Theagenion’ Cancer Center, THESSALONIKI, Greece; 2 ‘Theagenion’ Cancer Center, THESSALONIKI, Greece

Background. Stomach represents the most common site of primary extranodal diffuse large B-cell lymphoma (DLBCL). The ideal therapeutic strategy in gastric DLBCL remains controversial. Gastrectomy prior to chemotherapy is favored by some, while others prefer sole administration of chemotherapy. Aim. To evaluate and compare these two therapeutic strategies, gastrectomy plus chemotherapy and chemotherapy alone, in gastric DLBCL of early stage in the context of a retrospective study. Methods. Between 1979 and 2003, 78 patients with gastric DLBCL of early stage (I-II, no X) were diagnosed and treated in our department. Patients were divided in group A that comprised 46 (59%) patients, who underwent gastrectomy prior to chemotherapy and group B that consisted of 32 (41%) patients, who received chemotherapy alone. Chemotherapy in both groups included CHOP and CHOP-like regimens. Median number of chemotherapy cycles administered in group A and B was 6 (5-9) and 6 (2-9) respectively (p=0.05). Rituximab was also administered in 14 (30.4%) patients of group A and in 12 (37.5%) patients of group B (p>0.05). Five (11%) patients of group A and 2 (6.3%) of group B received additionally radiation therapy (p>0.05). The characteristics of our patients (gender, age, stage, IPI, presence of B symptoms and extranodal involvement other than primary), as well as response rates, were compared between the two groups using chi-square tests. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Results. Median follow-up time for patients in groups A and B was 70 (270) and 46 (2-155) months respectively. On an intention-to-treat basis, the complete response rate was 91.3% for group A and 87.5% for group B (p>0.05). DFS, OS and FFS rates at 4 years in groups A and B were 89.1% and 92.9%, 80.2% and 81.5%, 75.3% and 76.3% respectively (p>0.05). Conclusion. Gastrectomy plus chemotherapy failed to prove its superiority as treatment for gastric DLBCL of early stage in our study. Similar response and survival rates were achieved with chemotherapy alone, saving at the same time the patient from the morbidity impact of gastrectomy on quality of life.

**0211**

PREDICTORS OF SURVIVAL IN ELDERLY PATIENTS WITH AGGRESSIVE LYMPHOMA. A LONG-TERM FOLLOW-UP OF A RANDOMISED TRIAL

S.Y. Kristinsson,1 E. Ösby,2 H. Hagberg,2 S. Kvaloy,1 L. Teerenhovi,3 H. Anderson,3 H. Holte,4 E. Cavallin-Stahl,4 A. Ost,4 J. Myhr,4 D. Del Posto,4 H. Pertovaara,5 B. Nilsson,6 M. Björkholm7

1 Karolinska University Hospital, SOLNA, Sweden; 2 Uppsala Academic Hospital, UPPSALA, Sweden; 3 Det Norske Radiumhospital, OSLO, Norway; 4 Helsinki University Central Hospital, HELSINKI, Finland; 5 Lund University, LUND University Hospital; LUND, Sweden; 6 The Finsen Center, Rigshospitalet, COPENHAGEN, Denmark; 7 Tampere University Hospital, TAMPERE, Finland; 8 Medilab AB, Thy, Sweden

Background. We previously reported the results of a study in elderly patients (>60 years) with aggressive non-Hodgkin lymphoma (NHL) randomizing patients to receive CHOP (doxorubicin 50 mg/m²) or CNOP (mitoxantrone 10 mg/m²) with or without G-CSF (5 µg/kg from day 2 until day 10-14 of each cycle every 3 weeks; 8 cycles; Blood 2003;101: 3840). In that analysis 85% of patients were alive after a median follow-up time of 57 months. The main findings were 1) patients receiving CHOP fared better than those given CNOP chemotherapy and 2) the addition of G-CSF reduced the incidence of severe granulocytopenia and infections. We now report long-term follow up data with special emphasis on predictors of survival. Methods. The study included 455 previously untreated patients (median age 71 years; range 60-86 years) with stage III to IV aggressive NHL. Forty-seven patients previously hospitalized for class I to II congestive heart failure were randomized to receive CNOP with or without G-CSF (not included in the CHOP versus CNOP analysis). Results. After a median follow-up time of 115 months (18-151 months) 19% (88/455) of patients were alive. In univariate analysis CNOP treatment (p<0.001;figure), increasing age (p<0.001), poor performance status (p<0.001), high LDH (p<0.001), advanced stage (p<0.055), and the presence of more than one extranodal disease manifestation (p=0.045) negatively influenced overall survival from diagnosis. Gender (p=0.156), presence of B symptoms (p=0.079), bulky disease (p=0.085), and treatment with G-CSF (p=0.094) did not significantly affect overall survival. In multivariate analysis, all factors significant in univariate analysis except extranodal disease, remained significant and independent predictors of survival. Conclusion. At long-term follow-up of this large multicenter randomised study of elderly with aggressive NHL the projected 10-year survival of CHOP treated patients was in excess of 20%. Increasing age, poor performance status, high LDH and advanced stage independently predicted a poor survival.

Figure 1. Survival according to type of chemotherapy.

**0212**

UPDATE REPORT ON 78 PATIENTS WITH POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS FOLLOWED BY A SINGLE CENTER

E. Ravelli, V. Mancini, G. Mutti, P. Oreste, M. Nichelatti, E. Morra

Niguarda Ca’ Granda Hospital, MILAN, Italy

Background. Post transplant lymphoproliferative disorders (PTLDs) are a well recognized complication after solid organ transplantation, related to the chronic immunosuppressive regimen. The wide spectrum of histological features, clinical variability and high therapy-related toxicity make management of PTLD patients difficult. Aims. This abstract provides an update of clinical and pathological data of PTLD patients, followed at our Center between 1989 and 2006. Methods. Our study included 78 patients with a diagnosis of PTLD in solid organ transplant recipients (36 heart, 23 liver, 17 kidney and 2 lung). Morphological classification was made according to WHO criteria. In 72/78 patients tumour EBV status was tested by in situ hybridization for EBV encoded RNA (EBER). Results. It was not possible to assign 2/78 cases to any histological category because of inadequate specimens; 6/76 (8%) evaluable patients were classified as having Plasmacytic Hyperplasia (PH), 13/76 (17%) Polymorphic Lymphoproliferative Disorders (PLD) and 57/76 (75%) Malignant Lymphoma (ML). Fifty-nine of seventy-eight (75%) patients tested for EBER, 49/72 (68%) were EBV positive. While EBER positive PTLDs were heterogeneous with regard to time of occurrence and histological characteristics (6 PH, 11 PLD, 31 ML, 1 not classified), all EBER negative PTLDs were late onset ML. Diagnosis was obtained post-mortem in 9/78 patients. The treatment was tailored according to clinic-pathological features: 52/69 (75%) patients received a single agent or a combined regimen of chemotherapy, associated with antiviral drugs in EBER positive forms. Rituximab has been introduced in the therapeutic schedule of CD20+ PTLDs since 2000. It was administered to 24 patients, combined with chemotherapy in all but 2 cases.
Radiation and surgery were used when indicated. Eleven patients died early, before any treatment was completed (median time 13 days, range 5-56); 2/69 patients were lost at follow-up. Therefore, a total of 56 patients underwent their scheduled treatment and were evaluable for the outcome. We observed 10/56 deaths because of treatment related toxicity or disease progression. Complete remission (CR) was achieved in 45/56 (80%) patients. Of these, 8 (18%) relapsed, mostly responsive to second line therapy (7/8 patients), and 10 patients died because of late treatment-related toxicity or infection. One patient is still alive in partial remission (PR) at 23 months. The median survival time of evaluable patients was not reached at 3250 days (see Figure 1).

**Conclusions.** We can confirm that PTLD is a significant cause of mortality in solid organ transplant recipients: in our study the overall mortality rate was 45% (51/69) and the exitus mostly represents an early event, occurring within 6 months from diagnosis in 20/31 patients (64%). Nevertheless, timely and tailored treatment of the disease and its complications warrants long-lasting complete response with low relapse rate. PTLDs are characterized by wide clinico-pathological variability and represent a heterogeneous disease: better knowledge of biological parameters (e.g. EBV pathogenic role, donor or recipient PTLD origin, immunologic status of patients) could help to stratify our patients in different risk groups and could allow more appropriate treatment.

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**0213**

**RISK OF SECOND CANCER IN NON-GASTRIC MARGINAL ZONE B-CELL LYMPHOMA OF MALT: A POPULATION-BASED STUDY FROM NORTHERN ITALY**


*Department of Pathology, PAVIA, Italy; 2Division of Hematology, PAVIA, Italy; 3Department of Pathology, PAVIA, Italy*

**Background.** Marginal zone B-cell lymphomas (MZL) of MALT show a peculiar relationship with the triad autoimmunity-infection-immunosuppression. For this reason, these lymphomas have been studied for the risk of second cancers. Most series reported so far regard patients with gastric MALToma, while data on non-gastric MZL of MALT are lacking. **Aim.** To define the risk of second cancer in nongastric MZL of MALT in a population-based study from Northern Italy. **Methods.** We studied the prevalence of second cancers in a series of 157 patients with nongastric MZL of MALT consecutively diagnosed in two haematological Institutions of the Northern Italy region Lombardia. We compared the occurrence of second cancer with respect to the general population by calculating the standardized incidence ratio (SIR), with the age- and sex-specific incidence rates of the Cancer Registry of Lombardia as a reference. **Results.** A history of 30 additional neoplasms was documented in 29 patients (18%). 8 breast, 4 endometrium, 4 skin, 3 thyroid, 2 lung, 1 prostate, 1 colon, 1 small intestine, 1 salivary gland, 1 bladder, 1 ovary and 1 stomach. In 4 patients the site of cancer and lymphoma was the same. For the entire group, the SIR of an additional malignancy was 0.8 (95% CI: 0.55-1.17, p=0.2). The relative rate of an additional malignancy was 0.7 for males (95% CI: 0.59-1.26, p=0.2) and 0.89 for females (95% CI: 0.55-1.46, p=0.6). The comparison of risks between males and females was not significant (SIR ratio 1.28, 95% CI: 0.5-1.12, p=0.2). The relative rate of a second tumor was 0.6 for males (95% CI: 0.31-1.15, p=0.1) and 0.89 for females (95% CI: 0.54-1.47, p=0.6). The comparison of risks between males and females was not significant (SIR ratio 1.49, 95% CI: 0.65-3.4, p=0.8). After excluding all previous malignancies, the SIR of a second cancer was 1.52 (95% CI: 0.69-2.55, p=0.4). All concomitant and subsequent malignancies were invasive tumors. The relative rate of a second cancer was 1.46 for males (95% CI: 0.61-3.51, p=0.4) and 1.19 for females (95% CI: 0.44-3.16, p=0.7). The comparison of risks between males and females was not significant (SIR ratio 0.81, 95% CI: 0.22-3.02, p=0.8). Conclusions. These data demonstrate that patients with nongastric MZL of MALT are not at increased risk for second cancer compared to the general population of the same geographical area. However, since nongastric MALT lymphoma is a long-lasting disease of advanced age with high risk of relapse, a careful clinical follow up is always warranted.

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**0214**

**ACHIEVEMENT OF MOLECULAR REMISSION AFTER FIRST LINE TREATMENT PROLONGS SURVIVAL IN FOLLICULAR LYMPHOMA PATIENTS**


*Teaching Hospital, OLOMOUC, Czech Republic; 1Dept. of Hemato-oncology, Teaching Hosp., OLOMOUC, Czech Republic; 2Dept. of Pathology, Teaching Hosp., OLOMOUC, Czech Republic, 3Dept. of Radiology, Teaching Hosp., OLOMOUC, Czech Republic*

**Background.** However is follicular lymphoma (FL) still considered conventionally incurable disease, prolonged complete remissions were reported. Results of recent studies suggest, that patients who achieve complete remission (CR) with PCR bcl-2/IgH negativity (molecular remission, Crm) have better long term outcome. **Aims.** To evaluate whether achieving of molecular remission after first line treatment have an impact on disease free (DFS) and overall (OS) survival in all risk subgroups, previously untreated, follicular lymphoma patients. **Methods.** 104 pts with FL were diagnosed and treated in our department during last 8 years. All of them were examined with a qualitative PCR (bcl-2/IgH) from bone marrow (BM). Bcl-2/IgH (MBR, mcr or long-distance PCR) positivity was observed in 55 pts (57%) at the time of diagnosis. 91% of bcl-2/IgH+ pts had an advanced disease stage (III/IV), BM involvement was present in 43.5% bcl-2/IgH+ pts. First line treatment was stratified acording generally used risk factors (FLIPI, GELF, ≥2-m level), bulk disease. Patients under 60 (65) y.o. with high risk disease (FLIPI ≥3 or additional risk factors) were indicated to stem cell transplantation (SCT). 19 patients underwent autologous and 1 patient allogeneic SCT. 56 patients were treated conventionally (CHOP or fludarabine based regimens). Rituximab was administred as first line concomitant chemo-immunotherapy in 21 pts (equally in both groups). PCR (BM and/or peripheral blood) was reevaluated on the day +100 after SCT or at the point of restaging and during follow-up every 6 months.

![Figure 1. Event free survival: impact of residual disease.](image-url)
Results. After first line treatment 35/55 (64%) pts achieved CRm, 13/55 (24%) CR+, 9 (16%) PR+ (partial response). After SCT (81.8%), pts attained CRm (100% receiving rituximab in the 1st line). Median follow up is 50 months (mo). 23/55 (41.8%) pts relapsed or progressed (medi-an PFS 30 mo), 11 pts died - one in CRm due to acute graft versus host disease, the other due to progression of the disease. At present 32/55 CRm pts are still in CRm, whereas 6/11 pts in CR+ relapsed. Median disease free survival (DFS) was longer in CRm pts than in CR+ pts (medi-an EFS 33 mo vs not reached, p=0.0024). Median DFS in patients with or without autologous stem cell transplantation was not significantly different. Patients in CRm have longer overall survival (64 mo vs not reached, p=0.05) compared to pts in CR+ and PR. Summary. Molecular remission after first line treatment have an impact on disease free and overall survival in all risk subgroups of FL pts. Persistent PCR bcl-2/IGH positivity is associated with high risk of relapse and additional (maintenance) treatment should be considered.

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0215
CLINICO-BIOLOGICAL CHARACTERISTICS OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA AND HEPATITIS C VIRUS INFECTION
H. Ionta,1 F. Salvi,2 A. Chiappella,1 F. Giordano,4 M. Ceccarelli,4 A. Tucci,4 E. Pogliani,2 M. Iordache,1 E. Gallo1
1 Department of Pathology, ASO S. Giovanni Battista, TORINO, Italy. 2 Laboratory Department, CHIERI, Italy. 3 Department of Pathology, TORINO, Italy. 4 Laboratory Department, CUNEO, Italy.

Background. The infection with Hepatitis C Virus (HCV) is involved in the etiology of some subtypes of non Hodgkin’s lymphoma (NHL) as diffuse large B cell lymphoma (DLBCL). Aims. We tried to analyze the clinico-biological characteristics of a group of 82 patients with DLBCL associated with HCV infection. Methods. We did a retrospective analysis of the clinical and biological profile of 82 patients hospitalized in the Hematology Clinic during 1993-2003. All patients were HCV positive and they were positive at diagnosis for the HCV antibodies Elisa method. The statistical analysis was performed with the special programs EPI INFO 6 and INSTAT. Results. The characteristics of the diffuse large cell lymphoma group that we studied were: medium age - 52.2 years; males/females=1/1; extranodal determinations - 48 cases (58.5%); primitive extranodal 25 cases (30.5%) and secondary extranodal 23 cases (28%); B signs - 66 patients (80.5%); DK < 70 - 40 cases (48.8%); IPI at diagnosis was 16% low, 24% int.low, 35% int/high and 25% high; Bulky disease - 35 cases (42.7%); clinical stage I, II/III, IV=40/41; medullary determination - 14 cases (17.2%); LDH = 672, 024±598, 128 U/l; ESR = 50,45±37,73 mm/h; Kt=67 = 51,90±22,16. The transformation from a low grade lymphoma in a DLBCL was 10% and primary mediastinal DLBCL was 5%. The most important extranodal determinations were the stomach, the spleen, the liver, the skin. The treatment was CHOP or CHOP-like regimens. A small number of cases received CHOP and Rituximab. 11% of patients with severe liver dysfunction received monochemootherapy or radiotherapy only. In 5% of DLBCL and HCV positive patients the chemotherapy was discontinuous because of the hepatic failure. Medium follow-up was 48 month for the survivors and the overall survival at 5 years was 59%, while failure free survival at 5 years was 34%. Conclusions. It is important to recognize the clinical and biological features of DLBCL with HCV positive patients for bigger groups, which could clarify the connection between HCV infection and aggressive non Hodgkin lymphomas. It is also necessary to continue the study in order to evaluate the differentate survival of nodal and extranodal types of lymphomas.

0216
RITUXIMAB IN INDUCTION TREATMENT AND IN HIGH DOSE CHEMOTHERAPY PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION AS FIRST LINE THERAPY IN STAGE III-IV DIFFUSE LARGE B-CELL LYMPHOMA AT POOR PROGNOSIS
U. Vitolo,1 G. Rossi,2 M.G. Cabras,3 A.M. Liberati,2 A. Chiappella,2 E. Faveone,4 E. Angelucci,2 B. Bottò,5 M. Cecarcarelli,6 S. Cortelazzi,6 R. Freilone,6 E. Pogliani4 D. Rota Scalabrini,3 F. Salvi,3 A. Tucci,2 E. Gallo1 ASO S. Giovanni Battista, TORINO, Italy. 1 On the behalf of GIMIUREL, Hematology, TURIN, Italy.

Background. We investigated efficacy and safety of adding Rituximab (R) to induction and intensified HDC as part of first line treatment in pts with aa-IPI at Intermediate-High (IH) or High (H) risk with B-DLCL and comparing two groups of pts enrolled in randomized phase II clinical trials with up-front HDC and ASCT with or without R. Aims and Methods. 118 previously untreated pts <61 years with B-DLCL, stage III-IV at aa-IPI IH or H risk were treated: 41 pts were enrolled into HDC trial (control group; August 1991-August 1995) and 77 pts into R-HDC trial (study group; January 2001-December 2004). Treatment in R-HDC study group consisted in an induction treatment lasting two months with four courses of R-MegaCEOP chemotherapy (R 375 mg/m² day1, CTX 1200 mg/m² + EPI 110 mg/m² + VCR 1.4 mg/m² day8 and PBDN 40 mg/m² days3-7) every 14 days with G-CSF support; then two courses of intensified chemoinmunotherapy R-MAD (Mitoxantrone 8 mg/m² + ARAC 2000 mg/m²/12h + Dexamethasone 4 mg/m²/12h for 3 days and R 375 mg/m²/12h) followed by ASCT as conditioning regimen. Treatment in HDC group was an induction treatment lasting two months with MACOP-B x 8 weekly infusions followed by the same chemotherapy regimen and HDC regimen and HDT and ASCT. Results. In R-HDC trial we added R to HDC regimen in patients with aa-IPI at Intermediate-High (IH) or High (H) risk; 36% had bone marrow (BM) involvement, 80% LDH>normal and 42% extranodal sites>1. Complete Response at the end of the treatment was: 60 pts (78%) in R-HDC group and 28 (68%) in HDC group (p=0.25). Failures (17% vs 25%) and toxic deaths (5% vs 7%) were comparable between the two groups (R-HDC vs HDC). Short-term toxicity appeared similar. NO MDS or ANLL or solid tumours were reported. Three-year failure-free survival (FFS) was 64% vs 46% (p=0.016), OS 80% vs 54% (p=0.004). In the study group, 13 pts (17%) died before autograft: 10 died due to progressive lymphoma, 2 died due to infections, 1 died of unknown cause. In the HDC control group was an induction treatment lasting two months with MACOP-B x 8 weekly infusions followed by the same chemotherapy regimen and HDC regimen and HDT and ASCT. 11 pts died during the treatment (5 pts died due to progressive lymphoma, 5 pts died due to infections, 1 died of unknown cause). In the R-HDC group were: FFS 64% vs 46% (p=0.016), OS 80% vs 54% (p=0.004). In the study group, 13 pts (17%) died before autograft: 10 died due to progressive lymphoma, 2 died due to infections, 1 died of unknown cause. 3-year failure-free survival (FFS) was 64% vs 46% (p=0.016), OS 80% vs 54% (p=0.004). In the control group, 11 pts died (5 pts died due to progressive lymphoma, 5 pts died due to infections, 1 died of unknown cause). Conclusions. These results suggest that the addition of Rituximab to induction and intensified chemotherapy before BEAM and ASCT is effective and safe in B-DLCL at poor prognosis.

0217
EFFECTS OF PRE-TRANSPLANTATION TREATMENT WITH RITUXIMAB ON OUTCOMES OF AUTOLOGOUS STEM-CELL TRANSPLANTATION FOR DIFFUSE LARGE B-CELL LYMPHOMA
P.O. Obritsková,1 B. Vacková,2 H. Krejcová,3 P. Klener,1 M. Timeny
1 Charles University General Hospital, PRAGUE 2, Czech Republic 2, Charles University General Hospital, PRAGUE 1, Czech Republic.

Background. Rituximab (R) in combination with chemotherapy (CHT) has become standard treatment for patients (pts) with diffuse large B-cell lymphoma (DLBCL). However, there are limited data concerning the comparison of the use of R-CHT and CHT alone before high-dose therapy (HDT) either as a part of induction regimen or as a part of salvage treatment. Aims. We retrospectively analysed the efficacity of R-CHT versus CHT without R followed by HDT and autologous stem cell transplantation (ASCT) in patients with DLBCL. Methods. Out of 127 pts with DLBCL who underwent HDT with ASCT, 59 pts received R as part of high-dose therapy regimen (R-CHT group) and 68 pts received HDT in 1st CR. 68 pts received CHT without R (CHT group): 14/68 (21%) received HDT in 1st CR (p<0.0001). Patient characteristics were comparable in both groups with the exception of status at HDT, indi-
cation to HDT (induction vs salvage therapy) and type of salvage regimen. Higher proportion of patients received HDT as a part of induction therapy in R-CHT 69.5% vs 48.5% in CHT group (p=0.01). The majority of pts in R-CHT group received salvage regimen ICE. Regimens ESHAP and IVE were mostly used as a salvage therapy in CHT group. Median follow-up is 2.2 y (range 0.5-4.0) in R-CHT group and 7.3 y (range 2.1-11.0) in CHT group. Results. At 2 years from the date of transplantation, the estimated overall survival (OS) was 81% in R-CHT group vs 60% in CHT group (p=0.03) and the event-free survival (EFS) was 75% vs 56% (p=0.02). The results remain significant while analyzing data for pts transplanted in 1st CR in R-CHT vs CHT group, OS 87% vs 57% (p=0.04), EFS 84% vs 57% (p=0.04) at 2 years. The differences were however not significant for pts who underwent HDT for relapse in R-CHT vs CHT group: OS 69% vs 55% and EFS 53% vs 48% at 2 years. Conclusion. Our analysis suggests that rituximab plays a significant role in pretransplantation therapy in pts with poor risk factors treated with HDT in 1st CR, (EFS, p=0.04, OS, p=0.04). Rituximab seems to improve the outcome of CR pts, it might be important how is the CR reached (with or without antibody). The difference is not however significant for pts treated with HDT in relapse or progression. The role of rituximab in this subset of pts could be in the improvement of salvage therapy results in order to increase the number of pts who are able to undergo HDT and ASCT.

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**0218**

**COMPARATIVE ANALYSIS OF TREATMENT OUTCOMES WITH CHOP REGIMEN, ETOPOSIDE PLUS CORTICOSTEROID AND PREDNISOLONE IN ADULT PATIENTS WITH HEMOPHAGIC CYTOLYTIC LYMHOHISTIOCYTOSIS: BASED ON UNDERLYING DISEASES**

H.-J. Shin,1 J.S. Chung,1 Y. Choi,1 G.J. Cho,2 H.-J. Kim,2 Y.-K. Kim,2 D.-H. Yang,1 S.K. Sohn,1 J.G. Kim,2 Y.D. Joo,1 W.S. Lee1

1Pusan National University Hospital, BUSAN, South-Korea; 2Chonnam National University Hospital, Hwasun, South-Korea; Kyungpook National University Hospital, Daegu, South-Korea; Inje Bakt Hospital, BUSAN, South-Korea

Background. The outcome of CHOP treatment in the case of lymphoma-associated hemophagocytic lymphohistiocytosis (LAHLH) and EBV-associated HLH (EBV-HLH) has rarely been reported. Aims. The present study analyzed the treatment outcomes for CHOP chemotherapy as well as etoposide combined with corticosteroid (Eto-CS) and prednisolone (PRS) in adult patients with EBV-HLH and LAHLH. Methods. 46 adult patients older than 16 years of age were diagnosed with HLH. Among these patients, 30 treated with CHOP chemotherapy (n=18), Eto-CS (n=6), and PRS (n=6) were reviewed retrospectively. Results. With CHOP chemotherapy, complete remission (CR) was achieved in 5/18 patients (27.8%), partial remission (PR) in 5/18 (27.8%), and the overall response rate was 55.6%. With Eto-CS therapy, PR was achieved in 3/6 patients (50%), however no CR was achieved. With PRS therapy, CR was achieved in 1/6 patients (16.7%) and PR in 1/6 (16.7%). The median response duration (RD) was not reached and the 3-year estimated RD was 68.57% for the CHOP chemotherapy, while the median RD was three weeks for the Eto-CS therapy and one week for the PRS therapy, with a median follow-up of 132 weeks. The median duration for the overall survival (OS) was 16 weeks and the 3-year estimated OS rate 40.65% for the patients treated with CHOP therapy, yet only four and two weeks for the patients treated with Eto-CS and PRS, respectively (p=0.0016). Conclusions. CHOP chemotherapy seemed to be useful in adult patients with LAHLH and EBV-HLH. Additional treatment including stem cell transplantation may also be needed, especially for patients with poor prognostic factors.

**0219**

**PP2500 mRNA, A SPlice VARIANT OF THE MULTIPLE ANKIRIN REPEAT SINGLE KH DOMAIN (MASK), IS HIGHLY EXPRESSED IN PLASMA CELLS OF MULTIPLE MYELOMA**


Hemocentro- State University of Campinas, Campinas, Brazil

Background. The Ankyrin (ANK)-repeat is one of the most common protein sequence motifs, which leads itself to variation in overall domain size by simple sequence duplication or deletion. The Mask (Multiple Ankyrin Repeats Single KH domain) gene, which codifies an ANK-repeat protein, is located in chromosome 5(q31.3) and it is composed of 39 exons. It generates isoforms by alternative splicing. The first splice variant (hMask) lacks the 10A exon of the Mask gene, generating a mRNA containing 34 exons. The other, Mask-BF3ARF, results from fusion of splice variant hMask, with the two last exons of the gene Eif4Ebp3 (exons B and C) and an intermediate exon (exon 0), generating 36 exons. Recently, a new splice variant, denominated PP2500, was deposited in the data base Genebank, it presents the first 10 exons, homologous to Mask mRNA with a poly(A)+ signal and it is a new splice variant of Mask. In Drosophila, MASK protein seems to interact with members of the Receptor Tyrosine Kinase (RTK) signalling pathway and loss of this interaction increases programmed cell death, reduces cell proliferation, inhibits photoreceptor differentiation, affects RTK dependent processes but does not affect MAPK (Mitogen Activated Protein Kinase) activation. However, the biological functions of these proteins in humans remain still unknown. Aim: The aim of this study was to investigate the expression of Mask splice variants in multiple myeloma (MM). Methods. Fifteen patients with MM and 3 normal donors participated in this study. Total RNA was extracted from positively selected plasma cells in magnetic column, by Macs Microbeads anti-body anti-CD138 and the percentage of purity of plasma cells varied from 73.38% to 96.02% (average 87.95%). We used as control, total RNA from positively selected plasma cells, from a culture of B normal lymphocytes of bone marrow donors (purity 88.69%). The complementary DNA (cDNA) was analyzed by Real-time detection of amplification, performed in an ABI 7500 Sequence Detector System using SybrGreen PCR Master Mix (qPCR). The b-actin gene was used as endogenous control of the reaction. Results. The mean expression of the mRNA of the hMask and Mask-BF3ARF genes were 3 and 4 times increased, respectively, compared with control. The mean expression of the PP2500 mRNA was 14 times increased, compared with control. Quantification of hMask, Mask-BF ARF and PP2500 mRNA was not influenced by age, gender, ethnic origin, stage of the disease, B2-microglobulin, serum creatinine and lactate dehydrogenase values (Fisher’s exact test, p>0.05). Previously we demonstrated that MASK is associated with SHP2, a protein tyrosine-phosphatase. Conclusions. In MM, SHP2 mediates the anti-apoptotic effect of Interleukin-6 (Chauhan et al. JBC 275: 27845, 2000). Interleukin-6 triggers proliferation of MM cells via the MAPK cascade, which includes SHP2 activation. Thus, the increased expression of Mask splice variants in plasma cells of MM suggests that their proteins may be involved in this signaling pathway and provide an insight for novel treatment approaches in MM.

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**0220**

**A PHASE II STUDY OF THALIDOMIDE, DEXAMETHASONE AND PEGYLATED LYPOSOMAL DOXORUBICIN (THADD) FOR UNRETAINTED PATIENTS WITH MULTIPLE MYELOMA AGED OVER 65 YEARS**

M. Offidani,1 L. Corsvatta,1 M. Marconi,1 C. Polloni,1 M.N. Piantelli,1 G. Visani,1 F. Alessiani,1 M. Brunori,1 C.M. Bigazzi,1 M. Catarinì,1 M. Buratti,1 R. Centurioni,1 L. Morbidioni,1 A. Poloni,1 P. Leonì1

1Clinica di Ematologia, ANCONA, Italy; 2Divisione di Ematologia, PESARO, Italy; 3Unità Operativa Oncemato1gica, SEVERINO, Italy; 4Divisione Medicina, FANO, Italy; 5Divisione Ematologia, ASCOLI PICENO, Italy

Background. No standard therapy have been yet identified for elderly patients with multiple myeloma (MM) despite two third of cases affected by this incurable malignancy are older than 65 years. The combination melphalan-prednisone yields unsatisfactory results and high-dose therapy, despite feasible also in elderly patients, can be an unbearable option because of pre-existing medical comorbidities. Improvements in the outcome of elderly MM patients have been obtained using thalidomide as single agent or in combination with dexamethasone or conven-
tional chemotherapy. Aims. We report the results of a phase II study including 50 newly diagnosed patients with symptomatic MM older than 65 years regardless of comorbidities, performance status and renal function. Methods. All patients received thalidomide 100 mg/day continuously, pegylated liposomal doxorubicin 40 mg/m² on day 1 every 28 days, dexamethasone 40 mg on days 1-4 and 9-12 (ThDD). They also were given warfarin 1.25 mg/day as antithrombotic prophylaxis and ciprofloxacin 250 mg twice daily after a high incidence of infections was recognized. Median age was 71.5 years (range 65-78) and 64% were older than 70 years. Thirty-nine patients (78%) had clinical stage III, 37 (74%) ISS 2 or 3 and 7 patients (14%) a serum creatinine level > 2 mg/dl. Moreover, unfavourable cytogenetics were detected in 85% of patients with a value seen in 8% after ciprofloxacin had been added to the protocol. Grade 3-4 nonhematological side effects were mainly attributable to thalidomide and consisted of constipation (4%), fatigue (6%) and tremors (4%). Regarding toxicity due to pegylated liposomal doxorubicin, 2 patients experienced grade 3-4 mucositis and one grade 3 palmar-plantar erythrodysesthesia. Venous thromboembolic events occurred in 7 patients (14%) but only one patient experienced pulmonary embolism. Conclusions. Our study demonstrates that the combination low-dose thalidomide, pegylated liposomal doxorubicin and high-dose dexamethasone is very effective in the treatment of elderly patients with MM since it induces an ORR and particularly a CR rate higher than those reported with all other thalidomide-based regimes. It results well tolerated also by oldest fragile patients and thrombotic as well as infectious complications can be prevented by adequate prophylaxis.

0221
MONOKINE-INDUCED BY INTERFERON-γ SERUM LEVELS ARE A MARKER OF DISEASE LOAD AND CORRELATE WITH PROGNOSIS IN MULTIPLE MYELOMA
Wilhelmsuniversität, VIENNA, Austria; Medical University Vienna, VIENNA, Austria

Background. Monokine-induced by interferon-γ (MIG) is a chemokine known to be produced by monocytes and macrophages in response to interferon-γ, and acts as a chemoattractant to T-lymphocytes and other inflammatory cells. Besides its role in the host immune response to infections and neoplastic disease, MIG has also been implicated as chemokine acting in an autocrine loop to stimulate tumor cells through its receptor CXCR3. Myeloma cells are known to express CXCR3 (Pellegrino et al., 2004), however it is unclear if MIG is of biological significance in myeloma in vivo. Aims. We have shown recently that multiple myeloma oncogene 1 (MUM1) expression in myeloma cells correlates with prognosis in this disease (Heintel et al., 2005), and MUM1 is known to upregulate MIG gene expression in B cell malignancies (Urashini et al., 2005). This led us to evaluate the potential prognostic significance of MIG serum levels in a series of myeloma patients. Methods. MIG serum levels were determined by a commercially available ELISA (R&D Systems) in a series of 54 newly diagnosed myeloma patients. Serum from 5 healthy volunteers, 4 patients with osteoporosis, and 4 patients with chronic obstructive pulmonary disease (COPD) were used as controls. Results. Median MIG serum level was 58.3 pg/ml in healthy volunteers, range 22.7-52.79 pg/ml. In patients with osteoporosis and COPD the levels were higher (median 133.0, range 47.2-202.3 pg/ml; and median 54.9, range 25.8-200.2 pg/ml, respectively). In 54 newly diagnosed myeloma patients the median MIG level was 219.8, range 27.6-1966.0 pg/ml. When myeloma patients were stratified according to a MIG level < 200 and MIG > 200, a highly significant survival difference for the 2 cohorts was observed. While median survival was 88.2 months for patients with MIG < 200, patients with high MIG serum levels (> 200) had a survival of only 17.0 months (p=0.00409; see Figure). Serum-MIG levels correlated with markers of disease burden, including β2-microglobulin levels and extent of bone marrow plasma cell infiltration, MIG showed a negative correlation with hemoglobin and albumin levels. Interestingly, no correlation was found with C-reactive protein levels, indicating that MIG is not associated with an inflammatory response in myeloma. Preliminary experiments show that MIG mRNA is expressed in 1 out of 4 myeloma cell lines, with upregulation of expression seen after stimulation with interferon-γ in the positive line, but not in those without baseline MIG expression. Summary/Conclusions. MIG serum levels correlate with markers of disease burden in myeloma and high MIG levels are associated with a poor outcome in this disease.

0222
VAD-DOXIL VS. VAD-DOXIL PLUS THALIDOMIDE AS INITIAL TREATMENT IN MYELOMA PATIENTS: INTERIM ANALYSIS OF A MULTICENTER RANDOMIZED TRIAL OF THE GREEK MYELOMA STUDY GROUP
‘Theagenion Cancer Center, THESSALONIKI, Greece; ‘Theagenion Cancer Center, THESSALONIKI, Greece; Papageorgiou General Hospital, TESSALONIKI, Greece; ‘Agios Savvas Cancer Center, ATHENS, Greece; ‘Metaxa Cancer Center, ATHENS, Greece; ‘Alexandra General Hospital, ATHENS, Greece; ‘Evaggelismos General Hospital, ATHENS, Greece; ‘Lukas General Hospital, ATHENS, Greece; ‘Kratigos General Hospital, ATHENS, Greece; ‘251 General Airforce Hospital, ATHENS, Greece; ‘Agios Andreas General Hospital, PATRAS, Greece; ‘Errikos Dynan General Hospital, ATHENS, Greece

Background. VAD-doxi and VAD-doxi plus thalidomide have already been separately evaluated, as initial cytoreductive treatment in multiple myeloma, in two previous clinical trials of our study group. Both regimens proved effective yielding overall (complete and partial) response rates of 61.3% and 74% respectively, while toxicity remained acceptable in both studies. Aims: To compare the efficacy and toxicity of these two regimes in the context of a multicenter randomized clinical trial. Results of an interim analysis are presently reported. Methods. Patients randomized in arm A received vincristine 2 mg IV, liposomal doxorubicin 40 mg/m² IV in a single dose on day 1, and dexamethasone 40 mg PO daily for 4 days. The regimen was repeated every 4 weeks. Dexamethasone was also administered on days 15-18 of the first cycle. Patients randomized in arm B received additionally thalidomide 200 mg PO daily at bedtime. Response to treatment was the primary objective of the study and was evaluated after the completion of 4 cycles. Subsequently, patients were allowed to proceed to high dose chemotherapy or to receive two additional cycles of the same regimen. Response and toxicity were evaluated according to EBMT and NCI criteria respectively. Patients' characteristics, response and toxicity rates were compared using two-independent- samples tests and x2 tests. Results. Between June 2002 and December 2005, 230 patients entered the study, 115 randomized in each arm. To date, 195 patients are evaluable for toxicity and 160, 80 in each arm, for efficacy. The two treatment groups were well-balanced regarding the usual prognostic characteristics. On an intention- to- treat basis, overall response rate was 66.3% and 81.3% in arms A and B respectively (p=0.048). Neutropenia, thrombocytopenia, infections, mucositis, palmar-plantar erythrodysesthesia, deep venous thrombosis and early mortality were not significantly different (p>0.05) between arms A and B.
(13% vs. 15%, 8.5% vs. 10%, 7.5% vs. 5%, 5% vs. 4%, 6.3% vs. 5%, 3.8% vs. 9.5% and 6.3% vs. 5% respectively). Constipation, peripheral neuropathy, dizziness, insomnia, skin rash and edema were significantly higher (p<0.05) in arm B compared to arm A (57% vs. 10%, 46% vs. 15.8%, 54% vs. 0%, 13% vs. 0%, 10% vs. 2% respectively). Conclusions. VAD-doxil plus thalidomide compared to VAD-doxil alone, yields higher response rates in previously untreated myeloma patients. Nevertheless, the increased toxicity associated with the addition of thalidomide to VAD-doxil, should be counterbalanced against the increased response rate.

**O223**
THE COMBINATION OF BORTEZOMIB, MELPHALAN, DEXAMETHASONE AND INTERMITTENT THALIDOMIDE (VMDT) IS AN EFFECTIVE REGIMEN FOR RELAPSED/REFRACTORY MYELOMA AND REDUCES SERUM LEVELS OF RANKL, MIP-1A AND ANGIOGENIC CYTOKINES

E. Terpos,
A. Anagnostopoulou,
E. Kastritis,
D. Christoulas,
A. Zomas,
P. Poziopoulos,
A. Anagnostopoulou,
K. Tsionos,
M.A. Dimopoulos

1. 251 General Airforce Hospital, ATHENS, Greece; 2. University of Athens School of Medicine, ATHENS, Greece; 3. Gennimatas General Hospital, ATHENS, Greece; 4. General Army Hospital, ATHENS, Greece

Background. Interactions between myeloma (MM) cells and marrow microenvironment are crucial for myeloma growth and resistance to anti-myeloma therapy. Bortezomib (VELCADE®; V) and thalidomide (T) have proven anti-MM effect and exert their action partly through perturbation of the MM microenvironment. Furthermore, bortezomib enhances the cytotoxic potential of other agents, such as melphalan (M), and dexamethasone (D) in resistant cell lines. Aims. We hypothesize that combining VT (to target both MM cells and microenvironment) with M/D may help overcome resistance and increase clinical efficacy of these agents in relapsed/refractory disease. The aim of this phase II study was to determine the efficacy and safety of the VMDT regimen and its effect on angiogenesis and bone remodeling in relapsed/refractory MM patients. Methods. Bortezomib (1.0 mg/m²) was given iv, on days 1, 4, 8, and 11 of a 28-day cycle; oral melphalan (0.15 mg/kg) was administered on days 1-4, while thalidomide (100 mg/day) and dexamethasone (12 mg/m²) were given on days 1-4 and 17-20 every 4 weeks, for 4 cycles. Responders and patients with SD continued for up to 8 cycles. Effect of VMDT on angiogenesis was evaluated by measuring the serum levels of angiogenic cytokines, such as VEGF, angiogenin, angiopoietin-2, and basic fibroblast growth factor (bFGF) at baseline and after 4th and 8th cycle of therapy. Results. This study was conducted at the General Hospital of Athens, Athens, Greece. Forty-four pre-treated patients have been enrolled in this ongoing study including 25 patients treated during refractory relapse. Median time from 1st treatment to VMDT was 58 months. The median number of previous treatment was 2 (range: 1-6), including melphalan (47% of patients), thalidomide (56%), dexamethasone (100%), bortezomib (9%) and ASCT (32%). Results. Among 41 patients evaluable for response so far, 27 (65%) achieved an objective response (CR 9% and PR 56%). Furthermore, 5 patients (12%) achieved a MR and 5 SD. Median time to response was 56 days. Adverse events included fatigue (52%), thrombocytopenia (20% grade 3/4), neutropenia (8% grade 3/4), anemia (7% grade 3), neuropathy (47% grade 1/2, and 6% grade 3), infections (47%, including 4 HZV cases), and hyponatremia (18%). No patient experienced DVT, while 2 patients died due to sepsis and one due to necrotizing fasciitis. At baseline, MM patients had increased serum levels of sRANKL, sRANKL-OPG ratio, MIP-1α, CTX, VEGF, angiogenin, angiopoietin-2, and bFGF (p<0.01) compared with controls (21 healthy, age- and gender-matched, individuals), while serum levels of bALP and OC were reduced (p<0.0001). Our preliminary analysis has shown that sRANKL, sRANKL-OPG ratio, MIP-1α, CTX, and all angiogenic cytokines’ levels reduced after 4 (p<0.001) and 8 cycles of treatment (p<0.01) in all patients. Responders tended to have a higher reduction of both serum sRANKL and MIP-1α compared with non-responders. Conclusions. VMDT provided encouraging evidence of antitumor activity in relapsed/refractory MM, with manageable toxicities and alterations in cytokines conducting interactions between myeloma and stromal cells.

**O224**
BORTEZOMIB DEMONSTRATES SUPERIOR SURVIVAL COMPARED WITH HIGH-DOSE DEXAMETHASONE AND HIGHER RESPONSE RATES AFTER EXTENDED FOLLOW-UP IN THE APEX TRIAL IN RELAPSED MULTIPLE MYELOMA

P. Richardson,
P. Sonneveld,
M. Schuster,
D. Irwin,
E. Stadtmann,
T. Facon,
J.L. Harousseau,
D. Ben-Yehuda,
S. Lonial,
H. Goldschmidt,
D. Reece,
J. san Miguel,
J. Blade,
M. Boccadoro,
J. Cavenagh,
K. Anderson

Dana-Farber Cancer Institute, BOSTON, USA; 1. University Hospital Rotterdam; ROTTERDAM, Netherlands; 2. NY- Presbyterian Hospital, NEW YORK, USA; 3. Askia Bates Cancer Center, CALIFORNIA, USA; 4. University of PA Cancer Center, PA, USA; 5. Hospital Claude Huriez, LILLE, France; 6. Hotel Dieu Hospital, NANTE, France; 7. Hadassah University Hospital, JERUSALEM, Israel; 8. Emory University, GEORGIA, USA; 9. University of Heidelberg, HEIDELBERG, Germany; 10. Princess Margaret Hospital, ONTARIO, Canada; 11. Hospital University of Salamanca, SALAMANCA, Spain; 12. University of Barcelona, BARCELONA, Spain; 13. University of Turin, TORINO, Italy; 14. St Bartholomew’s Hospital, LONDON, United Kingdom

Background. In the open-label, international, multicenter phase 3 APEX trial, 669 patients with relapsed multiple myeloma (MM) following 1-3 prior therapies were randomized to receive bortezomib (VELCADE®) or dexamethasone (Dex). Patients with progressive disease on Dex were eligible to crossover to bortezomib. Patients receiving bortezomib achieved significant improvement in survival, time to progression (TTP), and response rate (CR + PR, EBMT criteria). Consequently, the Dex arm was halted early and all patients receiving Dex were allowed to cross over to bortezomib. Aims. To update survival data for both the bortezomib and Dex arms of the APEX trial, and to update efficacy data for the bortezomib arm. Methods. Updated overall and 1-year survival rates were analyzed for both arms based on median follow-up of 22 months of surviving patients and deaths in 44% of patients. Updated response rate, time to response (TTR), duration of response (DOR), and TTP were analyzed for the bortezomib arm with extended follow-up of approximately 14 months compared with the initial analysis. Timing of best response to bortezomib was analyzed in terms of EBMT criteria and M-protein reduction, and DOR was analyzed according to best M-protein reduction. Matched-pairs analyses were performed to compare survival and response rate in patients receiving bortezomib earlier (bortezomib arm) or later (Dex arm patients who crossed over to bortezomib arm). Results. Patients received a median of 6 cycles of bortezomib. Median survival was 29.8 months vs 23.7 months (p=0.0272), and 1-year survival rate was 80% vs 67% (p=0.0002), for bortezomib vs Dex, despite >62% of Dex patients crossing over to bortezomib. With extended follow-up, overall response rate by EBMT criteria with bortezomib improved from 45% at the initial analysis to 48%, and CR improved from 6% to 9%, with 56% of responders experiencing an improved response after cycle 2, and 54% of responders achieving first response after cycle 2. The proportion of patients achieving maximum M-protein reduction continues to increase over the entire course of study-defined treatment (up to 8 cycles). Median TTP (6.2 months), TTR (1.4 months), and DOR (7.0 months) with bortezomib were unchanged compared with initial analysis. Median DOR was 11.5 months in patients with 100% M-protein reduction, and 7.6 months in patients with ≥50% but <100% M-protein reduction. Overall response rate and median survival in patients receiving bortezomib earlier vs later were: 44% vs 34%, and not reached vs 16.4 months, respectively (Table). Conclusions. After extended follow-up, significantly longer survival with bortezomib compared with Dex was confirmed, despite substantial crossover from Dex to bortezomib. Response rates are higher, with many patients achieving best responses after longer duration of therapy. Patients achieving 100% M-protein reduction tended to have a longer DOR. Additionally, patients receiving bortezomib earlier appear to have a higher response rate and longer survival.

**Table**: Median survival, months

<table>
<thead>
<tr>
<th>Bortezomib earlier</th>
<th>Bortezomib later</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=102)</td>
<td>(n=102)</td>
</tr>
<tr>
<td>Response rate,%</td>
<td>44</td>
</tr>
<tr>
<td>CR</td>
<td>9</td>
</tr>
<tr>
<td>PR</td>
<td>35</td>
</tr>
<tr>
<td>near CR</td>
<td>6</td>
</tr>
<tr>
<td>Median survival, months</td>
<td>Not reached*</td>
</tr>
</tbody>
</table>

*pHazard ratio = 0.75; p= 0.1722

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The aim of Expression data of 173 newly diagnosed, untreated myeloma using gene expression profiling we have identified. We have retrospectively evaluated and after it IFN did not significantly affect OS after ASCT. The aim of this study is to test this hypothesis. Supervised cluster analysis of gene expression profiles will provide an improved molecular classification of MM patients compared to cytogenetics alone. The aim of this study is to test this hypothesis. Methods. Expression data of 173 newly diagnosed, untreated myeloma patients previously reported by Tian et al. (NEJM 2003;349:2483-2494) were downloaded from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo), accession number GDS531. The dataset contains expression data that were obtained using Affymetrix U95Av2 arrays and were normalized using the method of global scaling, provided in the Affymetrix MAS5.0 software. Unsupervised hierarchical cluster analysis was performed with complete linkage and Euclidean distance as similarity metric, using the Omniviz package. Supervised analyses were performed with the use of SAM software. Cluster-specific gene signatures obtained using the SAM method were independently estimated. Based on available annotations in Gene Ontology and the GenMAPP database we determined which pathways or processes were statistically overrepresented in the cluster-specific gene lists. Correctness for multiple testing was performed using the Benjamini-Hochberg method in EASE v2.0. Results. Unsupervised cluster analysis defined ten clusters based on overall correlation. Clusters displayed unique, non-overlapping gene expression signatures. Six of these clusters have not been described before. Three clusters corresponded to recurrent 14q32 translocations: t(4;14), t(11;14) and t(14;16)/t(14;20). One cluster appears to represent polyclonal plasma cell preparations. One of the novel clusters displayed specific expression of WNT10B, bone morphogenetic protein 4, and osteopontin as specific events in subgroups with low bone-disease frequencies. Summary/Conclusion. Using gene expression profiling we have identified a novel subgroup of t(11;14) myeloma patients and a new genetic cluster, representing only 37% of the patients were diagnosed with thalidomide. The unsupervised nature of our analysis has proven a powerful method for the classification of MM patients, showing improved discriminating capacity.

**0227**

**Efficacy of Single-Agent Bortezomib Versus Thalidomide in Patients with Relapsed or Refractory Multiple Myeloma: A Systematic Review**

E. Kamst, P. Sonneveld

ErasmusMC, ROTTERDAM, Netherlands

Background. Accumulation of malignant plasma cells in the bone marrow is a hematological malignancy referred to as multiple myeloma (MM). Herein we present and molecularly evaluate a supervised cluster analysis of gene expression profiles in newly diagnosed MM patients. The aim of this study was to test the hypothesis that MM contains expression data that were obtained using Affymetrix U95Av2 arrays and were normalized using the method of global scaling, provided in the Affymetrix MAS5.0 software. Unsupervised hierarchical cluster analysis was performed with complete linkage and Euclidean distance as similarity metric, using the Omniviz package. Supervised analyses were performed with the use of SAM software. Cluster-specific gene signatures obtained using the SAM method were independently estimated. Based on available annotations in Gene Ontology and the GenMAPP database we determined which pathways or processes were statistically overrepresented in the cluster-specific gene lists. Correctness for multiple testing was performed using the Benjamini-Hochberg method in EASE v2.0. Results. Unsupervised cluster analysis defined ten clusters based on overall correlation. Clusters displayed unique, non-overlapping gene expression signatures. Six of these clusters have not been described before. Three clusters corresponded to recurrent 14q32 translocations: t(4;14), t(11;14) and t(14;16)/t(14;20). One cluster appears to represent polyclonal plasma cell preparations. One of the novel clusters displayed specific expression of WNT10B, bone morphogenetic protein 4, and osteopontin as specific events in subgroups with low bone-disease frequencies. Summary/Conclusion. Using gene expression profiling we have identified a novel subgroup of t(11;14) myeloma patients and a new genetic cluster, representing only 37% of the patients were diagnosed with thalidomide. The unsupervised nature of our analysis has proven a powerful method for the classification of MM patients, showing improved discriminating capacity.
**0228**

**BORTEZOMIB IN RELAPSED MULTIPLE MYELOMA: RESPONSE RATES AND DURATION OF RESPONSE ARE INDEPENDENT OF CHROMOSOME 13q**

J. Drach, 1 E. Kienburg, 1 V. Sagaster, 2 N. Zojer, 3 A. Ackermann, 4 H. Kaufmann, 1 V. Odelga, 1 C. Ziebinski, 1 R. Wieser, 2 H. Ludwig 2

1Medical University Vienna, VIENNA, Austria; 2Wilhelmenspital, VIENNA, Austria

**Background.** Presence of a chromosome 13q-deletion confers a poor prognosis to patients (pts) with multiple myeloma (MM), even in the context of intensive treatment programs and thalidomide. Bortezomib is the first compound of a new class of agents (the proteasome inhibitors) showing activity in relapsed and chemotherapy-refractory MM. Results of SUMMIT and APEX trials suggested that bortezomib is active in MM with previously recognized unfavorable prognostic factors. Aims. To study the activity of bortezomib in relapsed MM and potential associations with prognostic factors (standard clinical parameters and chromosomal aberrations: deletion of chromosome 13q14 (del(13q14), 14q-translocations (t[14q32]), gain of 1q21). Patients and Methods. We evaluated 51 consecutive pts with relapsed/refractory MM (median number of prior therapies: 3; 92% had high-dose, pulsed dexamethasone, 71% thalidomide, 45% high-dose therapy; median time from first line therapy to bortezomib, 4.8 years). Treatment consisted of single agent bortezomib according to the standard regimen (1.3 mg/m2 on days 1, 4, 8, 11; 0.925 mg/m2 on days 1, 8, 15) for 6 cycles. The following variables were determined by means of interphase FISH. Results. Similar response rates to bortezomib were observed in pts with del(13q14) (13 of 26 pts = 50%) and with normal chromosome 13q (15 of 25 pts = 60%) (p=0.34). Of note, rates of CR/nearCR were also not different between the two patient populations (25% vs. 16%). Moreover, median duration of response was 10.4 months in pts with del(13q14) compared with 9.3 months in pts with normal 13q-status (p=0.29). Only those pts with del(13q14) who did not show a response to bortezomib experienced a rapidly progressive clinical course leading to overall shortened survival. For an improved identification of such pts, additional parameters were tested. In a subset of pts, analyses for gain of 1q21 (CKS1B gene) were performed. Among 10 pts with simultaneous del(13q14) and gain of 1q21, 8 failed to respond to bortezomib, and their median survival was only 3.3 months. We also observed that pts with low serum levels of albumin had a poor outcome after bortezomib. Thus, pts not benefiting from single-agent bortezomib were characterized by the combined presence of a del(13q14) and low serum albumin level (median survival 5.8 months vs. 12.7 months). Microlongobulin, however, was not important for treatment outcome after bortezomib. Finally, 3 pts were found to have a t(4;14) (p16;q32) in addition to a del(13q14), and all of them had a > 50% reduction of their paraprotein after bortezomib. Conclusion. Our results indicate that bortezomib has good clinical activity in MM patients with high-risk cytogenetic features. The simultaneous occurrence of a del(13q14) with gain of 1q21 and/or low serum albumin allows for the identification of pts not benefiting from single-agent bortezomib. It is suggested to evaluate bortezomib combinations in such pts.

**0229**

**INTEGRATIVE GENOMIC ANALYSIS OF MULTIPLE MYELOMA PATIENTS WITH 13q DELETION SUGGESTS A ROLE OF CHROMOSOMAL ABERRATIONS IN THE TRANSCRIPTIONAL FINGERPRINT**

L. Agnelli, 1 A. Callegaro, 2 G. Poretti, 3 S. Fabris, 1 D. Verdelli, 1 I. Kwee, 1 L. Baldini, 1 F. Morabito, 1 A. Zanello, 1 G. Lambertenghi-Delliliers, 1 F. Bertoni, 1 S. Bicciato, 1 L. Lombardi, 1 A. Nerli 1

1Fondazione IRCCS Ospedale Policlinico, MILANO, Italy; 2Dip Leg Clin Ing, Università degli Studi, PADOVA, Italy; 3Laboratory of Experimental Oncology,IOSI, BELLINZONA, Switzerland; 4U.O. Ematologia, A.O. Avanzista, COSENZA, Italy

**Background.** The chromosome 13 deletion [del(13)] represents one of the most frequent chromosomal alterations in multiple myeloma (MM), characterizing almost 50% of the patients. Several groups have reported an unfavorable prognostic role for del(13) in MM although, according to some authors, the prognostic value of del(13) should not be considered per se but has to be related to the ploidy or to the main chromosomal translocations involving 1q42q52 locus. Aims. To better characterize the biological meaning of the purpose of the present study was to provide a comprehensive analysis of the transcriptional profiles and the molecular features associated with del(13) on MM patients. Methods. The transcriptional profiles of 90 MM newly diagnosed MM patients have been generated from highly purified plasma cells by means of high-density oligonucleotide arrays (Affymetrix GeneChip U133A) and subsequently analysed using unsupervised and supervised approaches (two-dimensional hierarchical clustering and SAM, respectively). Chromosomal regions with modulation of the gene expression signals have been identified using a non-parametric model-free statistical method (LAP, locally adaptive statistical procedure). The aneusomy status was evaluated by the [1q21-1q42] (PISH) group, distinguished by fluorescence in situ hybridization (FISH) and the Chromosomal Abnormality Score Index recently proposed (Wuilleme S. et al., Leukemia, 2004). Genome wide profiling data for 10 MM samples have been generated on high-density SNP arrays (Affymetrix GeneChip Human Mapping 10k Xba 142.0 2.0 arrays) and analysed to investigate copy number alterations. Results. The differential expression of 87 transcripts (specific for 67 genes), all of them downregulated in del(13)+ group, distinguished del(13)+ from del(13)- MM cases; forty-four genes were localized along the whole chromosome 13, 7 on chromosome 11 and 4 on chromosome 19. The majority of the identified genes resulted involved in translational pathways. In addition, we identified the presence of the putative tumor suppressor gene RFF2, mapping at 13q14.3 within the minimally deletion region. An integrative genomic approach, based on the regional analysis of gene expression data, allowed detecting novel chromosomal regions whose modulation in global expression levels could differentiate the del(13)+ patients. In particular, we identified the upregulation of the 1q42 region and the downregulation of the 19p region and validated it on a whole chromosome array. To better characterize the molecular differences were investigated in our recent studies the specific chromosome regions by FISH, showing a strong relationship between del(13)+ and either the presence of 1q21-1q42 amplifications (p=6×10^-10) or the absence of chromosome 11 trisomy (p=5×10^-10). Finally, the genome wide profiling of 10 MM patients included in our study confirmed the patterns observed by FISH. Conclusions. Our genomic and integrative approaches in our integrative approach allowed us to characterize the whole chromosome 13 deletion in MM is specifically associated with distinct types of chromosomal aberrations, which may be responsible for the transcriptional differences between del(13)+ and del(15)- patients.

**0230**

**EFFICACY AND SAFETY OF MELPHALAN/ARSENIC TRIOXIDE/ASCORBIC ACID COMBINATION THERAPY (MAC) IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: A PROSPECTIVE, MULTICENTER, PHASE II, SINGLE-ARM STUDY**

R. Berenson, 1 R. Boccia, 2 D. Siegel, 2 M. Bozdech, 3 A. Bessudo, 3 E. Stadtmueller, 3 J.T. Pomeroy, 3 R. Steis, 2 M. Flam, 3 J. Lutzky, 3 S. Lilani 13 J. Volk 13 T. Lindgren, 1 R. Moss 13 H. Patel 13 H.S. Yeh 3

1Inst. for Myeloma & Bone Cancer Research, WEST HOLLYWOOD, CALIFORNIA, USA; 2Center for Cancer and Blood Disorders, BETHESDA, MARYLAND, USA; 3Hacksennack Medical Cancer Center, HACKENSACK, NEW JERSEY, USA; 4Redwood Regional Oncology Center, SANTA ROSA, CALIFORNIA, USA; 5San Diego Cancer Center, VISTA, CALIFORNIA, USA; 6University of Pennsylvania, PHILADELPHIA, PENNSYLVANIA, USA; 7Cancer Prevention & Treatment Center, SOQUEL, CALIFORNIA, USA; 8Atlanta Cancer Care, ATLANTA, GEORGIA, USA; 9 Hematology-Oncology Medical Group, FRENSNO, CALIFORNIA, USA; 10Mt Sina Comprehensive Cancer Center, MIAMI BEACH, FLORIDA, USA; 11Cancer Care Associates, TORRANCE, CALIFORNIA, USA; 12Palo Verde Hematology/Oncology Center, GLENDALE, ARIZONA, USA; 13Fountain Valley, FOUNTAIN VALLEY, CALIFORNIA, USA; 14Comprehensive Blood & Cancer Center, BAKERSFIELD, CALIFORNIA, USA

**Background.** Multiple myeloma (MM) is an incurable B-cell malignancy, and nearly all patients develop resistant disease. Most patients develop resistance to melphalan over comes resistance to this alkylating agent. Moreover, ascorbic acid (AA) enhances the cytotoxic effects of ATO. In preclinical MM studies, the addition of ATO to the cytotoxic agent melphalan overcomes resistance to this alkylating agent. To better characterize the molecular differences were investigated the presence of chromosome 13 deletion in MM is specifically associated with distinct types of chromosomal aberrations, which may be responsible for the transcriptional differences between del(13)+ and del(15)- patients.
received melphalan (0.1 mg/kg PO), ATO (0.25 mg/kg IV), and AA (1 g IV) on days 1-4 of week 1, ATO and AA twice weekly on weeks 2-5, and rest during week 6 of cycle 1; melphalan on days 1-4 and ATO and AA twice weekly on weeks 1-5, and rest during week 6 of cycles 2-6. Results. Patients (N = 65) had a median of 4 (range, 1-8) prior therapies, including melphalan, bortezomib, thalidomide/lenalidomide, glucocorticosteroids, and rituximab, and 38 (58%) showed improvement in renal function. Grade 3 or 4 anemia and/or neutropenia occurred in 3 patients and in 1 patient, respectively. Common grade 3 or 4 nonhematologic adverse events were fever/chills (15%), pain (8%), and fatigue (6%). Two patients had single occurrences of prolonged QTc interval (498 and 502 msec) resulting in a brief delay in ATO administration, but continued ATO dosing was not accompanied by any further episodes of QTc prolongation. One patient developed unstable bradycardia without a prolonged QTc following the first ATO infusion and was removed from the study. Conclusions. The MAC combination regimen was an effective treatment for patients with relapsed or refractory MM, producing objective responses in 60% of the patients in this heavily pretreated group. Patients with renal insufficiency at baseline showed improvements in renal function with MAC therapy. The MAC regimen was well tolerated, with relatively few grade 3 or 4 hematologic adverse events or cardiac events. These results show that the MAC combination regimen is an effective and well-tolerated new therapeutic option for patients with relapsed or refractory MM.

**0231**

INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) IS OVEREXPRESSED IN MULTIPLE MYELOMA PLASMA CELLS (PC) AND REGULATES THE EXPRESSION OF THE IGF-1 RECEPTOR

M.R. Renzulli,1 C. Terragna,1 N. Testoni,1 C. Nicci,1 C. Taccioli,1 T. Gra-1fone,1 P. Tosi,1 E. Zamagni,1 P. Taccetti,1 G. Perrone,1 M. Toccafondi,1 G. Martellini,1 M. Baccarani,1 M. Cavo1

1Institute of Hematology, BOLOGNA, Italy; 1University Cattolica, CAM-POBASSO, Italy; 1Ohio State University, COLUMBUS, USA

Background. IGF-1 plays an important role in regulating cell proliferation, differentiation, apoptosis, and transformation. Recent studies have shown that IGF-1 is an important survival and growth factor in multiple myeloma (MM). Moreover, IGF-1 down-regulates IGR-1 expression at the transcriptional level, by autocrine or paracrine mechanism. Method. The expression of IGF-1 and IGR-1 in cell fractions from CD138+ and CD138- cells was evaluated by real-time PCR in a series of 53 newly diagnosed MM patients primarily treated with thalidomide and dexamethasone. For each patient, we isolated the CD138+ cell fraction from bone marrow (BM) sample at diagnosis and, in 24/53 patients for whom material was available, also at the end of induction treatment (CD183+ and CD183- cells for each patient). A pool of donors was used as calibrator. The Mann-Whitney and the Wilcoxon tests were used to compare expression in BM and peripheral blood. Results. The expression of IGF-1 and IGR-1 was significantly higher in ID138+ compared to CD138- cells (p<0.0005, r= -0.33), thus suggest- ing a possible paracrine effect of PC-produced IGF-1 exerted on CD138- cells, which results in more enhanced proliferation properties. The relationship between IGF-1 and IGR-1 expression became significantly positive (p=0.03, r=0.64). After induction therapy, a median IGR-1 increase was observed among CD183+ samples (0.67 vs. 1.3, p=0.03); patients not harbouring del(13) and t(4;14) showed the most relevant increase (0.6 vs. 1.58, p=0.03 and 0.53 vs.1.13, p=0.0005, respectively). Conclusions. Our preliminary study confirmed the involvement of IGR-1/IGF-1 pathway in MM pathogenesis; we suggested a paracrine effect of PC-produced IGF-1 on CD138+ cells, which seemed to act only in responding patients. The ability to efficiently regulate IGR-1 expression may thus have an important prognostic value. Moreover, a different regulation of IGR-1/IGF-1 pathway may exist between different genetic subtypes. The study of post transduction modifications of the IGR-1 will be needed, in order to get more insight into the relationship between the IGR-1 and IGR-1 expressions and IGF-1R activation.

**0232**

AUTOMMUNITY IS ASSOCIATED WITH BETTER SURVIVAL IN MULTIPLE MYELOMA

O. Landgren,1 R.M. Pfeiffer,1 D. Bans,1 G. Gridley2, L. Mellenkjaer,3 K. Hemminki,1 L.R. Goldin,1 N.E. Caporaso1

1National Cancer Institute, NIH, BETHESDA, MARYLAND, USA; 2Danish Cancer Society, COPENHAGEN, Denmark; 3German Cancer Research Cen- ter, HEIDELBERG, Germany

Background. In Western countries, multiple myeloma (MM) is the second most common hematopoietic malignancy after non-Hodgkin lymphoma. MM remains yet an incurable B-cell malignancy with a median survival of 3 to 4 years. Autoimmunity is associated with improved outcome in patients with certain tumors suggesting that host-related immune response plays an important role in the pathogenesis. Given the association between MM and certain autoimmune diseases, documenting the impact of autoimmunity on risk of MM survival might provide clues to exploiting the host immune reaction for enhanced treatment strategies involving host immunity. Aims. To assess the prognostic significance of autoimmunity in patients with MM. Methods. Records on 10,557 population-based MM patients reported to the Swedish (1964-1990) and Danish (1977-1997) Cancer Registries were linked to the nationwide Inpatient Registries to capture hospital records including data on 32 defined autoimmune disease and the Cause of Death Regis-tries to retrieve mortality data. Using logistic regression models adjusted for gender, age, and country, we defined risk of MM mortality in relation to presence/absence of any autoimmune disease and for categories of autoimmune conditions: (Group A) autoantibodies (AAB) with sys-tematic involvement, (Group B) AAB with organ-specific involvement, and (Groups C) no AAB. We also evaluated risk of MM mortality for individual autoimmune conditions. Based on the expectation that secular trends in MM and autoimmune disease diagnostics/treatment could introduce heterogeneity we explored models stratified by calendar-period (<1987 and >1987) and by defined autoimmune disease categories. Results are presented by the approximate mid-point of year of MM diagnosis (<1987 vs. ≥1987) strata. Results. In the first calendar-period (<1987) we observed overall significantly decreased risk of MM mortality among persons with presence (n=576) of any autoimmune disease (OR=0.44, 95% CI 0.25-0.79). When we fit models by the 3 autoimmune disease categories, we observed decreased MM mortality for each: Group A (OR=0.34, 95% CI 0.15-0.75), Group B (OR=0.71, 95% CI 0.25-1.97), and Group C (OR=0.32, 95% CI 0.18-0.52), although only Groups A and C reached formal significance. The Group A effect was driven by the conditions rheumatoid arthritis and polymyositis/dermatomyositis; and the Group C effect was driven by conditions including ankylosing spondylitis, rheumatic fever, sarcoidosis, and polymyalgia rheumatica. In the second calendar-period (>1987), we found no statistical associa-tion between MM mortality and presence of any autoimmune disease (OR=1.20, 95% CI 0.92-1.56), Group A (OR=1.25, 95% CI 0.78-2.01), Group B (OR=1.59, 95% CI 0.87-2.21), Group C (OR=0.97, 95% CI 0.66-1.63), or individual autoimmune conditions. Estimates were similar when analyses were restricted to autoimmune diseases documented only prior to MM diagnosis (n=520). Summary/Conclusions. The decreased mortality among MM patients diagnosed in the first calendar-period with certain autoimmune diseases is intriguing and provides support for the role of host-related immunity as an anti-tumor agent in MM thera-py. The observed protective effect in the first (1964-1986), but not in the second (1987-1997), calendar-period might reflect variations in diagnos-tic procedures, clinical management, and/or treatment strategies for autoimmune disease and/or MM. Future studies are needed to clarify underlying mechanisms of our findings.
The combination of BOR and HDM was a logical F. Cavallo,
B. Bruno,
A. Falcone,
S. Bringhen,
CD34/kg) were infused on day 0. The dose of BOR M. Boccadoro,
) (RMP) and aspirin.
Venous thromboembolism (VTE) is a common complica-
M.T. Petrucci,
Combination approaches of new drugs with convention-

CR and 6 (46%) a VGPR. Two patients were non responders. Among the
4 patients receiving a second ASCT, 2 CR and 1 VGPR were observed
after HDM. The median duration of neutropenia (< 0.5
× 10^9/L) was 7 and 2 days respectively. Extra-
haematological toxicities were limited: grade 3/4 mucositis in 4 cases,
erythroderyemia in 6 cases and cardiac arrhythmia in 3 cases. Cutaneous
reactions were mainly reported in association with glycopeptid antibi-
octics. No toxic death was observed. Thirteen patients were assessed for
early response at 3 months after ASCT. Four patients (31%) achieved a
Response (VGPR= >90% of M component reduction) is the main prog-
nosis factor for survival after Autologous Stem Cell Transplantation (ASCT) in Multiple Myeloma (MM). High Dose Melphalan (HDM) (200mg/m^2) is the recommended conditioning (Regimen) before ASCT. However, the rate of CR+VGPR is only 40% to 50%. Bortezomib (BOR) has demonstrated a significant activity in relapsed/refractory patients, a
synergic effect with Melphalan (MEL) and a lack of haematological
toxicity. Aims. The combination of BOR and HDM was a logical
approach to improve the rate of CR+VGPR after ASCT. Methods. Between June 2005 and February 2007, 25 patients with stage II or III DS MM have been enrolled to receive an ASCT conditioned with both BOR
and HDM. BOR (1 mg/m^2) was delivered on days - 6 , - 3 , +1 and +4,
and thrombopenia (< 20
× 10^9/L) was 7 and 2 days respectively. Extra-
haematological toxicities were limited: grade 3/4 mucositis in 4 cases,
erythroderyemia in 6 cases and cardiac arrhythmia in 3 cases. Cutaneous
reactions were mainly reported in association with glycopeptid antibi-
octics. No toxic death was observed. Thirteen patients were assessed for
early response at 3 months after ASCT. Four patients (31%) achieved a
CR and 6 (46%) a VGPR. Two patients were non responders. Among the
4 patients receiving a second ASCT, 2 CR and 1 VGPR were observed
after BOR+HDM at 3 months. Conclusions. These preliminary results strongly suggest that BOR (1 mg/m^2 ×4) and HDM is a safe and highly
effective conditioning regimen in MM, requiring further investigations.
For the 13 patients assessed at 3 months, the rate of CR+VGPR was
77%.

**ENOXAPARIN OR ASPIRIN FOR THE PREVENTION OF RECURRENT THROMBOEMBOLISM IN NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH MELPHALAN AND PREDNISONE PLUS THALIDOMIDE OR LENALIDOMIDE**

A. Palumbo,
C. Rus,
F. Cavollo,
A. Bertolla,
M.T. Petrucci,
A.M. Liberati,
P. Musto,
E. Pisani,
S. Morandi,
J. Zeldis,
F. Rodeghiero,
M. Boccadoro

Background. Venous thromboembolism (VTE) is a common complica-
tion in cancer patients. The risk is particularly high after surgery, during
chemotherapy and in association with central vein catheters. Aggressive
antitumor therapy with thalidomide or lenalidomide increases the risk of
thrombosis. The underlying mechanisms are poorly understood, but
these therapeutic agents induce vascular damage. The risk of thrombo-
sis is higher for patients receiving thalidomide at diagnosis in compar-
ison with those treated at relapse. Low-molecular weight heparin is con-sidered the standard prophylaxis in these patients. Low-intensity war-
farin and aspirin have also been used. Patients received melphalan, pred-
nisone (MP) alone; or MP plus melphalan, prednisone, and thalidomide (MPT)
or MP plus lenalidomide (Revlimid®) (RMP) and aspirin. Aims. We evaluated the efficacy and safety
of enoxaparin or aspirin in the prevention of VTE, in newly diag-
nosed myeloma patients. Methods. In the MP group, no patient received
anticoagulant prophylaxis. In the MPT group, MP was combined with
Thalidomide (Pharmion Ltd., Cambridge, UK), no anticoagulant pro-
phylaxis was administered until December 2003. In a preliminary anal-
ysis, an high incidence of thrombosis was observed, therefore the proto-
col was amended and enoxaparin at 40 mg per day was introduced as
prophylaxis and delivered subcutaneously during the first four cycles of
MP. In the RMP group, all patients received oral aspirin 100 mg once
to a day continuously until any sign of relapse or progressive disease.
The time to occurrence of the first thromboembolism was calculated from
the start of chemotherapy. Results. In the MP group, VTE was
reported in 2 of the 144 patients; in the MPT group, symptomatic deep-
venous thrombosis, pulmonary embolism, or both occurred in 12 of the
MP patients who did not receive any anticoagulant prophylaxis. Throm-
boembolism was observed in 4 of the 78 MPT patients who received
enoxaparin prophylaxis; in RMP group, one of 50 patients, who received
aspirin, experienced pulmonary embolism. Median time for VTE was 4
months in the MP group, 3 months for MPT with and without antico-
gagulant prophylaxis. In the RMP group, the only episode of throm-
boembolism occurred after 1 months from start of therapy. In compar-
ison with MP, the hazard ratio for recurrent VTE in the MPT group with-
out any prophylaxis was 14.3 (95% CI, 3.2 - 64.3; p<0.0001); in the MPT
group with enoxaparin it was 3.76 (95% CI, 0.69 - 20.52; p=0.11); in the
RMP group, with aspirin it was 2.5 (95% CI, 0.15 - 19.3; p=0.67). No
significant interactions between treatment group and risk factors were
detected. No serious bleeding was observed during both aspirin and
enoxaparin prophylaxis. Conclusion. MPT with enoxaparin or RMP with
aspirin were safe and equally effective in reducing the risk of recurrent
VTE to levels observed in patients who received oral MP only.

**INTERMEDIATE-DOSE MELPHALAN (100MG/MD), THALIDOMIDE, DEXAMETHASONE AND STEM CELL SUPPORT IN PATIENTS WITH REFRACTORY OR RELAPSED MYELOMA**

P. Musto,
I. Avonto,
R. Scalzulli,
B. Bruno,
A. Falcone,
M.T. Ambrosini,
S. Bringhen,
E. Gay,
C. Rus,
F. Cavollo,
F. Palco,
M. Massaia,
M. Boccadoro,
A. Palumbo

‘Italian Myeloma Network, GIMEMA, Italy; ’Divisione di Ematologia Univ.
Torino, TORINO, Italy

Background. Combination approaches of new drugs with convention-
thal therapies have increasingly been adopted as savage or even first-line
treatment for multiple myeloma. High-dose or dose-intensive i.v. mel-
phalan followed by hematopoietic cell support induced higher response
rates and improved outcome compared to conventional oral melphalan
in several randomized trials. These findings formed the rational for the
combination of both bortezomib and thalidomide with intermediate-
dose melphalan (100mg/m^2) as conditioning regimen prior to autolo-
gous hematopoietic cell infusion. No data are available on the use of this
combination as conditioning regimen in the transplant setting. Aims. We
assessed the safety, tolerability and response rate of intermediate-dose
melphalan, Velcade, thalidomide and dexamethasone followed by stem
cell support in refractory or relapsed multiple myeloma (MM) patients.
Methods. Twenty-six advanced myeloma patients were treated with mel-
phalan at 50 mg/m^2 and bortezomib at 1.5 mg/m^2 on days -6 and -3
associated with thalidomide at 200 mg and dexamethasone at 20 mg on
days 6 through -3 (MVDt), followed by hematopoietic cell support on
day 0. Results. Between September 2004 and December 2005, 26 patients
with relapsed or refractory MM were enrolled in the study. Median time
diagnosis was 48.5 months (range 2.3-142 months). All patients were
induced with standard autologous transplants. Moreover, 14 (54%)
were treated with a combination of thalidomide and dexamethasone
and 13 (50%) with a second autologous transplant as salvage treatments.
Objective responses occurred in 17 of 26 patients (65%), including one
complete remission (CR 3%), 3 near complete remissions (nCR, 11%)
and 2 very good partial response (VGPR 7%); 3 patients (10%) showed
minimal response. Six patients (23%) showed no response (NR) and no
patients showed progressive disease (PD). Interestingly, of 5 patients
who had previously progressed while on thalidomide and prednisone,
1 reached nCR, 2 PR and 1 MR. After a median of 9 months (range 1-
16), 7 patients (27%) were alive in remission, 15 patients (58%) relapsed,
4 patients (15%) died from progression disease and one patient from
infective toxicity. Median progression-free survival for all patients was
6 months (range, 1 to 16 months). Response rate was higher than that
induced by the previous line of treatment in 12 patients (46%); response
duration was longer than in 6 patients (30%). Grade 3 thrombocytopen-
ia developed in 46% of patients, grade 4 in 54%. Forty-two percent of

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patients developed grade 3 anemia, 38% grade 4, whereas all patients showed grade 4 neutropenia. Five patients (19%) showed grade 1-2 neurologic toxicity, 1 patient grade 3. Infections consisted of pneumonia in 9 patients (35%), fatal for one patient and neutropenic fever (12%). Infections required iv broad spectrum antibiotic therapy in 50% of the patients. Conclusion. MVTD showed encouraging activity with manageable toxicity and represents a promising treatment for advanced myeloma patients.

0236
THALIDOMIDE-DEXAMETHASONE VS THALIDOMIDE-DEXAMETHASONE AND PEGYLATED LIPOSOMAL DOXORUBICIN: A CASE-MATCHED STUDY IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA

M.O. Massimo, 1 S. Bringen, 1 L. Corvatta, 1 M. Marconi, 1 P. Falco, 1 I. Avonito, 2 G. Visani, 2 E. Alesiani, 3 M. Brunori, 2 M. Ruggieri, 2 M. Catarini, 2 M.N. Piersantelli, 2 C. Polloni, 2 P. Leone, 1 A. Palumbo 3

1Clinica di Ematologia, ANCONA, Italy; 2Divisione di Ematologia, TORINO, Italy; 3Unita Operativa Onc hematologica, S. SEVERINO, Italy; 4Divisione Medicina, FANO, Italy; 5Divisione Ematologia, ASCOLI PICENO, Italy

Background. Thalidomide alone or in combination with dexamethasone and/or chemotherapy is the most extensively used compound in the treatment of relapsed/refractory multiple myeloma (MM). However, which thalidomide-based regimen is more effective and less toxic is still unknown. Recently we demonstrated that the combination of thalidomide, dexamethasone and pegylated liposomal doxorubicin (ThaDD) leads to high rate and high quality of response (ORR=92%; CR/TnCR= 32%) with a PFS of 47% and OS of 65% at 2 years. Aims. In the present study we compared ThaDD with the combination thalidomide-dexamethasone (T-D), frequently used in advanced MM patients as salvage therapy. Methods. A total of 47 relapsed/refractory patients treated ThaDD was compared with a control group of 47 pair matched patients for age, serum β2-microglobulin, previous chemotherapy and high-dose therapy. T-D regimen consisted of thalidomide 100 mg/day continuously, dexamethasone 40 mg on days 1-4 and 9-12, pegylated liposomal doxorubicin 40 mg on day 1 every 28 days. T-D regimen consisted of thalidomide 100 mg/day continuously and dexamethasone 40 mg on days 1-4 repeated monthly. Both groups included a lot of elderly patients, who had received 3 or more prior chemotherapy regimens and who had undergone stem cell transplantation. Results. ThaDD significantly increased overall response rate in comparison with T-D (92% vs 63.5%; p=0.008) and, importantly, induced significantly better quality of response (ePR 78.5% vs 59.5%, p=0.077; VGPR 36% vs 15%, p=0.018; CR/TnCR 30% vs 10.5%; p=0.021). Compliance to therapy was satisfactory in both groups of patients and grade 3-4 neurologic toxicity were limited (4.2% in patients treated with ThaDD vs 2.1% in those receiving T-D). On the contrary, grade 3-4 hematological toxicity (52% vs 0; p<0.0001), grade 3-4 infections (23% vs 0; p<0.0001) and vascular events (12.8% vs 6.4%; p=0.293) were more frequent in patients treated with ThaDD although no deaths were related to these complications. The rate of infections decreased below 10% when ciprofloxacin was added in the ThaDD regimen. The median PFS was significantly longer in ThaDD group (22 months vs 11.5 months, 36% vs 13% at 3 years; p=0.0008) as well as median EFS (21 months vs 11.5 months, 28% vs 13% at 3 years; p=0.007) and OS (NR vs 23.5 months, 52% vs 26% at 3 years; p=0.051). Conclusions. ThaDD, as salvage therapy for MM, regimen is superior to the combination thalidomide-dexamethasone since it induces a significantly higher and better quality response rate than T-D and this translates into a significantly better survival measures. The incidence of infections and deep venous thrombosis are more frequent in ThaDD group but they result manageable with adequate prophylaxis. We believe that ThaDD combination could be a valid candidate for comparison with bortezomib- or lenalidomide-based regimens in order to identify the optimal salvage therapy in advanced MM.

0237
DYSFUNCTION OF TOLL-LIKE RECEPTORS: A POSSIBLE IMPLICATION IN THE PATHOGENESIS OF IMMUNODEFICIENCY IN MULTIPLE MYELOMA

E. Andreakos, 1 M.A. Dimopoulos, 2 K. Tsionos, 1 C. Xirakia, 1 A. Anagnostopoulos, 1 E. Katodritou, 1 K. Zervas, 1 P. Sideras, 1 E. Terpos 1

1Academy of Athens, ATHENS, Greece; 2University of Athens School of Medicine, ATHENS, Greece; 3General Airforce Hospital, ATHENS, Greece; 4Papageorgiou General Hospital, THESSALONIKI, Greece; 5Thesigeion Cancer Center, THESSALONIKI, Greece

Background. Immune paresis, renal failure, neutropenia and anti-myeloma therapy can combine to cause severe immunodeficiency in multiple myeloma (MM). Thus, infections are a major cause of death in MM. There is limited information for the possible role of innate immunity in the pathogenesis of immunodeficiency in MM. Innate immunity provides a first line of host defence against infection through microbial recognition and killing while simultaneously activating a definitive adaptive immune response. Innate immune detection of pathogens relies on specific classes of microbial sensors, such as Toll-like receptors (TLRs). TLRs are principal mediators of rapid microbial recognition and function mainly by detection of structural patterns that do not exist in the host. Aims. The aim of this study was to evaluate the expression and function of TLRs in newly diagnosed MM. According to our knowledge, such information is not available in the literature. Patients and Methods. Twenty-two patients with MM at diagnosis (15M/9F; median age 68 years), 2 patients with MGUS and 11 healthy, age- and gender-matched controls were studied. Five patients had stage 1, 9 stage 2 and 8 stage 3 myeloma, according to ISS. After the collection of peripheral blood, mononuclear cells (PBMCs) were isolated by Ficoll centrifugation (Histopaque-1077, Sigma-Aldrich). These cells were measured for the expression of TLRs (antibodies from ebioscience) using fluorescence activated flow cytometry (FC500, Beckman Coulter). In addition, 1x10⁷ cells/mL were cultured in 5% FCS 1 pen/strep RPMI in the presence or absence of various TLR ligands and supernatants collected after 20h. These were examined for the presence of inflammatory cytokines (tumor necrosis-α, TNF-α, and interleukin-6, IL-6) by ELISA (Becton Dickinson). Results. We found that although patients with MM express TLRs in PBMCs, their response to certain TLR ligands is defective when compared to healthy controls. TLR2, TLR4 and TLR6 of PBMCs of healthy controls reacted normally to the presence of their respective ligands (LPS, Pam3Cys, poly I:C and R-848 (Imiquimod)) to secrete high levels of TNF-α; while their action in patients with MM was significantly reduced (median value of TNF-α: 200 pg/mL; range 100-500 pg/mL; p<0.001). On the contrary, TLR7 and TLR8 from MM reacted normally to their ligand R-848 (Imiquimod) to secrete high levels of TNF-α (median value for patients and controls: 4.3 and 4.5 ng/mL, respectively; p=NS). NOD1, another pattern recognition receptor that recognizes bacterial peptidoglycans also reacted normally in MM patients. Similar observations have been made for the expression of IL-6. Our preliminary analysis showed that there was no difference in terms of TLRs function between MGUS patients and controls and between myeloma patients of different disease stages. Conclusions. There is a significant defect in TLR function in patients with MM, especially of these involved in immunity against bacterial infections. Thus the immune system fails to receive early priming signal which may contribute to the increased infections observed in MM. The restoration of function of TLRs to their normal levels has the potential to improve bacterial immunity in MM patients.
In 128 patients (69%) PCs showed CD56 expression on BM and on those in peripheral blood.

CD56 expression on PCs varies among particular CD56 positive MM patients. Intensity of CD56 expression on PCs was in 118 patients, IgA-45, IgD-1, IgM-2, Bence Jones'18, N5-2 and 16 PCL patients. Controls were 10 healthy subjects. Immunophenotyping was done on freshly collected BM samples using triple staining combination of CD138/CD56/CD38 monoclonal antibodies analysed by flow cytometry (Cytoron Absolute and FACSCalibur-Becton Dickinson). Plasma cells were identified as cells showing high-density expression of CD38 and CD138 (syndecan-1). Antigen expression intensity was calculated as relative fluorescence intensity (RFI) and for direct quantitative analysis the QuantiBRITE test was applied. Mean channels of phycoerythrin fluorescence were defined and antibody binding capacity (ABC) was then calculated using QuantiCALC software. Results. In 128 patients (69%) PCs showed CD56 expression. Out of all CD38+/CD138+ BM cells mean proportion of PCs with CD56 expression, was 83±20%, median 93%. RFI values ranged from 7.6 to 27.4 in particular patients (18.0±4.5, median 17.8) and the number of CD56 binding sites (ABC) on MM plasma cells ranged from 2255 to 58469 (14199±15038, median 8866). A correlation was found between RFI and ABC values (r=0.76; p<0.001%). Normal PCs did not express CD56.

The aim of our study was to evaluate the role of fluorine-18 fluoro-deoxyglucose positron emission tomography (FDG-PET) in plasma cell malignancies. A total of 49 patients were enrolled including 13 patients with newly diagnosed multiple myeloma (MM) and negative bone radiographs, four patients with solitary plasmacytoma, 26 patients with MM in remission but with suspected relapse, and six patients with monoclonal gammopathy of unknown significance (MGUS) with suspected progression to MM or with suspected other malignancy. FDG-PET results were verified by conventional imaging methods, including plain radiographs, magnetic resonance imaging (MRI) and computer tomography (CT). Focally increased FDG uptake was observed in three (23%) of 11 newly diagnosed myeloma patients with negative bone radiographs. The findings were all confirmed by CT or MRI. FDG-PET was negative in two patients with newly diagnosed MM, negative bone radiographs, and without focal infiltration on MRI but with anemia, high monoclonal immunoglobulin and high bone marrow infiltration by plasmocytes. In all other cases FDG-PET negativity in asymptomatic patients was associated with favorable prognosis; these patients are without progression after the median follow-up of 14 months. Focally increased tracer uptake was found in five of 26 patients with MM in remission. In four cases it was due to MM relapse, in one case due to ovarian carcinoma. Only in one patient FDG-PET failed to recognize extramedullary progression. Of the 20 patients who had negative FDG-PET scans, only one relapsed 12 months after FDG-PET examination; the remaining 19 patients are without progression with the median follow-up of 15 months. FDG-PET was positive in two of six patients with MGUS. In one case a thyroid carcinoma was later detected, in the other an intestinal tumor was found. We conclude that FDG-PET might contribute to initial staging of MM patients with negative bone radiographs and is useful for the follow-up of patients in remission especially in non-secretory MM and in patients with large plasmacytoma (>5 cm) after radiochemotherapy.
effects were constipation (10 patients WHO grade 1, 8 patients WHO grade 2), polyneuropathy (14 patients WHO grade 1, 2 patients WHO grade 2) and somnolence (4 patients WHO grade 1). None of the 23 patients developed dose-limiting hematotoxicity as defined by an ANC < 1.0 Gpt/L for > 7 days or an ANC < 0.5 Gpt/L for > 3 days or platelet count < 25 Gpt/L. Short neutropenia was reported in 8 patients (WHO grade 3 and 4) but no thrombocytopenia was observed. BPT with a dose between 50 and 200 mg thalidomide daily is well tolerated in patients with relapsed or refractory MM.

**0241. CANTHARIDIN, A DERIVATIVE OF BLISTER BEETLES INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS VIA INHIBITION OF IL-6-INDUCIBLE STAT3 PATHWAY: NEW AGENT FOR SIGNAL TRANSDUCTION THERAPY OF MULTIPLE MYELOMA**

M.S. Sagawa, T.N. Nakazato, Y.I. Ikeda, M.K. Kizaki
KEIO University School of Medicine, TOKYO Japan

**Background.** Multiple myeloma remains incurable despite the use of high-dose chemotherapy with hematopoietic stem cell transplantation; therefore, novel therapeutic approaches are urgently needed in clinical settings. The understanding that has recently been gained into the biology of myeloma has led to the development of biological treatments, which target the myeloma cells and its microenvironment. These agents have shown remarkable activity against refractory myeloma in early clinical trials, but prolonged drug exposure may result in the development of drug resistance. Therefore, the identification and validation of novel targeted therapies to overcome drug resistance and improve patient outcome are necessary. Aims. Previous reports suggest that IL-6 promotes survival and proliferation of myeloma cells through the phosphorylation of STAT3. Thus, compounds that suppress STAT3 phosphorylation have the potential for the treatment of myeloma. Recent studies have shown that Chinese traditional medicine cantharidin (CTD), a derivative of Blister Beetles, induces apoptosis in hepatoma, colon cancer, and leukemia cells. Therefore, we assume that CTD has the potency to induce apoptosis in myeloma cells, and may lead to a novel targeted therapeutic approach.

**Methods.** To address our hypothesis, myeloma cell lines (U266, RPMI8226), and fresh myeloma samples from patients were treated with CTD. The effects of CTD on cell growth, apoptosis, cell cycle status, and the signaling pathway were studied.

**Results.** CTD inhibited cellular growth of myeloma cells as well as freshly isolated myeloma cells from 5 patients in dose (0-10 μM)- and time (0-48h)-dependent manners with IC50 of 4.3 μM. Cultivation with 5 μM CTD did not induce cell cycle arrest, but induced apoptosis of myeloma cells and primary cells from patients, but not bone marrow cells from healthy volunteers 24h after treatment. These results suggest that CTD-induced apoptosis is cell cycle-independent manner. Treatment with CTD induced caspase-3 activity in myeloma cells, and it was completely blocked by the pre-treatment with Z-VAD. To address the molecular mechanism of CTD-induced apoptosis in myeloma cells, we next examined the effect of CTD on IL-6 signaling pathway. CTD inhibited IL-6-induced gp130 activation in a time-dependent manner. STAT3 is a transducer of the IL-6 signaling pathway, therefore we examined whether CTD could inhibit the STAT3 pathway. CTD inhibited phosphorylation of STAT3 at tyrosine 705 residues as early as 30 min after treatment, and down-regulated the expression of anti-apoptotic Bcl-xL. It has reported that STAT3 directly binds and activates the transcription of Bcl-xL gene promoter, resulting in the induction of the expression of Bcl-xL in IL-6-treated myeloma cells. Our results suggest that the inactivation of STAT3 and the down-regulation of Bcl-xL may contribute to CTD-induced apoptosis in myeloma cells. Conclusions. In conclusion, we report here for the first time that CTD induces apoptosis in various myeloma cells and primary myeloma cells in cell cycle-independent manner. Down-regulation of Bcl-xL with modulation of STAT3 in IL-6-mediated signaling pathway plays an important role in CTD-induced apoptosis in myeloma cells. Therefore, CTD is one of the promising candidates for the new therapeutic agent as a signal transduction therapy of myeloma.

**0242. CITRULLINE CONCENTRATION AFTER HIGH-DOSE MELPHALAN IN AUTOLOGOUS HSCT RECIPIENTS**

N.M.A Blijlevens, J.P. Donnelly, A.V.M.B. Schattenberg, T.J.M. de Witte
UMC St Radboud, NijMegen, Netherlands

**Background.** Mucosal damage to the intestines induced by intensive myeloablative conditioning for an allogeneic HSCT can be determined by the concentration of citrulline which is a functional marker of small intestinal enterocytes. However, there are no data available about the kinetics of citrulline levels for high-dose melphalan used to prepare for an autologous HSCT. Aims We were interested to know whether and when the citrulline concentrations declined after starting myeloablative therapy. Methods We selected 29 patients who underwent an autologous HSCT following conditioning with HDM 100 mg/m² HSCT day -3 & -2. We collected plasma samples from each patient via a central venous catheter at 9:00 hour on the first day of conditioning therapy and 5 times per week (Monday, Wednesday, Friday) thereafter until discharge. The samples were stored frozen until citrulline concentrations could be determined by HPLC. Oral mucositis was registered using a Daily Mucositis Score. Results The baseline mean citrulline concentration was 28 mM which is lower than the 45 mM that is found normally. The mean citrulline concentrations declined rapidly thereafter reaching a nadir of 6.7 μmol/L 11 days after starting HDM which is HSCT day +7. Citrulline concentrations then only increased gradually and were still significantly low at 12 mM when patients were discharged. The most severe oral mucositis coincided with the nadir of citrulline. Conclusion Citrulline appears a valuable marker of small intestinal mucosal barrier injury induced by HDM to prepare for an autologous HSCT.

**Reference**

results were similar - for stage I, II and III values 6.2%, 4.9% and 4.3%, p<0.05. Finally, there was a significant decrease in the values of PC-PI and PC-AI within the course of 20 MGUS patients - there was no statistical significance between the values, either. Conclusion. Our measurements support the hypothesis of PC-PI and PC-AI being independent prognostic factors and also the indicator of early transformation of MGUS into MM. Within the course of MGUS there exists no significant change in either of the indicated factors. Transformation into a more aggressive myeloma, there is a significant increase in PC-PI together with decrease of PC-AI. The above results also confirm the major importance of proliferation in the process of transformation into MM - there is significant difference even between MGUS and stage I MM, on the other hand, decrease in apoptosis reduction points to the importance of this process. Measurement of proliferation and apoptosis contributes to the assessment of MM prognosis, and plays also a prominent role in the evaluation of the course of MGUS, especially as an early predictor of transformation into multiple myeloma.

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0244

EPIDEMIOLOGY OF ANEMIA IN 720 PATIENTS WITH MULTIPLE MYELOMA: RESULTS FROM EUROPEAN ANAEMIA SURVEY

H. Ludvig,1 P. Gascón,2 S. Van Belle,3 for ECAS Investigators1

1Wilhelmina spinal, VIENNA, Austria; 2Hospital Clinic, BARCELONA, Spain; 3University Hospital Ghent, GENT, Belgium; University Hospitals and Clinics, EUROPE, Switzerland

Background. Although anemia is a common complication of multiple myeloma (MM) patients (pts), information on the evolution of anemia during follow up, relation with age and performance status, risk factors for anemia and treatment practices was not available. Aims. Identify the incidence, prevalence and evolution of anemia during an up to 6 months follow up period, analyse possible correlations between anemia and clinical characteristics, identify risk factors for evolution of anemia (in pts with myeloma and lymphoma) and study patterns of anemia treatment in European myeloma pts. Methods. 720 patients with multiple myeloma (male 52% and female 48%) were enrolled into a prospective, epidemiologic survey, ECAS (European Cancer Anemia Survey), which included an additional 1640 pts with lymphoma (L) and a total of 15 370 pts with cancer at any stage of their disease. Survey data were collected for up to 6 data points or 6 months of scheduled visits (Ludvig, EJC 2004; 40 (15): 2293-2307). Results. Median age in MM pts was 65.7 years (range 31-94), with 28% of patients presenting with age <60 years (yrs), 52% with age 60-69 yrs, and 40% with age ≥70 yrs. 28% of the 720 pts with MM were newly diagnosed 55% had persistent/recurrent disease and 17% were in remission. In terms of cancer treatment, 50% were receiving chemotherapy (CT), 46% were not receiving any cancer treatment, with the remainder receiving radiotherapy or concomitant CT and radiotherapy. At enrollment, 69% of patients were anemic (Hb <12 g/dL), 30% had Hb <10 g/dL and 59% Hb of 10 to 12 g/dL. 85% were anemic at some time during the survey. 78% of those <60 yrs, 85% of those 60-69 yrs and 90% of those 70+ were ever anemic. 44% had a WHO score of 2-4. The incidence of anemia in MM who were not anemic at enrollment and who started CT during ECAS was 75%. Incidence of anemia increased with increasing age (60% in pts < 60 yrs, 88% in those 60-69 yrs and 100% in those 70+). Adverse WHO score correlated with low Hb (r=0.346). Despite the 59% of those who became anemic having a nadir Hb <10 g/dL, 41% received no anemia treatment, 2% received iron, 22% transfusion and 35% received epoetin. Logistic regression analysis of MM/L pts revealed 4 variables significantly predicting anemia development: Initial Hb (adjusted odds ratio (AOR) 4.2), persistent/recurrent disease (AOR 1.5), female gender (AOR 2.8), and treatment with platinum-based CT (AOR 5.5) were found to independently predict anemia (p<0.001). Conclusions. Frequency of anemia in MM pts remains substantial and important: prevalence of anemia (ever anemic) was high (85%) in MM pts, increased with age and correlated with poor PS. Follow up during the 6 month post-enrollment period indicated that 75% of initially non-anemic pts developed anemia after starting CT. Anemia treatment was given to 41% of ever anemic MM pts, although 59% had at least once Hb levels <10 g/dL. With the identification of important risk factors, anemia management in MM pts could be improved.

0245

A PHASE I/II STUDY OF ARSENIC TRIOXIDE, BORTEZOMIB, AND ASCORBIC ACID IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

R. Berenson,1 J. Matous,2 D. Ferretti,3 R.A. Swift,1 R. Mapes,1 B. Morrison,1 H.S. Yeh2

1Inst. for Myeloma & Bone Cancer Research, WEST HOLLYWOOD, CALIFORNIA, USA; 2Rocky Mountain Cancer Centers, DENVER, COLORADO, USA; 3Oncotherapeutics, Inc., WEST HOLLYWOOD, CALIFORNIA, USA; 4Millennium Pharmaceuticals, Inc., CAMBRIDGE, MASSACHUSETTS, USA

Background. Arsenic trioxide (ATO), a trivalent arsenite salt, is believed to exert its cytotoxic effect by causing DNA fragmentation characteristic of apoptosis. Clinical studies have shown that ATO has antitumor activity as a single agent in patients with relapsed or refractory multiple myeloma (MM). Bortezomib (B) is a proteasome inhibitor that is currently approved for the treatment of relapsed or refractory MM. Preclinical studies have shown that combining ATO and B results in synergistic antitumor activity against human MM cells in tissue culture and xenograft animal models. Furthermore, the addition of ascorbic acid (AA) can sensitize human MM cells to the cytotoxic effects of ATO. These observations suggest that the combination of ATO/B/AA may be an effective treatment regimen for patients with MM. Aims. The primary aim of this study was to determine the safety and tolerability of the ATO/B/AA regimen in patients with relapsed or refractory MM. The secondary aims were to determine overall response rate, time to response, time to progression, progression-free survival, and overall survival in these patients. Methods. Patients with relapsed or refractory MM were enrolled in this Phase I/II dose-escalation trial in 6 cohorts. Patients were given ATO (0.125 or 0.250 mg/kg), B (0.7, 1.0, or 1.3 mg/m²), and a fixed dose of AA (1000 mg) IV on days 1, 4, 8, and 11 of a 21-day cycle for a maximum of 8 cycles. Results. At the time of this interim analysis, 22 patients (median age, 63 years) have been enrolled, and accrual has been completed on all cohorts. This group had a failed median of 4 (range, 3-9) prior therapies. One occurrence of grade 4 thrombocytopenia was observed. One occurrence of asymptomatic arrhythmia led to patient withdrawal. All other adverse events were grade 1 or 2. For the 21 patients evaluable for efficacy, objective responses were observed in 9 patients (45%), including 2 complete (CR; 10%), 2 partial (PR; 10%), and 5 minor (MR; 24%) responses. Only 1 (1 MR) of 6 patients receiving the lowest dose of B (0.7 mg/m²) showed a response, whereas 4 (1 CR and 3 MR) of 6 patients receiving the middle dose of B (1.0 mg/m²) responded, and 4 (1 CR, 2 PR, and 1 MR) of 9 patients receiving the highest dose of B (1.3 mg/m²) responded. Conclusions. The ATO/B/AA regimen was well tolerated by the majority of patients and produced objective responses in 45% of the patients in this heavily pretreated group. Eight of 15 patients enrolled in the highest dose of B had clinical responses to this regimen. The results of this Phase I/II study warrant further clinical evaluation of the ATO/B/AA combination regimen for the treatment of patients with relapsed or refractory MM.

0246

PREVALENCE OF RAS GENE MUTATIONS IN THE CONTEXT OF A MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA

D. Intini1, L. Agnelli,2 S. Fabris,3 G. Ciceri,1 L. Nobili,1 L. Baldini,1 F. Morabito,2 S. Bicciato,1 L. Lombardi,2 A. Zanella,2 G. Lambertenghi-Dielli,1 A. Neri1

1Fondazione IRCCS Ospedale Policlinico, MILANO, Italy; 2U.O. Epatologia, A.O. Anzani, COSENZA, Italy; 3Dip Pol Chir Ing, Università degli Studi, PADOVA, Italy

Background. Earlier studies have reported that activating mutations involving RA genes, in particular NRAS and KRAS, occur frequently in multiple myeloma (MM). The reported prevalence of mutated tumors varies from 10% to 40% at presentation, raising to 70% at relapse, suggesting a role of this lesion in tumor progression. Notably, the occurrence of such mutation in MGUS and indolent tumors is very low. Mutations of KRAS, but not NRAS, have been found to be associated with higher bone marrow burden and shorter survival. Aims. In the present study we investigated the prevalence and type of RAS mutations in MM in the context of a proposed molecular stratification, namely as TC classification, based on the presence of IGH translocation and dysregulation of cyclin D genes in MM. Methods. The presence of NRAS and KRAS gene mutations was investigated in a panel of 82 MM at diagnosis, 13 patients with extramedullary myeloma or plasma cell leukemia, 9 patients with
Personal History of Repeated Pneumonia Is Associated with Increased Risk of Multiple Myeloma


National Cancer Institute, NIH, BETHESDA, MARYLAND, USA; Danish Cancer Society. COPENHAGEN, Denmark

Background. In Europe and the U.S. a total of more than 85,000 multiple myeloma (MM) cases are diagnosed annually. Although the etiology of MM remains unclear, associations between MM and past history of disorders characterized by chronic immune dysfunction such as pneumonia have been observed in limited clinical and epidemiological studies. Aims. To evaluate risk of MM associated with a personal history of airway infections. Methods. Using population-based linked registry data from Denmark, we conducted a case-control study including 4,476 MM cases diagnosed 1977-1997 and 16,727 age and gender matched controls. All individuals were linked with the Danish Inpatient (1977-1997) and Outpatient (1994-1997) Register to gather information on discharges listing any of the following coded airway infections: tuberculosis, pneumonia, bronchitis, unspecified lower airway infection, laryngitis, nasopharyngitis/pharyngitis, unspecified upper airway infection, sinusitis, otitis media, and influenza. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) as measures of relative risks for each condition using logistic regression. Airway infection data were restricted to those that occurred more than one year before MM diagnosis for cases and their corresponding controls. In models including multiple prior airway infections, we examined the association between MM risk and number of events (1, 2, and 3+) and time from discharge listing a defined airway infection until MM diagnosis (1-5, 5-10, and 10+ years latency). Observed associations were stratified by age at MM diagnosis (≤65 vs. >65 years). Results. We found significantly increased risk of MM associated with personal history of pneumonia (OR=1.7, 95% CI 1.5-2.0; 207 cases, 207 controls). Significant risk was also found for bronchitis (OR=1.5, 95% CI 1.2-1.9; 149 cases, 152 controls) and especially for sinusitis (OR=2.0, 95% CI 1.3-2.8; 49 cases, 53 controls). Odds ratios increased with number of years of latency. Among persons with 1 or 2 prior pneumonia events, we found significantly increased risk of MM (OR=2.2, 95% CI 1.4-3.4; 10 cases, 21 controls). This risk was significantly higher among older (≥65 years) individuals (OR=2.1, 95% CI 1.2-3.7). Among subjects with sinusitis or bronchitis, the increased risk was observed in the 1-5 years latency interval only. Conclusion. Personal history of repeated pneumonia is associated with increased risk of MM.

Mineral Residual Disease Can Be Detected In Almost All Multiple Myeloma Patients in Remission Using A Combined Approach of Five-Colour Flow Cytometry and Interphase FISH on Subsequently Sorted Plasma Cells


Hematology, BARI, Italy; Virga Jesse Hospital, HASSELT, Belgium

Background. Translocations involving IGH, del(13q) and del(17p) are commonly found in MM and are excellent markers for residual disease. However, these findings are only insufficiently reliable and do not allow the identification of differentially expressed transcripts. Aims. Our study confirms the previous evidences reported by us and others and indicates that RAS mutations did not correlate at significant levels with specific genetic lesion or molecular features in MM.

Results. Mutations were found in 16/82 (20%) myeloma patients, in 2/13 (15.4%) PCL samples and in none of the MGUS patients. In 11 MM patients the mutation involved the NRAS gene at codon 13 (8 patients) and 61 (5 patients), and the KRAS gene at codon 12 (4 patients) and 61 (1 patient), respectively. PCL patients were both harboring a NRAS mutation at codon 61. Mutations were found in patients included in all TC groups: 4 patients in TC1 (25.5%), 5 in TC2 (38%), 3 in TC3 (17.5%) and 2 patients in both TC4 (12.5%) and TC5 (50%) groups. Although the higher frequency of mutations observed in TC1 and TC2 groups, this finding did not reach a significant statistical level. No significant correlation was found with chromosome 13q deletion, trisomy of chromosome 11, or Ig amplification. Unsupervised analysis of gene expression profiles of the 82 patients did not show any particular evidence of clustering of tumors with RAS mutations. A supervised analysis approach, comparing the RAS mutated MM (16 cases) versus wild-type (66) tumors in the complete dataset as well as in the TC1, 2, 3 or 4 groups, did not allow the identification of differentially expressed transcripts. Conclusions. We found significantly increased risk of MM associated with 3+ previous pneumonia events in the 1-5 years latency interval suggesting that pneumonia might be a potential late trigger for MM development, rather than a risk-factor for the precursor of MM, monoclonal gamopathy of undetermined significance (MGUS). Alternatively, pneumonia could be a manifestation of immune disturbances in late-stage MGUS. Future studies examining underlying mechanisms of the observed findings may provide insights to the etiology of MM.

Bortezomib Transiently Inhibits Osteoclast Activity in Cell Culture: Conditions Mimicking in Vivo Intermittent Treatment

P. Boissy, T. Pesner, J.M. Delaissé

Vie Hospital, VEJLE, Denmark

Background. Bone disease induced by multiple myeloma (MM) leads to severe pain, high risk for collapse of vertebral bodies and fractures of the major weight-bearing bones. It is due to acute degradation of bone matrix by osteoclasts, not coupled with new bone formation by osteoblasts. MM-induced bone disease is currently treated with bisphosphonates, highly effective bone resorption inhibitors, which do not stimulate but rather inhibit bone formation. Furthermore bisphosphonates may cause renal damage and osteonecrosis of the jaw. Therefore,....
it is important to reconsider the management of MM bone disease in long-term treatment. Recently, preclinical studies have reported that the proteasome inhibitor Bortezomib used for the treatment of MM patients can stimulate bone formation, and that in MM patients treated with Bortezomib serum levels of bone formation markers are increased. Aims. In this study, we have investigated whether Bortezomib may inhibit osteoclast activity. Methods. Osteoclasts were differentiated from pure populations of CD44+ cells with M-CSF and RANKL for 6-7 days. Cells were treated with Bortezomib at different concentrations in a continuous mode. It has been reported that prolonged inhibition of proteosome activity may be toxic for any cell type and in vivo pharmacodynamic studies have shown Bortezomib to be toxic to the vascular compartment as 30min after intravenous injection, displaying maximal inhibitory activity of the proteasome within 24 hours subsiding rapidly thereafter. Therefore, Bortezomib was also given intermittently to mimic the in vivo situation. Osteoclast differentiation and activity were assessed by measuring Tartrate-Resistant Acid Phosphatase (TRACP) activity in the medium. Cell viability was determined with Celltiter Blue measuring metabolic activity. To extend our observations to the clinical situation, serum levels of CTX-I, a bone resorption marker, were measured during the 3 days following therapeutic Bortezomib administration in a single patient. Results. Continuous treatment with Bortezomib at 4nM and higher concentrations proved to be highly toxic for differentiating osteoclasts (cultures in presence of M-CSF+RANKL) but also monocytes (cultures in presence of M-CSF only) during a 7-day culture. However, a 6-hour-pulse treatment with Bortezomib every third day, was not toxic to primary monocytes, even at a concentration as high as 25nM and a culture period as long as 7 days. In this condition, TRACP activity of osteoclasts was strongly inhibited with Bortezomib at first 24 hours with 65% inhibition at 25nM Bortezomib) but the activity returned to the control level after 72 hours. In the patient serum patient serum levels of CTX-I decreased during the first 48 hours after each Bortezomib injection (n = 5), and tended to increase again after 72 hours suggesting a partial recovery of osteoclast activity between each dose. Conclusion. Our results suggest that Bortezomib temporarily inhibits osteoclast activity in vitro and in vivo. This transient inhibition of osteoclasts could be an advantage compared to the more persistent inhibition of osteoclast activity by bisphosphonate since recent reports suggested that formation of new bone requires at least a transient activity of osteoclasts. Further clinical studies are warranted to validate our findings.

Combination Therapy Effects of Lenalidomide in FGFR3 Multiple Myeloma Cell Lines

A. Gandhi, J. Kang, L. Capone, P. Schafer, W. Sherman, D. Stirling
Celgene Corporation, SUMMIT, USA; Columbia-Presbyterian Medical Center, NEW YORK, USA

Background. Lenalidomide (Revlimid®) has recently been approved for the treatment of a subset of myelodysplastic syndromes, and is being evaluated as treatment for a broad range of other hematologic and oncology conditions, including multiple myeloma (MM), chronic lymphocytic leukemia and solid tumor cancers. Data from Phase II and Phase III studies of lenalidomide plus dexamethasone in MM patients show significant benefits of combination therapy versus dexamethasone alone, in terms of median time to disease progression and overall survival. A subset of MM patients (10-20%) are known to have a t(4;14) translocation that results in the overexpression of oncogenes FGFR3 and MMSET/WHSC1. Results that FGFR3 is a tyrosine receptor kinase that is activated by the pro-angiogenic growth factors aFGF and bFGF. FGFR3 is not normally expressed in MM cells but is overexpressed and somatically hyperactivated in constitutively activating mutation in multiple myeloma cells with t(4;14). We hypothesized that FGFR3+ cells may have enhanced sensitivity to lenalidomide inhibition. Aims. Given the involvement of FGFR signaling in angiogenesis and the anti-angiogenic activity of lenalidomide, we studied the effect of lenalidomide on proliferation signals in FGFR3+ MM cells. Methods. Three FGFR3+ (NCI-H929 (t(4;14)), wt FGFR3), LP-1 (t(4;14), FGFR3 F384L), and OPM-2 (t(4;14), constitutive FGFR3 K650E)) and six FGFR3- multiple myeloma cell lines (JNJ3, SK-MM-2, EJM, RPMI-8226, Karpas-620 and KMS-12) were tested. Cells were incubated with compounds for 72 hours and assayed by 3H-thymidine incorporation. IC50s were calculated by nonlinear regression analysis with GraphPad Prism. Results. The lenalidomide sensitivity in cell proliferation assays is NCI-H929 > Karpas-620 > LP-1 > SK-MM-2 > EJM, OPM-2 > JNJ3 > RPMI-8226 > KMS-12 indicating no correlation between lenalidomide anti-proliferative activity and FGFR3 expression. Thalidomide had little if any effect (IC50>100 nM) in all cells tested. To study the effect of lenalidomide in combination with other chemotherapeutic agents in FGFR3+ MM cells, three FGFR3+ MM cell lines (NCI-H929, LP-1 and OPM-2) were treated with lenalidomide in combination with dexamethasone (Dex), doxorubicin (Dox), and vincristine (vinc). In FGFR3-negative cells (RPMI-8226), lenalidomide was only partially additive with Dex, and completely non-additive with the other three agents. In FGFR3+ cell lines, partial additivity with Dex and Vinc was observed in 2 out of the 5 cell lines tested. In the constitutively active FGFR3+ cells, the len-Dex and len-Vinc combinations were fully additive. In the len+Dex combination was also examined in 5 addition FGFR3- multiple myeloma cell lines and partial additivity was observed in 2 out the 5 cell lines tested. In the constitutively active FGFR3+ cells, the len-Dex and len-Vinc combinations were fully additive. The len+Dex combination was partially additive in two out of three FGFR3+ MM cell lines. Conclusions. No correlation was found between lenalidomide anti-proliferative activity and FGFR3 expression. In four MM cell lines (RPMI-8226, NCI-H929, LP-1, OPM-2), the len+Dex combination is better than either agent alone, which correlates with the clinical observation that this combination in MM patients is better than Dex alone. Our data suggest that the len+Dex combination may also be beneficial in an FGFR3+ MM population. These data also provide new evidence to suggest that the len+Vinc combination may be better than either agent alone against multiple myeloma clone proliferation.

A NOVEL IN VIVO ANIMAL MODEL FOR HUMAN MULTIPLE MYELOMA BASED ON BIOLUMINESCENCE IMAGING OF TUMOR CELL GROWTH

H. Rozemuller, I. Koggel, R. Spaapen, A. Hagenbeek, A.C.M. Martens
University Medical Center, UTRECHT, Netherlands

Preclinical testing of new therapeutic strategies or new cytotoxic drugs for the treatment of multiple myeloma (MM) requires animals models that closely resemble human disease and that allow quantitative evaluation of the applied therapy. Here we present a novel in vivo MM model by engraftment with U266 or RPMI-8226/S cells, both of human origin, into RAG2gc double knock-out mice (RAG2GC). These mice are immune deficient because they lack T-, B and NK cells and the mice easily accept human cells (van Rijn et al., Blood 2005, Rozemuller et al., 2004). In this model we introduce the use of luciferase gene marking of the MM cells and applying Bioluminescence imaging (BLI) in living animals for measuring the initial growth of the MM cells and the response to treatment. After intravenous injection of 2x10^6 MM cells engraftment and outgrowth occurred in all mice but it was limited to the bone marrow compartment, thus resembling human MM. FACS analysis revealed the presence of human CD45, CD138 and CD38 positive myeloma cells in a variety of examined bone specimen. Infiltration into other organs was not observed. MM cells were transduced with a GFP- Firefly luciferase (Fluc) fusion gene. When luciferase converts the substrate luciferin, photons are emitted that can be registered by using sensitive CCD cameras. The absolute number of photons that are produced correlates (in our application) with the local tumor mass. Mice were injected i.v. with GFP-Fluc 2x10^4 cells MM cells (U266 or RPMI8226/S) and then imaged weekly using BLI. Within 2 weeks after injection significant BLI signals were detectable. Per mouse 5-10 foci showed luciferase activity, predominantly in the pelvic region, skull, limbs and the spine. All mice were examined weekly with BLI. We observed that the amount of light produced at the various foci of tumor growth, within an individual mouse as well as between mice, showed a comparable increase. After 9-12 weeks all mice were killed due to excessive tumor growth. Growth curves that were made on the basis of subsequent BLI images revealed exponential growth of the total tumor mass per mouse as well as for the individual foci of MM growth in each mouse. All curves show similar growth kinetics with an average population doubling time of approximately 5-6 days. The range in which tumor growth can be monitored with BLI (and as a consequence the reaction to treatment) spans 3-4 decades. The BLI signals could post-mortem be confirmed by flow cytometry of GFP+ cells in affected bones. The major advantage of this model is the option for quantitative evaluation of the effect of a given treatment has on the tumor load. In conclusion, we have developed a novel in vivo model to study the characteristics of homozygous and outgrowth of MM and we show that it can be used for quantitative evaluation of the efficacy of the therapeutic intervention.
0252

RELATIVE QUANTIFICATION OF TUMOR ASSOCIATED ANTIGENS MAGE-A1 AND MAGE-A3 IN MULTIPLE MYELOMA

J.V. Vagaskova, J. Kadlecova, R. Spesna, R. Gayllova, M. Penka, J. Michalek, R. Hajek

Faculty Hospital Brno, BRNO - BOHUNICE, Czech Republic; 2Dept. of Medical Genetics, FH, BRNO, Czech Republic; 3Dept. of Clinical Hematology, FH, BRNO, Czech Republic; 4DIK, Faculty Hospital, BRNO, Czech Republic; 5I'HOK, Faculty Hospital, BRNO, Czech Republic.

Background. Multiple myeloma (MM) is a malignant plasma cell neoplasm that often is preceded by a common pre-malignant monoclonal expansion of plasma cells called monoclonal gammapathy of undetermined significance (MGUS). MGUS is reported to be present in 1% of the adult population and to progress to MM at a rate of 1% per year. MM is an incurable tumor characterized by clonal expansion of malignant plasma cells in the bone marrow. The MAGE genes encode antigenic peptides that are presented by HLA class I molecules and that are recognized on human tumors by T lymphocytes. They are activated in a variety of malignant neoplasms while remaining silent in normal tissues with the exception of testis and occasionally placenta. Presence of RNA transcripts encoding members of the MAGE gene family in myeloma tumor cells and cell lines has been documented. Aims. The aim of this study is to evaluate the possibility of using these genes as molecular markers of progression of MGUS to multiple myeloma and the early relapse of the MM. This abstract covers our pilot and preliminary Results. Total of 50 samples from bone marrow were evaluated: 25 samples from myeloma patients, 8 samples of patients with early stage of MM who did not require treatment (smoldering MM 2x and stage 1A 6x), 5 samples of MGUS patients, 9 samples of normal healthy donors served as control group. Total RNA was evaluated by RT-PCR and then by real-time PCR using FRET probes on the LightCycler instrument (Roche). For relative quantification we used G6PDH housekeeping gene as an external standard. As positive control we used myeloma cell line U266. Results. None from samples of 9 healthy donors did show expression of MAGE. Only 1 of 5 (20%) samples from MGUS patient showed expression of MAGE-A1. Five (62.5%) from 8 patients with early stage of MM (IA and smoldering) showed expression of MAGE. On the contrary 11 (44%) of 25 samples from MM patients showed expression of at least one gene MAGE-A1 or MAGE-A3 or both (7 cases). Summary/Conclusions. We have confirmed that expression of MAGE is not present in samples of healthy donors. There is an obvious correlation between expression of the MAGE genes and early late stage of the disease as our preliminary evaluation confirmed the detection of low expression levels of MAGE-type mRNA in bone marrow from patients with MGUS and early stage of MM. It is possible that MAGE antigen monitoring may predict the evolution towards more advanced disease as well as this method should be used for monitoring minimal residual disease in patients with MM. The prospective evaluation is under way. The actual results covering total of 15-50 evaluated patients in conclusive groups will be presented. This work is supported by grant of the Ministry of Education, Czech Republic, LC06027.

0253

POST RELAPSE AND OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA THAT PROGRESSED AFTER DECEMBER 1998


1Sheba Medical center, TEL-HASOMER, Israel; 2Department of Hematology, Medical Univers, LUBLIN, Poland

Background. The prognosis of multiple myeloma (MM) patients progressing after autologous BMT (ABMT) was documented to be poor, ranging between 14-18 months in various reports. Since December 1998, a variety of novel methods were introduced for the salvage therapy of MM, namely Thalidomide, reduced intensity allogeneic stem cell transplantation (RISTCT) and later on Bortezomibe and Lenalidomide. Aims. To evaluate the impact of the introduction of novel methods in progressing MM. Methods. We report the outcome of a non-selected group of MM patients that progressed from ABMT after December 1998 and were treated in our center. The treatment strategy for this group of patients was based on the nature and the risk score of the relapse, according to the following milestones: 1. Treatment only at clinical indication; 2. Thalidomide with or without steroids: A. As first line of salvage therapy. B. At relapse from RISTCT in a combination with donor lymphocyte infusion (DLI); 3. RISTCT or ABMT: A. for consolidation of response in patients resistant to thalidomide (after an induction of response with platinum containing regimen) and/or with high risk responding relapse. B. At escape from thalidomide effect; 4. Bortezomibe and Lenalidomide: A. in patients resistant to or escaping from Thalidomide effect, with an attempt to consolidate response by high dose therapy with allogeneic or autologous stem cell support. B. At progression from RISTCT that did not respond to DLI and Thalidomide. Results. 84 patients (pt’s) that their disease progressed after ABMT between December 1998 and May 2004 were enrolled. All patients were treated with Thalidomide as first salvage therapy at a clinical indication, followed by the various options according to the scheme. At a later stage, 32 patients underwent RISTCT (22 from related and 10 from unrelated donors) and 18 patients had an ABMT. 16 patients were treated with Bortezomibe and 8 patients received Lenalidomide, for further progression. The median interval from detection of progression to initiation of therapy was 5.5 months. Response rate to thalidomide + steroids was 59% with a median duration of response (for responders not transplanted immediately at response) being 15 months (the longest exceeding 5.5 years). Transplant related mortality in RISTCT was 22%. The 3 years overall survival (OS) for all the patients that underwent RISTCT is 42%, and for those transplanted at response 61%. The median OS rate from progression, of the entire group of 84 patients, is 59 months. The median OS from first ABMT of this group is 84 months. Summary. The introduction, since 1998, of novel tools for the treatment of progressing MM, significantly prolongs the post relapse and the overall survival of patients with MM that undergo ABMT as a part of the initial therapy.

0254

OPG/ RANKL SYSTEM IN MULTIPLE MYELOMA

V.S Goranova-Marinova, S.E. Goranov, P.I. Pavlov, A.D. Bojadzhieva

1University Hospital Sv.Georgi, PLOVDIV, Bulgaria; 2University Hospital Sv.Georgi, PLOVDIV, Bulgaria; 3Department of Clinical Laboratory, Bulgaria

Background. According to the contemporary ‘convergent’ hypothesis the major osteoerostive and antiresorptive factors converge to the system osteoprotegerin (OPG)/receptor activator of nuclear factor-kB ligand (RANKL) and influencing its delicate balance they affect osteoclast proliferation, activation and apoptosis. Clinical results concerning the importance of the system in myeloma bone disease (MBD) are controversial. Aims. To analyse the serum levels of OPG and RANKL in patients with multiple myeloma (MM) and their correlations with clinical stage (Durie et Salmon), degree of MBD (according to the Merlini scale) and basic parameters of disease activity. Methods. We studied 66 newly diagnosed patients with MM, 29 male, 37 female, median age=61; 8±6.5; range 45-81 years. In I / II / III clinical stage were 15.7%, 30.3% / 58.0% patients, renal failure (RF) was found in 40.9%, MBD in 84.9%, hypercalcemia in 31.8%. Serum levels of OPG and RANKL (ELISA kits Bio-medica, Vienna) were compared to a control group of healthy individuals (n=30). Statistics were performed by SPSS for Windows v. 11.0. Results. OPG levels were higher in myeloma patients: 5,36±0,46 pmol/l vs 3,77±0,33 pmol/l (p<0,001) but OPG/creatinin ratio (thus eliminating the influence of RF) does not differ between the groups. The lowest OPG levels were measured in patients with MBD grade 2-3; 4,26±0,56. A positive correlation between β2-microglobulin and OPG was found (p<0,001; r= -0,375). We found strong negative correlations of OPG/creatinin with clinical stage (p<0,001; r=-0,616), MBD (p<0,001; r= -0,521) and bone marrow plasmocytosis (p<0,001; r= -0,509). Levels of RANKL were higher in MM compared to controls: 0,458±0,046 pmol/l vs 0,205±0,051 pmol/l (p<0,001).

Table 1. Rankl and Rank/OPG ratio-clinical correlations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rankl</th>
<th>Rank/OPG</th>
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<tbody>
<tr>
<td></td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Clinical Stage</td>
<td>&lt;0.001</td>
<td>0.524</td>
</tr>
<tr>
<td>Myeloma Bone Disease</td>
<td>&lt;0.001</td>
<td>0.524</td>
</tr>
<tr>
<td>Bone marrow plasmocytosis</td>
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</tr>
<tr>
<td>β2-microglobulin*</td>
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<td>0.577</td>
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<tr>
<td>LDH</td>
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<td>0.397</td>
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<tr>
<td>CRP</td>
<td>NS</td>
<td>0.24</td>
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</table>

*Patients without RF.
SAFETY AND EFFICACY OF BORTEZOMIB FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA IN DAILY ONCOLOGY PRACTICE

H. Freiman,¹ A. Calderoni,² P. Cornu,² R.A. Olie³

¹KSSW Kantonales Spital Sursee-Wolfhusen, SURSEE, Switzerland; ²Oncologia Varini & Calderoni, LUGANO, Switzerland; ³Hôpital Riviera/La Providence, VEVEY, Switzerland; ⁴Janssen-CEVA, BAAR, Switzerland

Background. Bortezomib is a novel first-in-class anti-cancer agent, a proteasome inhibitor. Several publications reported on the safety and efficacy of bortezomib in the treatment of relapsed/refractory multiple myeloma in clinical trials. Complementary data on the experience with bortezomib in daily oncology practice are needed. Aim. Evaluate the safety and efficacy of bortezomib in the treatment of relapsed/refractory multiple myeloma in routine clinical practice. Methods. Patients having undergone >2 prior lines of therapy were treated within a Compassionate Use Programme proposing 6 cycles of bortezomib. While treatment modalities were at physician’s discretion, the Programme recommended 1.3 mg/m² as bortezomib administration on days 1, 4, 8, and 11 of each 21-day cycle. Addition of oral dexamethasone (20 mg the day of and the day after bortezomib administration) was recommended after 2 or 4 cycles in case of PD or SD, respectively. Post-hoc safety and efficacy analysis of patient records was performed using predefined criteria. Best response achieved was evaluated by the attending physician or by marker (M-protein or light chain) nadir level. Responders were patients with at least MR (a decrease ≥25% in marker level vs. baseline). CR/nCR was defined by physician’s criteria or by a decrease >95% in marker level vs. baseline. Patients that received <2 cycles and irregular injections were excluded from the main analysis. However, a response analysis on an intention-to-treat (ITT) basis also included these patients. Results. Eighty-eight patients entered the Programme involving 62 oncologists/hematologists. Data from 5 patients were unavailable. Main analysis focused on 69 patients and ITT response analysis included 88 patients. Median patient age was 66 years (44-86), median time since diagnosis was 4 years (0.5-14) and median number of previous treatments was 3 (2-6). Median number of bortezomib cycles at data collection was 4 (2-19) and 37.9% of patients were co-treated with corticoids during the Programme. In 73.9% of patients (51/69) at least an MR was observed (61.4% in the ITT analysis). In 37.7% of cases, this response occurred within the first cycle and in 95.5% within 3 cycles. Best response was achieved within the first cycle in 11.1% of patients and within 3 cycles in 68.9%. In the other responders, continuation of treatment improved the quality of response. Eighteen weeks after treatment initiation, corresponding to the anticipated duration of the Programme, 76.2% of the responders were still responding (≥MR). At the time of data collection, median 4.7 months (0.5-15) after last bortezomib injection, 44.9% of patients (51/69) were still responding (37.3% of ITT patients), according to the physician’s evaluation. The most frequently reported adverse events were peripheral neuropathy (54.3%), thrombocytopenia (29.9%), diarrhea (25.9%) and fatigue (22.4%). Among the cases of peripheral neuropathy, 65.2% were due to aggravation of a pre-existing condition. No cases of bortezomib-related haemorrhage were reported. Conclusions. The use of bortezomib in daily clinical practice resulted in encouraging high response rates with a predictable adverse event profile in patients with relapsed/refractory multiple myeloma. Efficacy and safety data were similar to those reported in clinical trials.


dent with MM (MM) establishes the necessity for a staging system with prognostic reliability. For more than 30 years, the Durie-Salmon Staging System (DSSS) remained the system of reference despite its drawbacks. In the meantime, the prognostic significance of the combination of β2 microglobulin (β2m) and albumin (alb) has been recognized and subsequently employed by Bataille et al., the South West Oncology Group (SWOG) and most recently the International Myeloma Working Group (IMWG), in attempts to develop simpler staging systems with a stronger prognostic impact. Aims. To evaluate and compare the prognostic significance of these 4 staging systems in a large number of previously untreated MM patients.

Methods. Between January 1989 and January 2006, 470 consecutive patients were diagnosed with MM in our department. Ninety-two (19.6%) patients received high dose therapy followed by autologous stem cell transplantation and the rest 378 (80.4%) were treated with conventional chemotherapy. All patients were classified according to the following staging systems: 1) DSSS. 2) Staging System of Bataille et al. (BSS). Stage I: β2m < 6 mg/L and alb ≥3 g/dL. Stage II: β2m ≥ 6 mg/L and alb <3g/dL. Stage III: alb ≥3g/dL. 3) Staging System of the SWOG (SWSS). Stage I: β2m <2.5 mg/L. Stage II: 2.5 mg/L ≤β2m <5.5 mg/L. Stage III: β2m ≥5.5 mg/L and alb ≥3g/dL. Stage IV: β2m ≥5.5 mg/L and alb <3g/dL. 4) International Staging System (ISS) of the IMWG. Stage I: β2m <3.5 mg/L and alb ≥3.5g/dL. Stage II: neither stage I nor III. Stage III: β2m ≥5.5 mg/L. Overall survival (OS) was estimated according to Kaplan-Meier method. Differences in survival were assessed using the log-rank test. Results. The distribution and median OS of the patients according to each staging system are displayed in Table 1. Classification according to DSSS, BSS and SWSS yielded a significantly heterogeneous distribution of our patients, with the majority being classified in stage III, I and II respectively. ISS achieved the most homogeneous patient distribution. There was no statistically significant difference (p<0.05) in survival between stages II and III of DSSS, II and III of BSS, as well as between stages III and IV of SWSS. ISS alone yielded significant difference (p<0.0001) in survival between all three stages. Conclusion. Our study confirms the superiority of ISS over DSSS and previous prognostic classifications based on the combination of β2m and albumin. ISS proved to be a simple, reproducible alternative with high prognostic power, definitely able to gain wide clinical applicability.

Table 1.

<table>
<thead>
<tr>
<th>Stage</th>
<th>DSSS</th>
<th>BSS</th>
<th>SWSS</th>
<th>ISS</th>
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<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>OS months (95% CI)</td>
<td>N (%)</td>
<td>OS months (95% CI)</td>
</tr>
<tr>
<td>I</td>
<td>38 (8.1)</td>
<td>75 (62-78)</td>
<td>270 (57.4)</td>
<td>53 (44-62)</td>
</tr>
<tr>
<td>II</td>
<td>131 (27.9)</td>
<td>47 (37-57)</td>
<td>79 (16.8)</td>
<td>25 (21-31)</td>
</tr>
<tr>
<td>III</td>
<td>301 (64)</td>
<td>36 (52-40)</td>
<td>121 (25.7)</td>
<td>19 (13-23)</td>
</tr>
<tr>
<td>IIIA</td>
<td>231 (40)</td>
<td>38 (35-41)</td>
<td>121 (13-23)</td>
<td>98 (20.8)</td>
</tr>
<tr>
<td>IIIB</td>
<td>70 (13)</td>
<td>24 (16-32)</td>
<td>70 (14.8)</td>
<td>21 (15-27)</td>
</tr>
</tbody>
</table>

E. Verrou, E. A. Banti, E. Terpos, D. Mihou, T. Zelelouis, D. Krikelis, K. Vathsevanos. Theagenion Cancer Center, THESALONIKI, Greece; Theagenion Cancer Center, THESALONIKI, Greece; Airforce General Hospital, ATHENS, Greece.

Background. Bisphosphonate (BP)- associated osteonecrosis of the jaw (ONJ) is a new and distinct clinical entity. Cases of ONJ associated with the administration of BPs, that is characterized by decalcification of the oral mucous membranes and exposure of necrotic underlying mandible or maxilla, were first reported in 2003. Aim. To study retrospectively a large number of multiple myeloma (MM) patients treated with BPs, in order to estimate the incidence and identify possible risk factors for the development of ONJ. Methods. Administration of BPs was initiated in our department in 1991. A review of the medical records of all patients diagnosed with MM since 1991 was performed. We evaluated the type of BP administered, the time of exposure to BP and the cases of ONJ. The diagnosis of ONJ was based on the presence of symptoms and signs of introral bone necrosis, the findings of panoramic x-rays and the results of bone biopsies. Patients were divided into 3 groups according to the type of BP administered. Group A received pamidronate, Group B zoledronate and Group C pamidronate and zoledronate sequentially. The χ2 test was used for comparisons of proportions across levels of categoric variables. Mann Whitney U test and One Way ANOVA test were used to compare the median and mean time of exposure to BPs among groups respectively. Kaplan Meier method was used to estimate the actuarial risk of ONJ in each group. Differences were assessed using the log-rank test. Throughout the analysis a level of 5% was used to denote statistical significance. Results. Between 1991 and 2005, 303 patients with MM were diagnosed in our department. Bisphosphonates were administered to 254 (83.8%) patients with median time of exposure 15 months (4-77). Group A included 78 patients (50.7%), Group B, 91 (58.8%) and Group C, 85 (53.5%) with median time of exposure 10 (4-38), 12 (4-52) and 36 (6-77) months respectively. p(A,C) <0.000, p(B,C)<0.000. Forty- nine (16.2%) patients did not receive BPs. Twenty eight cases (11.02%) of ONJ were observed among patients treated with BPs. None of the patients without exposure to BPs developed ONJ. The median time of exposure to BPs in patients who developed ONJ was 55 months (12-68), whereas the respective time for patients who did not, was 14 months (4-77) (p<0.001). One case of ONJ was observed in group A (1.28%), 6 cases in group B (6.5%) and 21 in group C (24.7%). p(A,B =0.084). All ONJ cases in Group C occurred during treatment with zoledronate. The actuarial risk of ONJ after 18 months of administration was 3.7% for group A and 7.8% for group B (p=0.25). At 36 months the actual risk of ONJ was 37.2% and 12.4% for group B and C respectively (p=0.036). Conclusions. The incidence of ONJ in patients with MM treated with BPs is high. Time of exposure and probably the type of BP seems to contribute to the occurrence of ONJ.
Chronic lymphocytic leukemia and related disorders – Clinical/Experimental

0259 DETECTION OF RISK-IDENTIFYING MARKERS AND ADDITIONAL ABERRATIONS BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) IN CHRONIC LYMPHOCYTIC LEUKEMIA
A. Benner, L.M. Pedersen, S. Stilgenbauer
UMIC Utrecht, UTRECHT, the Netherlands

B cell chronic lymphatic leukemia (B-CLL) is the most common form of leukemia in adults. Recently, deletions of ATM, TP53 and trisomy of chromosome 12 have been identified as unfavorable markers using interphase FISH. FISH analysis is labor-intensive, expensive and limited to the number of probes analyzed. We developed a robust method based on multiplex ligation-dependent probe amplification (MLPA) of target sequences for 40 different tumor-associated genes, including unfavorable risk-identifying deletions of ATM and TP53 and trisomy of chromosome 12. MLPA data of 53 cases with CLL and one case with follicular lymphoma (FL) were validated using conventional karyotyping and interphase FISH analysis, revealing high sensitivity and specificity of the assay for these risk-identifying mutations. DNA profiling using MLPA showed recurrent gain of PMAIp1 and BCL2 (18q21.3), known targets in B-cell non-Hodgkin lymphoma (NHL). A recurrent deletion of CDKN2A/B locus (9p21) was found, that was associated with aggressive disease progression. A trisomy chromosome 19 was identified that went undetected by cytogenetics. MLPA confirmed trisomies of chromosomes where we demonstrated copy number changes in purified cases. MLPA can be used for rapid analysis of known risk-identifying markers and detection of additional numerical cytogenetic unbalances in B-CLL.

0260 QUANTITATIVE GENE EXPRESSION ANALYSIS OF SURROGATE MARKERS FOR GENETIC RISK GROUPS AND ADDITIONAL ABERRATIONS IN B-CLL
D. Kienle, A. Benner, A. Kröber, D. Winkler, P. Lichter, R. Dalla-Favera, H. Döhner, S. Stilgenbauer

University of Ulm, ULM, Germany; Central Unit Biostatistics, DKFZ, HEIDELBERG, Germany; DKFZ, HEIDELBERG, Germany; Columbia University, NEW YORK, USA

Background. The genetic factors VH mutation status, V2-31 gene usage, and genomic deletions at 11q22-q23 and 17p13 have been shown to be important prognostic markers in CLL. Given the high complexity of these analyses in the recent years several molecular surrogate markers were developed and are used in the facilitation of routine prognosis assessment. Aims. To assess the value of potential surrogate markers for the prediction of genetic risk groups and survival. Methods. Real-time RT-PCR (RQ-PCR) of candidate genes was performed in a CD19-purified and a non-purified CLL cohort each comprising the relevant genetic subgroups (VH mutated, VH unmutated, V2-31 usage). Several markers were validated in unpurified cases (11q-1p, 17p, 1p, 17q, 17p-1p, 17q-) and a non-purified CLL cohort each comprising the relevant genetic subgroups. Results. VH sequencing and FISH screening for genomic aberrations were carried out for all cases. Survival information was available for 80 (puriﬁed) and 88 cases (non-puriﬁed). Logistic regression was performed to test the predictive value of gene expression for genetic risk groups, Cox proportional hazards statistics for survival analysis. Results. The genetic risk groups in both cohorts showed the expected correlation with survival with significantly shorter survival of VH unmutated, 17p-, and 11q- cases indicating a representative composition of the cohorts under study. In non-purified cases, the best predictive marker for VH status was LPL (p = 0.001). While no reliable predictive markers were identiﬁed for V2-31 usage or 17p-, the ATM expression was predictive for 11q-. In survival analysis including all candidate genes, only TCF7 was identiﬁed as a signiﬁcant factor. In contrast to ZAP70, which was of borderline signiﬁcance (p = 0.061), TCF7 expression was positively correlated with survival times. In multivariate analysis, the parameters 17p-, 11q-, V2-31 usage, TCF7 and ZAP70 expression were identiﬁed as independent prognostic factors. Summary. Several results from our case studies obtained in CD19-purified cases could not be reproduced in unpurified cases strongly arguing for a tumor cell selection prior to expression analysis. In puriﬁed cases, ZAP70, LPL, and TCF7 were the best predictors for VH mutation status. Additional markers such as ATM and ZNF2 may help to identify genomic risk groups since 11q- and 17p-. Multivariate survival analysis suggests TCF7 as a strong survival predictor and points to a pathogenic role for this gene in CLL.

0261 DISTINCT EXPRESSION LEVELS OF NOXA IN PERIPHERAL VERSUS LYMPH NODE CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE LINKED WITH SURVIVAL CAPACITY
Academical Medical Center, AMSTERDAM, Netherlands

Background. The relentless accumulation of chronic lymphocytic leukemia cells is presumed to derive from proliferation centers in lymph node and bone marrow. To what extent the properties of these leukemic cells are linked with the often stated characteristic anti-apoptotic phenotype of CLL is unknown. Recently, we have described that in peripheral blood and lymph node proliferating CLL cases NOXA expression was not limited to protective changes but also included increased levels of pro-apoptotic Noxa and Bmf. The functional consequence of this finding is not known, nor whether this aberrant apoptosis gene profile is also present in CLL proliferation centers. Aim. To perform a functional comparison of apoptosis gene profiles from peripheral blood CLL versus lymph node CLL proliferation centers. Methods. Immunofluorescence microscopy, RT-Multiplex-Ligation-dependent Probe Amplification (RT-MLPA), Western blot, Transfection, RNA interference. Results. Lymph node material from 9 B-CLL patients and peripheral blood samples from 16 B-CLL patients were included. All B-CLL expressed CD5, CD23 and CD40. Direct manipulation of Noxa protein levels was achieved by protesome inhibition in CLL and via RNAi in model cell lines. In all these instances, the viability of the cells was directly linked with Noxa levels. Noxa overexpression in CD40-activated cells is linked with increased cell death. NOXA knockdown prolonged cell survival in vitro. Conclusions. In contrast to ZAP70, which was of borderline significance (negative association), in survival analysis including the expression of all candidate genes, only TCF7 was identified as a significant factor. In contrast to ZAP70, which was of borderline significance (p = 0.061), TCF7 expression was positively correlated with survival times. In multivariate analysis, the parameters 17p-, 11q-, V2-31 usage, TCF7 and ZAP70 expression were identified as independent prognostic factors. Summary. Several results obtained in CD19-purified cases could not be reproduced in unpurified cases strongly arguing for a tumor cell selection prior to expression analysis. In purified cases, ZAP70, LPL, and TCF7 were the best predictors for VH mutation status. Additional markers such as ATM and ZNF2 may help to identify genomic risk groups since 11q- and 17p-. Multivariate survival analysis suggests TCF7 as a strong survival predictor and points to a pathogenic role for this gene in CLL.

0262 SIGNIFICANT CORRELATION BETWEEN OBJECTIVE RESPONSES AND EXPOSURE TO HUMAX-CD20 IN CHRONIC LYMPHOCYTIC LEUKEMIA
Centre Hospitalier Lyon Sud, PIERRE-BENITE CEDEX, France; Centre Hospitalier Lyon Sud, POissy, France; Kas Herlev, HERLEV, Denmark; Vele Hospital, VEJLE, Denmark; Odense University Hospital, ODENSE, Denmark; University of Amsterdam, AMSTERDAM, Denmark; The University of Iowa, IOWA CITY, USA; Klinika Hematologii, BIALYSTOK, Poland; Klinika Hematologii i Transplantacji Szp, KATOWICE, Poland; Klinika Hematologii Akademii Medycznej, GDANSK, Poland; Mscmc, Warsaw, Poland; Genmab A/S, COPENHAGEN, Denmark; Medical University of Lodz, LODZ, Poland

Background. The fully human monoclonal IgG1 antibody HuMax-CD20 targets a novel epitope of the CD20 molecule on B-cells. HuMax-CD20 stops growth of engrafted B-cell tumors in SCID mice more effi-
CD40 LIGATION SENSITIZES PS3 DYSFUNCTIONAL CLL CELLS TO CHEMOTHERAPY INDUCED APOPTOSIS VIA THE P73 PATHWAY

A.F. Kater,1 E. Dicker,2 C.E. Prada,2 J.E. Castro,2 T.J. Kipps2
1Academic Medical Center, AMSTERDAM, Netherlands; 2MIL Munich Leukemia Laboratory GmbH, MUNICH, Germany; 3Moores Cancer Center, UCSD, LA JOLLA, USA

CD40 activation of chronic lymphocytic leukemia (CLL) cells enhances their capacity to induce an immune response. Although CD40-activation has been shown to enhance sensitivity to both cytotoxic T cell mediated killing and death receptor mediated apoptosis, its effect on chemotherapy is much less clear. We showed recently that CD40 activation of CLL cells resulted in induced expression of pro-apoptotic factors like death receptors, p21 and the BH3-interacting-domain death activation has been shown to enhance sensitivity to both cytotoxic T cell mediated killing and death receptor mediated apoptosis, its effect on chemotherapy is much less clear. We showed recently that CD40 activation of CLL cells resulted in induced expression of pro-apoptotic factors like death receptors, p21 and the BH3-interacting-domain death receptor mediated apoptosis. This phenomenon could be suppressed by specific c- abl inhibition through imatinib treatment. These results demonstrate that CD40 ligation may sensitize leukemia cells not only to extrinsic but also intrinsic apoptotic stimuli via a c- abl-dependent pathway and that CD40-based therapy may be helpful in overcoming the resistance of p53-dysfunctional CLL to anti-cancer therapy.

0264 RHAMM/CD168 IS A NOVEL LEUKEMIA ASSOCIATED ANTIGEN WITH PROGNOSTIC VALUE FOR PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

K.G. Giannopoulos,1 A. Kröber,2 A. Dmoszyńska,3 J. Rolinski,2 H. Dönhöfer,3 S. Stilgenbauer,3 M. Schmitt1
1Medical University of Lublin, LUBLIN, Poland; 2Dept. of Internal Medicine III, ULM, Germany; 3Dept. of Hematooncology, LUBLIN, Poland; 4Dept. of Clinical Immunology, LUBLIN, Poland

Background and Aims. Differential expression of molecules in patients with B-cell chronic lymphocytic leukemia (B-CLL) might define suitable targets for T cell based vaccines and/or antibody approaches. Methods. We assessed the mRNA expression of the tumor-associated antigen (TAA) RHAMM/CD168 defined earlier by serological analysis of cDNA expression libraries (SEREX) from leukemic cells. Results. Peripheral blood mononuclear cells from 40 B-CLL patients and 20 healthy volunteers (HVs) were examined by quantitative RT-PCR. A leukemia-restricted expression of the antigen RHAMM/CD168 was observed in 39/40 B-CLL and in 19/20 HVs. The expression was absent in 1/40 B-CLL and 1/20 HV. We evaluated the immunogenicity of this novel LAA, mixed lymphocyte peptide cultures (MLPCs), followed by enzyme-linked immunosorbent spot (ELISPOT) and flow cytometry assays were performed to detect antigen-specific CD8+ T cells. RHAMM/CD168 specific responses by CD8+ T cells in 40% of responders of 27 (C) patients with relapsed or refractory chronic lymphocytic leukemia (B-CLL) received 4 weekly i.v. infusions of HuMax-CD20 and will be followed for 12 months. The first infusion was 100 mg, 300 mg and 500 mg in cohort A, B and C, and the following 3 infusions were of 500, 1000 and 2000 mg, respectively. Patients were premediated with oral acetaminophen and i.v. antihistamine and received i.v. glucocorticoids before first and second infusions. The endpoints were B-cell depletion, adverse events, objective response according to the NCI working group guidelines for CLL, time to progression, duration of response, time to next anti-CLL treatment, and pharmacokinetics. Results. Median age was 61 years; median time since diagnosis was 6.3 years. Maximum tolerated dose was not reached. All patients in the highest dose group had pronounced reduction of the leukemic CD19+CD5+ cell counts. Objective response rate was 46% (12 of 26 evaluable patients in cohort C) with 2 nPR and 10 PR. By analyzing pharmacokinetic parameters, a statistically significant increased AUC were demonstrated in responders (median: 1256 µg/mL*h, range: 1780-1580) compared to non-responders (median: 940 µg/mL*h, range: 540-1260), p=0.011. Significant similar differences were found for Cmax and Cmin. Conclusion. This preliminary analysis of data from the first 33 CLL patients treated with HuMax-CD20 demonstrated significant depletion of CD19+CD5+ cells and provided an indication of clinical efficacy that correlates with the exposure to HuMax-CD20. These data encourage further development of HuMax-CD20 in CLL.

PARTHENOLIDE INDUCES REACTIVE OXYGEN SPECIES AND SELECTIVE APOPTOSIS OF B-CHRONIC LYMPHOCYTIC LEUKEMIA CELLS VIA A P53 INDEPENDENT PATHWAY

J. Steele,1 G. Prentice,2 A.V. Hoffbrand,3 B. Mehta,1 K. Ganeshaguru,4 R.G. Wickremasinghe5
1Royal Free and UCL Medical School, LONDON, United Kingdom

Background. CLL is incurable using conventional therapy. While the disease is controlled by treatment with chlorambucil (CHL) or fludarabine (FLU), extended exposure results in resistance, which may result from impaired operation of the p53-mediated apoptotic pathway. It is therefore important to identify novel agents that are capable of effectively inducing apoptosis in early stage B-CLL patients, especially with worse prognosis (IgVH unmutated). Aims. (a) whether PTL is selectively toxic to CLL cells, (b) whether PTL is toxic to CLL isolated from refractory CLL cells, (c) whether target may be important in overcoming the resistance of p53-dysfunctional CLL to anti-can

EORTC Lymphoma Group

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CD40 LIGATION SENSITIZES PS3 DYSFUNCTIONAL CLL CELLS TO CHEMOTHERAPY INDUCED APOPTOSIS VIA THE P73 PATHWAY

A.F. Kater,1 E. Dicker,2 C.E. Prada,2 J.E. Castro,2 T.J. Kipps2
1Academic Medical Center, AMSTERDAM, Netherlands; 2MIL Munich Leukemia Laboratory GmbH, MUNICH, Germany; 3Moores Cancer Center, UCSD, LA JOLLA, USA

CD40 activation of chronic lymphocytic leukemia (CLL) cells enhances their capacity to induce an immune response. Although CD40-activation has been shown to enhance sensitivity to both cytotoxic T cell mediated killing and death receptor mediated apoptosis, its effect on chemotherapy is much less clear. We showed recently that CD40 activation of CLL cells resulted in induced expression of pro-apoptotic factors like death receptors, p21 and the BH3-interacting-domain death agonist (Bid), even in CLL cells with dysfunctional p53. Since the effect of fludarabine is highly mediated by p53 dependent response genes we hypothesized that CD40 activation could sensitize p53 dysfunctional CLL cells to fludarabine mediated apoptosis. In ex vivo studies, we show that stimulation of CLL cells by co-culture with CD34+ expressing cells induced leukemia-cell expression of p73, a p53-related transcription factor that is regulated by the c- Abl tyrosine kinase in both p53 functional and dysfunctional cases. Transduction of CLL cells with an adenovirus encoding p73/5 also induced Bid. Next we showed that p53-dysfunctional CLL cells resistant to fludarabine treatment could be sensitized to fludarabine upon CD40 ligation or p73 transduction. This phenomenon could be suppressed by specific c- abl inhibition through imatinib treatment. These results demonstrate that CD40 ligation may sensitize leukemia cells not only to extrinsic but also intrinsic apoptotic stimuli via a c- abl-dependent pathway and that CD40-based therapy may be helpful in overcoming the resistance of p53-dysfunctional CLL to anti-cancer therapy.
In conclusion, the rapid, selective, p53-independent cytotoxic action of PTL and a lesser role of CLL isolates factor as a surrogate marker for IgVH mutation status in predicting response success patient response in chronic lymphocytic leukemia. Aims. In order to determine its accuracy at predicting response and survival, drug sensitivity is being tested both at initial entry (closed October 2004) and at second randomisation (still open) in the UK Leukaemia Research Fund (LRF) CLL4 trial. Methods. At first randomisation, blood specimens were sent to Bath for drug sensitivity testing: initially by DiSC (Differential Staining Cytotoxicity assay); subsequently by its development, the TRAC (Tumour Response to Anti-neoplastic Compounds) assay. Ten drugs were tested including chlorambucil, fludarabine and mafosfamide (used in vitro in place of cyclophosphamide). LC90s were calculated. Patients were randomised into Trial arms to receive chlorambucil (Chl), fludarabine (Flu) or Fludarabine+cyclophosphamide (FluCy) in the ratios 2:1:1. Numbers of patients with any versus no response were compared. P is by Fisher’s exact test. Results. From 777 randomised, LC90 results from 442 patients could be compared with subsequent patient response. Definitions of test-sensitive were LC90s of ≤6.5 ug/mL for chlorambucil, and ≤10.0 ug/mL for both fludarabine and mafosfamide. No difference in average drug sensitivity was found between Trial arms. Results are presented in the Table. All differences between response rates in the test sensitive and resistant groups were highly statistically significant. For instance, for those treated with Flu or FluCy, 90.7% (95% confidence interval (CI) = 86.8-94.6) of test-sensitive patients responded compared with only 60.0% (95% CI) of resistant patients. Conclusions. At diagnosis of CLL, even within a group of patients with a high clinical response rate, ex vivo drug sensitivity can be used to identify a proportion of patients with a significantly poorer probability of clinical response. TRAC results predict better for patient response to fludarabine (± cyclophosphamide) than for response to chlorambucil.

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0268
INTRACELLULAR CYTOKINE EXPRESSION BY B AND T CELLS DIFFERS IN ZAP-70 POSITIVE AND ZAP-70 NEGATIVE B-CLL PATIENTS

M. Fodhorecka, A. Bojarska-Junak, J. Kolinski, A. Dmoszynska
Medical University of Lublin, LUBLIN, Poland

Background. Changes in cytokine network between malignant cells and residual T lymphocytes may be responsible for accumulation of malignant cell clone and for immune abnormalities in B-cell chronic lymphocytic leukemia (B-CLL). B-CLL is the most frequent type of adult leukemia in Western countries and it seems to be a heterogeneous disease with a highly variable clinical course and prognosis. Recently the role of ZAP-70 (zeta associated protein, a member of the Syk-ZAP family) as a surrogate marker for IgVH mutation status in predicting response success patient response in chronic lymphocytic leukemia. Aims. In order to determine its accuracy at predicting response and survival, drug sensitivity is being tested both at initial entry (closed October 2004) and at second randomisation (still open) in the UK Leukaemia Research Fund (LRF) CLL4 trial. Methods. At first randomisation, blood specimens were sent to Bath for drug sensitivity testing: initially by DiSC (Differential Staining Cytotoxicity assay); subsequently by its development, the TRAC (Tumour Response to Anti-neoplastic Compounds) assay. Ten drugs were tested including chlorambucil, fludarabine and mafosfamide (used in vitro in place of cyclophosphamide). LC90s were calculated. Patients were randomised into Trial arms to receive chlorambucil (Chl), fludarabine (Flu) or Fludarabine+cyclophosphamide (FluCy) in the ratios 2:1:1. Numbers of patients with any versus no response were compared. P is by Fisher’s exact test. Results. From 777 randomised, LC90 results from 442 patients could be compared with subsequent patient response. Definitions of test-sensitive were LC90s of ≤6.5 ug/mL for chlorambucil, and ≤10.0 ug/mL for both fludarabine and mafosfamide. No difference in average drug sensitivity was found between Trial arms. Results are presented in the Table. All differences between response rates in the test sensitive and resistant groups were highly statistically significant. For instance, for those treated with Flu or FluCy, 90.7% (95% confidence interval (CI) = 86.8-94.6) of test-sensitive patients responded compared with only 60.0% (95% CI) of resistant patients. Conclusions. At diagnosis of CLL, even within a group of patients with a high clinical response rate, ex vivo drug sensitivity can be used to identify a proportion of patients with a significantly poorer probability of clinical response. TRAC results predict better for patient response to fludarabine (± cyclophosphamide) than for response to chlorambucil.

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intracranial heterogeneity, indicating ongoing mutational activity. We recently showed that CLL light chain repertoire is skewed and characterized by CLL-biased features and also provided evidence for the complementary role of light chains in antigen recognition by CLL malignant cells. In the present study, we evaluated the intracranial diversity status of IGKV/IGLV genes in 32 CLL cases; 25 cases expressed IgMδD, whereas 7 cases expressed IgMγD. IGKV-J and IGLV-J rearrangements were amplified by RT-PCR, purified, ligated into the pCR 2.1 vector and transfected in E. coli TOP10F' cells. Sequence data were analyzed using the V-QUEST/IMGT and Clustalw/EMBL tools. Mutations observed in only one of the IGK/IGL molecular clones from the same sample were characterized as non-confirmed, whereas mutations observed more than once in the IGK/IGL molecular clones from the same sample were characterized as confirmed. The Taq DNA polymerase error rate in our laboratory is 0.052%, which may amount to 0.17 mutations/IGK or IGL clone. Overall, the cloning process was followed for 22 IGKV-J rearrangements (2/22 from lambda-expressing cases) and 10 IGLV-J rearrangements. Twelve out of 32 rearrangements (37.5%) carried IGKV/IGLV genes with greater than 98% homology to germline (unomitted); 5/12 unomitted IGKV/IGLV genes had 100% homology to germline. Information on the intracranial variation was obtained by sequencing a minimum of 7 colonies per rearrangement. No differences were found between individual clones of 10/52 (51.2%) IGKV-J (or IGLV-J) rearrangements. The remaining rearrangements (20/52, 38.5%) exhibited intracranial variation. The number of different subclones per cloning sample ranged from 3 to 5. Eight out of 32 rearrangements (25%) carried only non-confirmed mutations. Ten IGKV-J and four IGLV-J rearrangements (overall, 14/52, 43.8%) carried confirmed ongoing mutations. All confirmed mutations were single base substitutions and consisted of (S) and replacement (R) mutations; nucleotide insertions or deletions were not observed. The number of nucleotide variations ranged from 1 to 7. Overall, 66 ongoing mutations (29 confirmed/37 nonconfirmed) were observed: 24 S mutations and 42 R mutations. Twenty-nine out of 42 R mutations encoded for functionally similar amino acids. Most mutations were located in FR1/FR3/CDR1; occasional mutations were also detected in CDR2 and the IGL variable part of CDR3. Ongoing confirmed mutations were observed not only in mutated cases but also in 7/12 unomitted rearrangements, of which two had 100% homology. Mutations targeted A/G/C/T in a ratio of 17/19/17/13. Transitions predominated over transversions (47 vs. 19); pyrimidines were targeted slightly more often than purines (36 vs. 30). These results indicate that IGK/IGL genes in CLL can undergo intracranial diversification in a considerable percentage of cases and provide further support for the active contribution of light chains in antigen recognition. Mutations among subclones had specific molecular traits. Finally, intracranial diversity did not correlate with the original mutational load, since it was observed both in CLL cases with little or no somatic mutations as in cases with considerable mutations.

**0271 QUANTITATION OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA USING LNA-MODIFIED FLUORESCENTLY LABELED PROBES AND REAL-TIME PCR TECHNOLOGY**

P.S. Pekova, S.L. Saudkova, S.L. Smolje, J.T. Kozak

1 Hospital Na Homolce, PRAGUE, Czech Republic; 2 Dept. of Clin. Hematol., Faculty Hospital, PRAGUE, Czech Republic

**Background.** Patients with chronic lymphocytic leukemia (CLL) relapse even after aggressive therapies and stem cell transplantation. As the therapeutic goal today is to clear off the tumor cell burden as much as possible (by stem cell transplant or intensive chemoimmunotherapy), high-sensitivity assays for minimal residual disease (MRD) evaluation and monitoring are needed. At present, many patients with not only germline IgVH sequences, but also with hypermutated IgVH genes are being treated, with the need for a sensitive and specific MRD monitoring. The original notion of MRD follow-up in CLL was based on the usage of IgH-gene specific and non-specific; hybridization or PCR products due to the vast diversity of B-CLL clonal rearrangements to be detected, the original idea has been challenged and the methodology should be modified. Aims. Since the hypermutation process does not restrict itself to the VH segments only and might afflict the JH segment as well, the molecular tools for the monitoring of B-CLL clonal rearrangements must be versatile enough to allow for the detection and quantification of virtually any sequence possible. Moreover, the technique must meet the criteria for high sensitivity and specificity. We present here a novel methodology for MRD monitoring in CLL, based on LNA technology (Locked Nucleic Acids) and quantitative Real-Time PCR. Methods. Thirty-nine patients with the diag
nosis of CLL were enrolled into our MRD monitoring study (16 females, 25 males, median age 69.5 ± 9.6 years). 21 of 39 individuals had unmutated IgVH genes (5 females, 18 males), 18 out of 39 patients had mutated IgVH genes (13 females, 5 males). For each patient, clone-specific primers were designed and their clonal IgVH sequences were molecularly cloned to construct the quantitation standards. In one patient, allelic inclusion has been identified (VH1-8 and VHS-30, both mutated), and for this individual, clonotypic primers and standards have been constructed for both rearrangements. To quantify the individual clonal IgVH transcriptions, LNA-modified fluorescently labeled probes targeted against individual VH gene segments were employed. For any of 6 (7) IgVH families with unmutated IgVH genes, family-specific consensus LNA-modified probes were used. For those CLL cases with heavily hypermutated genes, ProbeLibraryTM was employed. For quantitation experiments, ABL was used as the control gene. Results. The LNA-modified probes are distinguished by a very high specificity and sensitivity (reaching to 10-5, in contrast to flow cytometry with its detection limit being 10-4). The LNA-based assays allow for precise monitoring of the residual tumor cell burden in CLL patients, especially during those periods of time, when other, less sensitive techniques fail to trace the malignant clone (during chemoimmunotherapy, after stem cell transplant). Conclusions. LNA-modified probes and Real-Time PCR technology represent a highly versatile, specific and extremely sensitive methodology for the monitoring of MRD, characterized by a very high sensitivity. We strongly advocate their use in the molecular follow-up of MRD in the setting of CLL (and possibly other B-cell malignancies with hypermutated VH gene sequences as well). CLL and related disorders - Clinical / Experimental I

0272

ADAPTIVE IMMUNOTHERAPY OF B-CELL MALIGNANCIES WITH A TRIFUNCTIONAL, BISPECIFIC ANTIBODY (ANTI-CD3 X ANTI-CD20) AND ALLOGENIC DONOR LYMPHOCYTE TRANSFUSION

R. Buhmann,1 M. Stanglmaier,2 B. Simoes,3 T. Yang,4 P. Ruf,2 H. Lindhofer,2 H.J. Kolb4

1GSE MUNICH, Germany; 2TRION Research GmbH, Martinsried, MUNICH, Germany; 3Klinikum Grosshadern, LMU, MUNICH, Germany.

Background. CD20-directed treatment approaches turned out to be highly effective in patients with B cell non-Hodgkin’s lymphoma (NHL). But although the chimeric anti-CD20 antibody rituximab induced overall response rates (ORR) of nearly 50% with median response durations of approximately 1 year in relapsed or refractory indolent lymphoma it is not curative and new immunotherapeutic treatment approaches have to be validated. Aim. In compassionate use, 5 patients with refractory B-cell chronic lymphocytic leukemia (B-CLL) and 5 patients with refractory B-cell high grade non-Hodgkin lymphoma (B-NHL) were treated with the combination of the trifunctional antibody Bi20 and donor lymphocyte transfusions (DLT). Methods/Study-Design. The Bi20 antibody is trifunctional, it binds to CD20 and CD3 and activates phagocytosis of the leukemia cell by accessory cells via the Fc part. Bi20 was applied in escalating doses from 10 µg up to 2000 µg and followed by DLT (1 × 107/kg body weight). Patient 2 and 3 received repeated courses of antibody and DLT. Results. In 4 out of 6 patients, we observed a prompt, but only transient clinical response. Two patients died from HG-NHL did not respond. In cases of B-CLL, a dose-dependent decrease of the leukemic cells was observed even within hours after antibody infusion. Moreover, enlarged lymph nodes and B-symptoms disappeared transiently. Side effects were restricted to fever, chills and bone pain that could be easily controlled. These effects peaked at a concentration of 80 µg and did not increase or even decreased at higher concentrations. The cytokine profile was characterized by a transient increase of IL-6, IL-8 and IL-10. With respect to the transaminases, only a transient and modest increase of γGT was observed. HAMAs (human anti mouse antibodies) were not detectable; their absence allowed repeated application of the trifunctional antibodies. Remarkably, graft-versus-host disease (GVHD) was not observed. Unfortunately relapse of the disease occurred in all cases. In two cases of B-CLL and one case of HG-NHL repeated application of Bi20 and T-cells induced repeated response. Conclusion. Bi20 can induce a prompt anti-tumor response in even extensively pretreated patients. The toxicity of treatment is tolerable. However, until now the response is of short duration and further studies are necessary to improve the outcome by e.g. optimizing the application schedule.

0273

TELOMERE LENGTH IS A PROGNOSTIC FACTOR STRONGER THAN VH-MUTATIONAL STATUS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

I. Ricca,1 M. Ladetto,1 A. Rocci,1 R. Francesc1, D. Drandi,1 C. Lobetti Bodoni,1 M. Compagno,1 E. Genuardi,1 M. Rosace,1 M. Astolfi,2 P. Omede,3 C. Mainone,2 L. Bergui,2 M. Boccadoro,2 C. Tarella,2 A. Gallamini1

1University of Turin, TORINO, Italy; 2ASO S. Giovanni Battista, TORINO, Italy; 3ASO S. Croce e Carlo, CUNEO, Italy.

Background. Telomere restriction fragments (TRF) length has a prognostic impact in B-CLL. Some studies suggest that this is a mere reflection of its association to VH-mutational status (VH-MS). However, the cut-off of the two parameters has not been set, particularly in cases in which they are discordant. Aim. To compare, in a large population of B-CLL patients, the prognostic impact of TRF length and VH-MS, in terms of overall survival (OS), time to first treatment (TTFT) and progression free survival (PFS). PATIENTS AND METHODS. 184 B-CLL patients have been analyzed for TRF length and VH-MS. All samples were taken before treatment start. Males were 118, females 66. Median age was 62 years (range 34-87). According to Binet staging system, 117 were stage A, 34 B and 33 C. Cytogenetics, CD38 and ZAP-70 expression were available in 80% of patients. Median follow-up was 36 months (range 6-291). Eighty-seven patients have been already treated. TRF length was evaluated by Southern blot and VH-MS by direct sequencing. The standard cut-off of 2% deviation from any germ line VH sequence was employed to define VH-MS. Survival analyses were performed using the Kaplan-Meier method. Cox multiple regression was used to analyze the independence of the following potential prognostic parameters: sex, age, Binet stage, CD38 and ZAP70 expression, cytogenetic features, VH-MS and TRF-length. Results. Median TRF length was 6000bp (range 1465-14837bp). There was no correlation between TRF length and patient age, sex or stage. TRF length had a major impact on prognosis with best results observed with a cut-off of 4250bp. Patients with TL<4250bp had a worse outcome than patients with TL>4250bp (median OS: 83 vs 269 months, p<0.0001; median TTFT: 21 vs 63 months, p<0.0001; median PFS: 12 vs 36 months, p<0.0001). VH-MS analysis was successful in 91%. Overall, discordance between VH-MS and TRF length was observed in 16% of patients. Discordance was common among VH-unmutated patients (58%) but rare among VH-mutated patients (6%). Discordant and concordant patients could not be distinguished based on VH usage or degree of homology (H) to the germline IgH sequence (i.e. H=100% vs H>100% and >99% vs H<99% and >98%). In addition they could not be distinguished based on stage, cytogenetics, CD38 and ZAP70 expression. The 24 discordant patients with VH-unmutated status and TRF length>4250bp had a clinical outcome that was significantly different from VH-unmutated patients with TRF length<4250bp (median OS: 83 vs 215 months, p<0.05 and median PFS: 12 vs 33 months, p<0.05) and similar to that of VH-mutated patients (median OS 269 months and median PFS 54 months, p=n.s.). Finally, the multivariate analysis indicated that TRF length and Binet stage were the most important prognostic indicators in B-CLL. Conclusions. Our data demonstrate that: 1) TRF length is a major prognostic indicator in B-CLL in terms of OS, TTFT and PFS; 2) when discordance exists between VH-MS and TRF length the latter better predicts outcome.

0274

HIGHLY SENSITIVE DETECTION OF MINIMAL RESIDUAL DISEASE IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION ON FLOW SORTED CELLS


Virga Jesse Hospital, HASSELT, Belgium

Background. The introduction of new therapeutic agents such as fludarabine and alemtuzumab, with or without autologous or allogeneic stem cell transplantation, has resulted in increased complete remission rates in B-cell chronic lymphocytic leukaemia (CLL). Preliminary data have suggested the absence of minimal residual disease (MRD) is an end point of therapy that, if achieved, translates into an improved survival. Future prospective clinical trials that aim toward achieving long-lasting complete remissions should include a test to assess MRD. However, current assays for assessing MRD in CLL show various sensitivity levels and lack standardization. Aim. We have developed and validated a combined method to assess MRD in CLL using fluorescence-activating
cell sorting (FACS) and interphase fluorescence in situ hybridization (FISH) for the detection of numerical chromosomal aberrations that occur in up to 80% of CLL cases. Methods. CLL cells were purified from the peripheral blood of CLL patients by FACS-Aria (BD, US) based on the CD19+CD5+ co-expression, with a purity of > 95%, as assessed by microscopy and by reanalysing with flow cytometry. These CLL cells were sorted either deletion 11q (ATM) or deletion 13q14 in > 95%, by using dual colour FISH. Peripheral blood samples from normal individuals were spiked with the purified CLL cells with dilutions of 10^{-6} to 10^{-10} white blood cells (WBC). WBC from these spiked samples were subsequently labelled with CD19 and CD5 moAbs and analysed by FACS. CD19+CD5+ cell fractions were purified by FACS-Aria and analysed by FISH for either deletion 13q or deletion 11q. Results. FISH detection of the specific chromosomal aberration in CD19+CD5+ purified cells allowed discrimination of CLL cells from normal precursor B-cells. Reproducible positive results, above cut-off levels of the probe, were demonstrated in all dilutions up to 10^{-6} or 10^{-10}. Quantification was feasible using the percentage of CD19+CD5+ cells and the percentage of aberrant purified cells. Conclusions. This approach for the detection and quantification of MRD in CLL reaches a sensitivity at least as high as and even higher than other methods, such as four-color flow cytometry or quantitative allele-specific PCR. It can be used for at least 80% of CLL patients, including all CLL patients with poor prognosis as assessed by the presence of the deletion 11q (ATM) or the deletion 13q (p53). Furthermore, it allows easy standardization among laboratories, applying FACS cell sorting, as it is based on a two-colour labelling only and on FISH assays using commercially available probes. We are now clinically validating the method by assessing MRD levels in intensively treated CLL patients and we propose this method as a candidate approach for assessing the clinical impact of MRD detection in prospective clinical trials on CLL.

**0276**

**MRD KINETIC AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN CHRONIC LYMPHOCYTIC LEUKAEMIA CAN PREDICT INDIVIDUAL TIME TO RELAPSE AND IS ASSOCIATED WITH CLINICAL OUTCOME AND IG VH MUTATIONAL STATUS**

M. Ritgen, S. Boettcher, S. Stilgenbauer, A. Larkin, M. Clynnes, T.C.M. Morris

‘Universitätsklinikum Schleswig-Holstein, KIEL, Germany; †University Clinic of Ulm, ULM, Germany; †AK St. Georg, HAMBURG, Germany; †Technical University, MUNICH, Germany; †College University Hospital, COLOGNE, Germany; †University clinic of Heidelberg, HEIDELBERG, Germany

Introduction. Minimal residual disease (MRD) short after autologous SCT in pts with CLL is known to be close to the detection limit in patient and laboratory remission. Therefore early MRD after SCT is not suitable to predict outcome in patients in remission after SCT. Nevertheless, outcome after SCT is rather heterogeneous even in this population and it has been shown, that the IgH mutational status and other risk factors are of prognostic value after SCT. We therefore analysed MRD in 61 patients with high risk CLL after autologous conditioning regimen of TBI and high-dose cyclophosphamide and consecutive autologous SCT. We established a mathematical model to describe the individual kinetics of MRD-increase after SCT and correlated this to known risk factors as IgVH mutational status, cytogenetics, Lymphocyte doubling time, STK, leucocyte count and clinical outcome. We therefore plotted LOG-MRD levels in each individual patient against time after SCT for an observation period between 12 and 36 months after SCT and calculated patient individual standard curves by linear regression. Significant MRD increase was defined by a change of more than 0.5 orders of magnitude within this observation time, all other cases where regarded as MRD stable or decreasing. 31 of 61 patients showed increasing MRD level with a median slope of 0.08 (0.04-0.88). Assuming that MRD level of 0.5 would be diagnosed as hematologic relapse, we could predict the individual clinical relapse by extrapolation with acceptable accuracy in the majority of CLL pts post SCT, whereas decreasing or stable MRD levels are associated with long lasting remission. Absolute MRD levels after SCT are homogeneous low regardless known risk factors, but fast increase of the relapsing CLL clone is correlated to VH unmutated cases. This indicates that the dismal outcome of VH unmutated cases is based on higher proliferating capacity compared to VH mutated cases and not on chemo-resistance.

**0277**

**ANTILEUKEMIC ACTIVITY OF LENALIDOMIDE (REVLIMID) IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA**

A. Chanan-Khan, K. Miller, L. Musial, S. Padmanabhan, D. Lawrence, Z. Bernstein, K. Takeshita, D. Spamer, C. Byrne, C. Crystal, M. Czuczman

‘Roswell Park Cancer Institute, BUFFALO, United States of America; ‘Celgene Corporation, SUMMIT, United States of America; ‘Toronto Sunnybrook Regional Cancer Centre, TORONTO, Canada

Introduction. The ImIDs are a new class of immunomodulating agents with antitumor activity against various malignancies. We previously reported the antileukemic activity of thalidomide (T) in combination with Fludarabine in CLL pts. Based on this experience we investigated Lenalidomide (L), a more potent analog of T in pts with rel/ref CLL. Here we report the results of this ongoing phase II clinical trial. Patients and Methods. All pts...
with rel/ref CLL requiring treatment are eligible. Oral L is given at 25mg/day for 21 out of a 29-day cycle. Treatment is continued until complete response (CR) or progressive disease (PD). NCI-WG 1996 criterion is used to determine response. Three pts had PD and rituximab was added to L. All patients have achieved a PR. (reported separately).

Results. Nineteen of the 29 pts (median age 64 years; range: 47-75) were 136 males and 97 females, aged 34 to 84 years (median 63). At presentation 184 (79%) were Binet stage A, 38 (14,3%) stage B and 7 (4,7%) stage C. Median follow-up was 62 months (range 12 to 387). Overall, AIC was observed in 23 patients (9,8%); 15 were AIHA, 5 AITP, 2 ES and 1 PRCA. In 7 cases (30%) the complication was present at diagnosis (3 AIHA, 2 AITP, 1 ES, 1 PRCA), in the remaining it appeared subsequently, mostly after treatment (alkylating agents in 13, fludarabine in 2). ZAP-70 was expressed in leukemic cells of 18/23 (78%) patients with AIC. The actuarial cumulative incidence of AIC at 10 years was 33±10% in ZAP-70 positive vs 7±5% in ZAP-70 negative cases (p=0.0004). In B-CLL patients developing AIC, survival was lower (24±14% vs 80±4% at 10 years, p=0.0003). Overall survival of all ZAP-70 positive B-CLL patients was significantly shorter (44±9% at 10 years vs 88±4% of ZAP-70 negative cases p=0.0004). ZAP-70 expression was the only significant factor for developing AIC at multivariate analysis (p<0.02). No significant association with age, sex, Binet stage, lymphocyte count or previous B-CLL treatment was found. Conclusions. our data suggest that ZAP-70 expression in leukemic cells is independently strongly associated with the risk of autoimmune cytopenias in B-CLL. A possible pathogenic suggestion might be related to the enhanced signalling via BCR complex induced by ZAP-70.

0279

IN VITRO TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS WITH FLUDARABINE, ETOPOSIDE AND ALEMTUZUMAB (CAMPATH-1H) LEADS TO SPECIFIC MECHANISMS AND RATES OF CELL DEATH IN DIFFERENT GENETIC SUBGROUPS

D. Winkler, C. Schneider, D. Nitsch, A. Habermann, H. Döhner, S. Stilgenbauer

Uniklinik Ulm, ULM, Germany

Background. Treatment of CLL with fludarabine, etoposide and the monoclonal anti-CD52 antibody alemtuzumab leads to cell death and clinical responses. The mechanisms by which these processes occur are poorly understood. Aims and Methods. In order to gain insight into these mechanisms CLL cells from 41 patients were collected and individually treated with fludarabine (500 μM), etoposide (60 μM) and alemtuzumab (10 mg/ml; cross-linking f(ab')2 fragments) for 24 and 48 hours respectively. Each sample treated with alemtuzumab was also cultured with and without allogeneic serum as a source of complement. In 21 cases T-cell and NK-cell depletion was done using negative selection with anti-CD2 and anti-CD14 magnetic beads. Of 38 cases investigated 25 were VH unmutated and 13 of 39 had del 11q and/or del 17p (n=3). FACS analysis was used to measure rates of cell death with double staining for Annexin V/7AAD and caspase-3 activation. Results. Treatment with fludarabine and etoposide induced apoptosis in all but 2 cases, which carried del 11q and del 17p respectively. The rates of apoptosis were lower in cases with genetically high-risk (del 11q or del 17p) CLL, although these cells showed stronger caspase-3 activation than low-risk CLL when incubated with fludarabine (see table). Response to alemtuzumab was highly dependent on the presence of serum in the culture. 7% Annexin-V/7AAD-positive cells in serum-free cultures vs 67% in cultures with serum. Addition of f(ab')2 fragments increased the percentage of Annexin-V/7AAD-positive cells even in serum-free cultures. Response to alemtuzumab was independent of the genetic subgroup of the case. Notably, treatment with alemtuzumab in serum containing cultures did not produce cells that stained Annexin-positive/7AAD-negative, a typical feature of early apoptosis, whereas treatment with fludarabine, etoposide and alemtuzumab in serum-free medium resulted in a significant number of Annexin-positive/7AAD-negative cells. This was also observed in T-cell-depleted cultures. In the presence of serum, alemtuzumab did not induce caspase-3 activation, neither did the addition of f(ab')2 fragments. However, in serum-free cell cultures, active caspase-3 was clearly detectable after alemtuzumab treatment, and caspase-3 activity was further up-regulated when f(ab')2 fragments were also added. Summary. After in vitro treatment of CLL cells with fludarabine, etoposide and alemtuzumab mechanism and rate of cell death differed significantly depending on the genetic subgroup affiliation. CLL cells with high-risk aberrations were more capable of caspase-3 activation when treated with fludarabine or alemtuzumab. Alemtuzumab killed CLL cells independently of serum as a source of complement, but the mechanism of response was different and more effective when serum was added. In serum-free CLL cultures, alemtuzumab induced apoptosis with activation of casepase-3, and addition of cross-linking f(ab')2 fragments increased the rate of apoptosis, whereas in the presence of serum treatment with alemtuzumab induced no typical features of apoptosis, even in T-cell depleted cultures. These findings favor a combination of both CDC and apoptosis but not of ADCC as the cell kill mechanisms activated by in vivo alemtuzumab.

Table 1.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>mean% of cells Annexin V+/7AAD+</th>
<th>caspase-3 activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH unmutated</td>
<td>fludarabine (48 hrs)</td>
<td>etoposide (48 hrs)</td>
</tr>
<tr>
<td>GH unmutated</td>
<td>33%</td>
<td>29%</td>
</tr>
<tr>
<td>del 11q/del 17p</td>
<td>58%</td>
<td>35%</td>
</tr>
<tr>
<td>13q/normal karyotype</td>
<td>11%</td>
<td>32%</td>
</tr>
</tbody>
</table>
ANALYSIS OF EXPRESSED AND NON-EXPRESSED IMMUNOGLOBULIN LAMBDA LOCUS REARRANGEMENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA


In normal individuals, nearly all lambda expressing B cells have rearranged immunoglobulin kappa (IGK) genes and carry IGKV-J junctions. We have recently reported IGK locus rearrangements in the vast majority (97%) of lambda-CLL cases. In the present study, IGL locus rearrangements were analyzed in parallel on cDNA/genomic DNA in 166 kappa- and 104 lambda-CLL cases. In all cases, the tumor load was greater than 70%. All experiments were repeated at least three times with identical Results. Furthermore, in 156/267 cases repeat samples (obtained at different times) were analyzed and also gave identical Results. In lambda-CLL, 110 IGLV-J transcripts were amplified in 104 cases. Two cases carried double in-frame (IF) transcripts: in such cases, the possibility that leukemic cells expressed more than one lambda chain cannot be excluded. Four out of 110 IGLV-J transcripts were out-of-frame (OF); 2/4 OF transcripts were heavily mutated and carried stop codons. DNA-PCR identified additional, non-transcribed IGLV-J rearrangements in 6/104 lambda-CLL cases, of which only one was in-frame. The most frequent genes in transcribed, in-frame rearrangements were IGLV3-21/IGLV2-3/IGLV2-14/IGLV3-1/IGLV1-40. LCDR3 median length was 11 amino acids (range, 8-13). N nucleotides were detected in 50/106 (47.2%) IGLV-J joints; 84/106 cases (79.2%) used the IGLJ2/3 genes, whereas the remainder (22/106; 20.8%) used the IGLJ1 gene. Non-transcribed and out-of-frame rearrangements utilized 9 different IGLV genes and had a median LCDR3 length of 11 amino acids (range, 10-13). N nucleotides were detected in 57/10 IGLV-J joints; 8/10 cases (30%) used the IGLJ2/3 genes. In kappa-CLL, IGLJ-V rearrangements were amplified in 10/163 patients (6.1%); 6/10 rearrangements were in-frame. Eight different IGLV genes were identified. Somatic mutations were introduced in 8/10 IGLV sequences. Four out of ten IGLJ rearrangements in kappa-CLL were also transcribed; 3/4 IGLV-J transcripts were in-frame. In the three kappa-CLL cases with transcribed, in-frame IGLJ-V rearrangements, flow cytometry and immunohistochemistry demonstrated that monotypic Ig expression was still maintained. In particular, malignant B cells were negative for either cytoplasmic or surface lambda light chains, suggesting post-transcriptional regulation of allelic exclusion. IGLV-J rearrangements in kappa-CLL had a median LCDR3 length of 10 amino acids (range, 9-12); N nucleotides were detected in 5/10 IGLJ-V joints; 4/10 cases (40%) used the IGLJ2/3 genes, whereas 6/10 cases (60%) used the IGLJ1 gene. In conclusion, biallelic IGL locus rearrangements are infrequently detected in lambda-CLL. A small subset of lambda-CLL patients have cells that may express more than one lambda chain allele, implying that allelic exclusion of light chains is not absolute. IGLJ locus rearrangements are infrequent in kappa-CLL, suggesting that the light chain rearrangement hierarchy in chronic lymphocytic leukemia (CLL) is not inherently different from normal cells. Differences in IGLJ gene usage between kappa vs. lambda CLL indicate negative selection of the IGLJ1 gene in the expressed CLL repertoire.

ACTIVATION-INDUCED CYTIDINE DEAMINASE EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA


‘Athens University School of Medicine, ATHENS, Greece; ‘Nikiara General Hospital, PIRAEUS, Greece; ‘G. Paparrnicou Hospital, THASSALONIKI, Greece; ‘Sismanogleion Hospital, ATHENS, Greece; ‘G. Gennimatas Hospital, THASSALONIKI, Greece; ‘St. Luke’s Hospital, THESSALONIKI, Greece

Mutation analysis of immunoglobulin heavy chain variable region (IGHV) genes has enabled a subdivision of chronic lymphocytic leukemia (CLL) patients in two subsets, with and without somatic mutations, associated, respectively, with an indolent or a more aggressive clinical course. Nevertheless, regardless of IGHV mutation status, recent data suggest that all CLL cells resemble antigen-experienced and activated B cells. Activation-induced cytidine deaminase (AID) plays a key role in somatic hypermutation (SHM) and class switch recombination (CSR). Given that AID is an essential component of the canonical SHM process in healthy B cells, its expression in CLL might potentially be relevant to the disease. In the present study we evaluated AID mRNA expression in CLL and explored possible associations between AID mRNA expression and surface immunoglobulin (slg) isotype expression, IGHV mutation status and outcome. Our study group included 130 CLL patients; ten healthy individuals served as normal controls. Slg expression was studied by flow cytometry and/or immunohistochemistry: 13/95 analyzed patients (13.7%) expressed slgG, whereas the remainder (62/95; 66.3%) expressed slgM/slgM,slgD. Clonal IGH rearrangements were amplified by RT-PCR, gel-purified and directly sequenced; sequence data were analyzed using the IMGT database (http://imgt.cines.fr). Using the 98% cut-off value for homology to germline, 12/130 patients (9.3%) carried mutated IGHV genes, whereas 48/130 patients (37%) carried unmutated IGHV genes. AID cDNA sequences were amplified by RT-PCR with primers covering the entire coding region. Sixty-nine out of 150 patients (53%) carried all three alternatively spliced AID transcripts; 21/130 patients (16%) carried one or two out the three splice variants; finally, 40/130 patients (31%) and all ten healthy individuals were negative for either AID transcript. At least one AID mRNA isoform was detected in all IgG-switched cases vs. 57/82 IgM/IgM,D cases (p=0.02). Detection of all three AID mRNA isoforms was observed in 36/48 IGHV-unmutated cases (75%) vs. 53/82 (40.2%) IGHV-mutated cases (p=0.001). Seventy-eight patients were evaluable for disease progression; 58/78 carried mutated IGHV genes, whereas 20/78 carried unmutated IGHV genes. Among 52 patients with progressive disease, 15 were IGHV-unmutated; the difference in disease progression rate by IGHV gene mutation status was statistically significant (p=0.0005). AID mRNA transcripts were detected in 25/52 (78%) patients with disease progression and 28/46 (60.9%) patients with stable disease (p=0.11, not significant). In conclusion, AID mRNA expression is more frequent in CLL patients with unmutated IGHV genes. IGHV-unmutated CLL cases positive for AID mRNA may be considered to originate from antigen-experienced B cells with inactivated SHM processes or under pressure to maintain their B cell receptor in the unmutated state.
Hodgkin Lymphoma - Clinical trials

0282
RESULTS OF ABVD AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN ADVANCED STAGE, HIV-RELATED HODGKIN’S LYMPHOMA (HL)
Gesida and Gelab Groups, BADALONA, Spain

Background and objective. In the HAART era, the results of therapy of HIV-related lymphomas are similar to those observed in non-immunosuppressed patients. There is scarce information on the results of therapy of HIV-related HL using standard chemotherapy together with HAART. The aim of this study was to analyze the results of ABVD regimen + HAART in a multicenter series of 62 Spanish patients with HIV-related HL in advanced stages. Patients and Methods. From 1996 to 2005, 62 HIV-infected pts with newly diagnosed HL were treated in 15 Spanish hospitals. HAART was given to all patients from diagnosis if they were not already receiving it. Six to eight cycles of ABVD were planned. G-CSF support was administered according to institutional practices. Response to chemotherapy as well as prognostic factors for response, OS and DFS were recorded. Results. Median age 37 yr (range 24-61), 54 (87%) males, 29 (47%) with previous known diagnosis of HIV infection (median from HIV infection diagnosis to HL: 5 yr, range 0-10), Risk activity for HIV infection: IV drug abusers 33 (53%), heterosexual 15 (24%), homosexual / bisexual 13 (21%), unknown 1 (2%). Median CD4 lymphocyte depletion 12 (20%), range 0-200, Median CD4 count <100/mL 22 (35%), median HIV load: 1.4x10^3 copies/mL (range 0-3.9x10^5), undetectable HIV load: 21/56 (37%). Forty seven (76%) patients were receiving HAART at the time of HL diagnosis (median 15 mo, range 1-109). HL subtype: nodular sclerosis 17 (27%), mixed cellularity 25 (41%), lymphocyte depletion 10 (16%), non-specified HL 10 (16%) (the main reason was diagnosis in extranodal areas). ECOG score ≥2: 52/56 (43%), B symptoms: 55 (89%), stage III: 21 (34%) stage IV: 41 (66%). BM involvement: 53/60 (55%). Patients with the scheduled 6-8 ABVD cycles was completed in 81% of cases. Induction death: 5 pts (8%), CR: 54 (87%), resistance 3 (5%). After a median follow-up of 44 mo, 5 pts have relapsed, with a DFS probability at 5 yr of 74% (95%CI 46-100), and 15 patients have died, being 5-yr OS probability 76% (95%CI 23-89). Causes of death: lymphoma progression 10, HIV-related 3, traffic accident 1, unknown 1. Virologic response to HAART at 6 months after the completion of treatment was observed in 24/26 (67%) evaluable patients. Only lower number of ABVD cycles than scheduled (<6) was a prognostic factor for CR achievement. DFS and OS (OR: 0.155, 95% CI: 0.051-0.462, p=0.001 for CR, OR: 0.137, 95% CI: 0.019-0.979, p=0.05 for DFS, and OR: 0.153, 95% CI: 0.051-0.462, p=0.001 for OS). Conclusion. Patients in advanced stage, HIV-related HL treated with ABVD+HAART have a response rate and survival similar to that of immunocompetent patients. The completion of the scheduled therapy was the only factor influencing response and survival in this series.

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0283
HODGKIN’S LYMPHOMA IN ADOLESCENTS - RESULTS FROM THE GERMAN HODGKIN STUDY GROUP
H. Bredenfeld,1 A. Engert,1 V. Diehl,2 E. Gilman,2 B. Pfister2
1University Hospital Cologne, COLOGNE, Germany; 2German Hodgkin Study Group, COLOGNE, Germany

Background. Both pediatric and adult patients with Hodgkin lymphoma (HL) are commonly treated in separate treatment protocols. Adolescents are treated with either pediatric or adult protocols depending on study group policies and local legislation. Between 1988 and 1998, the German Hodgkin Study Group (GHSG) did include younger patients from age of 15, and 16, in identically therapy regimens with adult Hodgkin’s lymphoma patients. Aims. With a focus on treatment outcome and the recording of secondary malignancies, this analysis aimed to demonstrate wether adolescent patients with HL represent a patient group distinct from adults, possibly requiring a separated therapy strategy. Methods. In two GHSG trial generations (G2, and G3), a total of 573 adolescents (15-21 years) in early, intermediate, and advanced stages HL were compared with 4544 adults (22-65 years) for complete remission rate (CR), 5 years survival rate (SV), 5 years freedom from treatment failure (FTF), and secondary neoplasias (2nd NHL). Results. For both ado-

blescents and adults, treatment outcome showed no differences in all stages in terms of CR, SV, and FTF. A higher rate of 2nd NPLs in the adults patient cohort was detected consistently for early, intermediate, and advanced stages in both trial generations. However, the absolute number of 2nd NPLs in the adolescent group was generally low. Conclusion. Adolescent and adult patients suffering from Hodgkin’s lymphoma show similar therapy outcome when treated with the same regimens. With regard to the small number of cases, a longer follow-up is needed to assess the risk of 2nd NPLs particularly in adolescents. Based on this analysis, adolescents seem not to be a distinct patient group with the need for a treatment strategy apart from adult Hodgkin’s lymphoma patients.

0284
INC-EU PROSPECTIVE OBSERVATIONAL EUROPEAN NEUTROPENIA STUDY: PRELIMINARY HODGKIN AND NON-HODGKIN LYMPHOMA RESULTS
R. Pettengell,1 A. Bosly,1 T.D. Szucs,1 C. Jackisch,1 R. Leonard,1 R. Pardlaens,2 M. Constenla,2 M. Schwenkglenks3
1St. George’s Hospital, LONDON, United Kingdom; 2UCL Mont-Godinne, YVOIR, Belgium; University of Basel, BASEL, Switzerland; 3Klinikum Offenbach, OEFENBACH, Germany; 4South West Wales Cancer Institute, SWANSEA, United Kingdom; 5U.Z. Gasthuisberg, LEUVEN, Belgium; 6Complejo Hospitalario de Pontevedra, PONTEVEDRA, Spain

Background. Chemotherapy of malignant lymphomas is often accompanied by major side effects including grade IV chemotherapy-induced neutropenia (CIN) and febrile neutropenia (FN), which may have life threatening consequences in the short term and affect treatment delivery. Aims. To assess the incidence, determinants and impact of CIN and FN in routine practice in Western Europe and, ultimately, to develop multivariate risk models of CIN and FN occurrence. Methods. A prospective observational study was conducted in 54 centres spread across 5 European countries (Belgium, France, Germany, Spain and UK).

Table 1. Association of RDI ≤ 90% with CIN/FN

<table>
<thead>
<tr>
<th></th>
<th>Hodgkin lymphoma</th>
<th>Non-Hodgkin lymphoma</th>
<th>Combined</th>
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<tr>
<td>Pts. with RDI ≤ 90 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- if no events</td>
<td>37.5</td>
<td>40.8</td>
<td>40.0</td>
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<tr>
<td>- if grade IV CIN</td>
<td>45.0</td>
<td>53.1</td>
<td>51.5</td>
</tr>
<tr>
<td>- if FN</td>
<td>60.0</td>
<td>70.6</td>
<td>68.9</td>
</tr>
</tbody>
</table>

Relative risk (95%-CI) of RDI ≤ 90% (compared to pts. with no events)

- if grade IV CIN 1.4 (0.8-2.4) 1.4 (1.1-1.8) 1.4 (1.1-1.7)
- if FN 1.9 (0.6-6.2) 2.3 (1.3-3.9) 2.2 (1.4-3.6)

A total of 307 lymphoma patients were enrolled and observed during their chemotherapy treatment. Treatment was as per usual clinical practice and not influenced by the protocol, except for one blood count at cycle 1 neutrophil nadir. Results. Sixty-five patients (21%) were diagnosed with Hodgkin lymphoma (HL) and the remaining 242 (79%) with non-Hodgkin lymphoma (NHL, 66% large B-Cell; 15% follicular; 19% other). Mean age at diagnosis±SD was 59±17 years for HL and 63±13 years for NHL. Men accounted for 56% of the sample in both groups. Ann Arbor stages were distributed I 18%; II 26%; III 16%; IV 40%, and 47% had B symptoms. Chemotherapy regimens for HL patients were ABVD-like (51%), BEACOPP-like (14%) and Stanford V (5%). The regimens used for NHL patients were mainly three-weekly CHOP-like (71%), followed by two-weekly CHOP-like (17%), ACVB/like (3%) and DHAP/ESHAP-like (3%). Primary prophylaxis with colony-stimulating factors (CSFs) was used in 15% of HL and 26% of NHL patients. Secondary prophylaxis with CSFs occurred in 38% and 26%, respectively. CIN was observed in 21% of HL and 34% of NHL patients. CIN occurrence by regimen type was ABVD-like 39%; BEACOPP-like 78%; Stanford V 33%; three-weekly CHOP-like 22%; two-weekly CHOP-like 5%; ACVB/like 100%; and DHAP/ESHAP-like 67%. FN occurred in 15% (CI 8-26%) of HL and 21% (CI 16-27%) of NHL patients. Dose reductions of ≥ 10% were seen in 51% and 54%, respectively. Mean relative dose intensity (RDI) compared to plan±SD was 89±18% for HL and 86±16% for NHL. Low RDI ≤ 90% was frequent and associated with CIN and FN occurrence (Table). Neu-

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A high proportion of lymphoma patients experience CI and FN and suffer the consequences of hospitalisation and impaired chemotherapy delivery, with a potential for short and long-term sequelae. Further analysis of this data will help to identify new, as well as validate existing risk models. Such models will help to target high-risk patients for prophylactic treatment, in order to decrease the incidence of CI and FN and allow full-dose chemotherapy to be delivered on schedule.

**0285**

**IFOSFAMIDE, VP16 AND OXALIPLATINE (IVOX) CHEMOTHERAPY FOR PATIENTS IN FIRST PROGRESSION OF HODGKIN LYMPHOMA (HL) AFTER ABVD CHEMOTHERAPY, A SINGLE CENTER PROSPECTIVE STUDY OF 21 PATIENTS**

P. Brique,¹ N. Mounier, M. Ertault,¹ P. Franchi,² J. Briere,¹ P. Colin,² N. Parquet,³ G. Sergent,² C. Gisellebrecht

¹Hopital Saint Louis, PARIS CEDEX 1 ², France; ²Policlinique de Courlancy, REIMS, French Guyana

Background. Despite a high cure rate, 10 to 40% of patients with HL may relapse after complete remission and 5% are refractory to ABVD. Many regimens have been used as salvage chemotherapy with response rates ranging from 40 to 90% depending on patients status (relapsing or refractory). Since 1998, MOPP/ABV chemotherapy has been abandoned and most patients are treated with first line ABVD with cumulative dose of doxorubicin often over 300 mg/m². Aims. For these reasons we developed a second line treatment without doxorubicin including ifosfamide/ etoposide and eloxatine (a platinum component without nephrotoxicity) to induce disease before high-dose therapy (HDT) and ASCT. Methods. the IVOX (ifosfamide 1500 mg/m² etoposide 150 mg/m²D1D2D3 and eloxatine 130 mg/m² D1) was given every 21 days with GCSF day 6 for 6 days. Twenty one patients with progressive HL (median age 29y) have been prospectively treated from 06/05 to 06/06. Characteristics of patients: initial stage III/IV (n=11) bulky mediastinum (n=8), all patients had received ABVD or etoposicin in 5 cases (EBVP) with radiotherapy in 10 cases. At progression 5 patients were induction failure and 16 were in unfavourable relapse (mean time to relapse at 8 mo., stage III/IV at relapse 60%). Patients were evaluate after 2 or 3 IVOX with a PET CT and had PBFC collection before HDT. Results. according to standard staging criteria 7 patients were in CR/CRu and 7 in PR> 50% giving a response rate of 66.6% and according to PET evaluation, 10 patients had a positive PET CT before intensive therapy. The toxicity was low without hospitalisation for febrile neutropenia, no transfusion, no mucositis. 19 patients had a successful PBFC (2 patients were excluded from PBFC collection, one 66 y with refractory disease and one for viral hepatitis). Among the 19 patients planned for HDT, one died with refractory disease and 18 received HDT (Tandem in 9 cases and RIC allogeneic in one case). At the last follow-up 16 patients are in CCR (negative PET CT), 2 died from HL and 3 are alive with disease. Conclusion IVOX is a very well tolerated chemotherapy regimen but doesn’t appear superior to previous published regimens in progressive HL.

**0286**

**LYMPHOCYTE-PREDOMINANT HODGKIN’S LYMPHOMA IN CHILDREN: THERAPEUTIC ABSTENTION AFTER INITIAL LYMPH NODE RESECTION IN STAGE I PATIENTS. A REPORT FROM THE SOCIETE FRANCAISE DES CANCERS DE LENFANT (SFCE)**

S. Gorde,¹ O. Oberlin,¹ T. Leblanc,¹ Y. Perel,¹ A. Robert,² D. Plantaz,² C. Schmitt,² Y. Bertrand,² H. Paquemont,² C. Edan,¹ A. Lambilliotte,¹ P. Luiz,¹ G. Michel² F. Demeneoç² C. Beari² G. Vaudre,¹ S. Fasola,¹ A. Baruchel,¹ G. Leverger,¹ J. Landman-Farker

¹Hopital Trousseau, PARIS, France; ²Institut Gustave Roussy, VILLEJUIF, France; ³Hopital Saint Louis, PARIS, France; ⁴CHU Bordeaux, BORDEAUX, France; ⁵CHU Toulouse, TOULOUSE, France; ⁶CHU Grenoble, GRENOBLE, France; ⁷CHU Nancy, VANDOEUVRE LES NANCY, France; ⁸Hopital Debrousse, LYON, France; ⁹Institut Curie, PARIS, France; ¹⁰CHU Rennes, RENNES, Lille, LILLE, France; ¹¹CHU Strasbourg, STRASBOURG, France; ¹²Hopital La Timone, MARSEILLE, France; ¹³CHU Clermont-Ferrand, CLERMONT-FERRAND, France; ¹⁴Hopital American, REIMS, France

Background. Lymphocyte-predominant Hodgkin’s lymphoma (LPHL) is characterized by early stage, indolent course, excellent prognosis but high risk of second tumors in part treatment-related. In order to clarify the treatment strategy, SFCE has reported its initial experience with a wait and see strategy after adenectomy in a limited number of patients (J Clin Oncol Pellegrino et al., 2003). Aims : to further document patients LPHL s evolution when they received no treatment beyond initial adenectomy. Methods. From 1990 to December 2005, 59 patients with LPHL confirmed after pathological review were available for the study. Clinical presentation was: 47 male; median age 10 years (4-17); stage I n=45, stage II n=6, stage III n=5, stage IV n=1. Based on physician decision, 22/45 stage I patients received no further treatment after initial surgery (group SA). 23 patients (group CT) received: combined treatment (n=10), involved field radiotherapy alone (n=3) or chemotherapy alone (n=10). None of them received monoclonal anti-CD20 antibody. The 2 groups were comparable for clinical status and follow up. Results. 45/48 achieved CR. All patients with residual lymph node relapsed. With a median follow up of 41 months (6-156), overall survival is 100%. Overall DFS stage I patients is 57% ± 10, DFS group SA: 52%±14 and DFS group CT: 63%±13 (p=0.2). Only two patients had TEP-FDG for post surgical evaluation. Median relapse time is 11 months (SA group 5 months/CT group 25 months p<0.2). Stage at relapse was SA group: 5/7 in the same node area and 2/7 stage II; CT group: 4/6 stage I, 1/6 stage III and 1/6 stage IV. Conclusions. No further therapy after complete lymph node resection is a valid approach in LPHL comparable to more aggressive approaches. Nevertheless, as most of the relapses involve the same site than the diagnostic a better evaluation of the quality of remission after surgery is to be recommended with TEP and CT/MRI. This could help for an adapted therapeutic approach.

**0287**

**ADMINISTRATION OF FULL DOSE DOXORUBICIN, BLEOMYCIN, VINBLASTINE, DACARBAZINE CHEMOTHERAPY IRRESPECTIVE OF GRANULOCYTE COUNT IN PATIENTS WITH HODGKIN LYMPHOMA: MAINTENANCE OF DOSE INTENSITY WITHOUT GROWTH FACTORS**

E. Boleti, R. Cathomas, T.R. Geldart, P.W.M. Johnson, G. Mead

Southampton University Hospital, SOUTHAMPTON, United Kingdom

Background. Review of existing trials revealed that in most cases administration of ABVD for Hodgkin Lymphoma is subject to dose modifications and the use of growth factors to avoid treatment delays and minimise neutropenia. Aim. To investigate whether administration of ABVD irrespective of the granulocyte count causes treatment delays or increases the number of injective episodes in patients with HL. Methods. All patients had confirmed HL and were treated with ABVD outside clinical trial protocols, either because they were not deemed eligible, or they declined to take part. Consecutive cases were reviewed for a 5 year period and all were treated on an outpatient basis. Results. Thirty-eight patients were treated with ABVD. Median age was 34 (17-68) with 19 males and 19 females. Thirty patients (78.9%) had nodular sclerosis histology, 7 (18.4%) mixed cellularity, and 1 (2.6%) patient had nodular lymphocyte-predominant HD. Twelve patients had stage I (81.5%), 21 (55.2%) had stage II, 2 (5.2%) had stage III and 3 (7.9%) had stage IV disease. Twenty-five patients (65.8%) received 3-4 cycles of ABVD for early relapse. The median number of chemotherapy visits per patient was 4.55 and the total number of chemotherapy visits was 346 (173 cycles). Growth factors were not used in any case. There were in total 14 days of treatment delay (0.2%) and 2 episodes of neutropenic pyrexia during the chemotherapy visits (0.57%). Thirty (78.9%) patients had at least one episode of neutropenia (<1.0×10⁹/L) during chemotherapy. The mean number of neutropenic episodes per patient was 3 while the mean granulocyte count in neutropenic patients was 0.6×10⁹/L. No dose modifications were performed. Three patients had recurrent disease (7.8%), of which one has received high dose therapy with autologous progenitor cell support and is disease-free, while two are currently undergoing salvage chemotherapy. Conclusions. ABVD administration irrespective of granulocyte counts did not lead to a higher number of injective episodes and allowed the treatment to be given at full dose without delays. There was no need for growth factor support, minimising treatment costs. The use of full dose ABVD irrespective of granulocyte count should be evaluated in future protocols for HD.

**0288**

**THE IMPACT OF THE SOCIOECONOMIC STATUS IN PATIENTS WITH HODGKIN’S DISEASE IN BRAZIL**

I. Biasoli,¹ A. Soares,¹ A. Scheliga,² R.R. Luiz,¹ S. Roman,³ M.A. Costa,¹ J.C. Morais,¹ N. Spector³

¹Federal University of Rio de Janeiro, RIO DE JANEIRO, Brazil; ²National Cancer Institute, RIO DE JANEIRO, Brazil

Background. The impact of socioeconimic status in Hodgkin’s Disease (HD) in Brazil is unknown. We aimed to evaluate the impact of socioeconimic status in patients with Hodgkin’s Disease in Brazil. Aims. To evaluate the impact of socioeconimic status on diagnosis and treatment of Hodgkin’s Disease in Brazil.

Material and Methods. 11th Congress of the European Hematology Association
Background. Socioeconomic status (SES) is a determinant of clinical outcome of Hodgkin’s disease (HD). Aim of the study was to analyse the impact of the socioeconomic status in patients with Hodgkin’s disease (HD). Methods. From November 2001 to January 2005, 194 consecutive patients were prospectively followed in five institutions (three public and two private) in Rio de Janeiro. Data regarding disease and treatment features were collected, and patients were classified according to the National Prognostic Score (NPS). Each patient answered a questionnaire about their socioeconomic status, including educational level, household income, ownership of household goods (radio, TV, refrigerator, washing machine, VCR/DVD and car), presence of household and housing features. Most of these items were used to calculate an index of socioeconomic status, the ‘Criteria for Economic Classification’, which has been validated in public and private hospitals in Brazil. Patients were divided in two groups according to their socioeconomic status: higher SES (classes A1 to C) and lower SES (classes D and E). The IPS score risk was also categorized in low risk (2 or less risk factors) or high risk (more than 2 risk factors). Results. There were 151 patients (78%) with a higher SES and 43 patients (22%) with a lower SES. The overall CR rate was 82%, and it was higher in patients with a low risk IPS (78% vs. 72%, p=0.04). Patients with a higher SES had a higher CR rate than those with a lower SES (85% versus 72%, p=0.006, 95% CI of difference:0.45% to 26.22%). The median albumin level at diagnosis was lower in the lower SES group (5.55 versus 5.9, p=0.065) and in the higher SES group (5.4 versus 5.9, p=0.018). There were no statistically significant associations between the SES group and other relevant variables, including stage, bulky disease, performance status, and time from the beginning of symptoms to diagnosis. Ten patients (5%) died during treatment. The causes of death were tumor progression, concomitant advanced disease in 3, and cachexia in one patient. Death during treatment was associated with a lower SES (16% vs. 2%, p=0.001), a lower performance status (p=0.0001), a lower lymphocyte count (p=0.012), and weakly with a lower albumin level (p=0.065). With a median follow-up of 1.7 years (0.07-43.3), a higher SES was associated with a better 2-year overall survival (79.5% versus 63.6%, p=0.01). Conclusion. Lower socioeconomic status was associated with an increased rate of fatal events during treatment, and with a trend towards a lower complete remission rate. Overall survival was lower in the socially deprived patients, apparently due to the higher fatality rate during treatment. Factors indicative of a poor health status at the time of diagnosis appear to explain the observed differences in outcome. In underprivileged countries, patients with a lower socioeconomic status require a more careful monitoring during treatment, possibly with specific support measures. Regimens more intense then ABVD could pose a prohibitive risk of complications in this group of patients.

0280

LACE (LOMUSTINE, ARA-C, CYCLOPHOSPHAMIDE, ETOPOSIDE) CONDITIONED AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: TREATMENT OUTCOME AND RISK FACTOR ANALYSIS IN 67 PATIENTS FROM A SINGLE CENTRE

J.B. Perez,' C. Giles,' R. Szydlo,' D. MacDonald,' D. Siepi,' R. Szydlo,' G. Desantis,' A. Kameo,' I. Néri,' F. Costa,' I. Lorand-Metze

State University of Campinas, CAMPINAS, Brazil

Background. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a recognised treatment option for patients (pts) with relapsed Hodgkin lymphoma. Patients and Methods. We analysed 67 pts (46m, 21f, median age at diagnosis 29y, range15-67) who underwent autologous stem cell transplantation (ASCT) after LACE [omustine (80 mg/m²) cytarabine (2000 mg/m²), cyclophosphamide, etoposide] conditioning for relapsed (n=61) or primary refractory (n=6) Hodgkin lymphoma. The predominant diagnostic histology was nodular sclerosis (n=42), whilst disease stage was I in 2 pts, II in 29pts, III in 22pts and IV in 14pts. Median age at ASCT was 32y (range: 17-70). Prior to ASCT, 40 pts were in complete or partial remission, but 27 pts had less than partial response. Results. The 100 day treatment-related mortality was 3%. With a median follow-up of 43.5 months (range 0.5-145.5) the probabilities of overall survival (OS) and progression-free survival (PFS) at 5 years for all 67 patients were 72% and 61%, respectively. Probabilities for OS and PFS at 5 years for patients with chemo-sensitive relapse (n=40) were 79% and 76%, versus 42% and 39% respectively for patients (n=27) with chemo-resistant relapse (p=0.056 for OS, p=0.008 for PFS). In univariate analysis gender, age at diagnosis or at ASCT, extranodal disease, bulk or bone marrow involvement at diagnosis, initial treatment type, response to first line chemotherapy, duration of first remission, time from diagnosis to ASCT, number of treatment lines before ASCT did not have an impact on OS. Three risk factors for worse OS were identified in multivariate analysis (mixed cellularity or lymphocyte-depleted histology, stage III or IV disease at diagnosis, and haemoglobin ≤ 10g/dl at ASCT). Patients with 0 (n=12), 1 (n=26), or 2-3 (n=29) of these three risk factors had 5 year OS probabilities of 100%, 75% and 52% respectively. Conclusions. We conclude that LACE followed by ASCT is an effective treatment for the majority of patients with chemo-sensitive relapsed Hodgkin lymphoma. A proportion of apparently chemo-refractory patients may also benefit.

0289

POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASE MU1 AND TETA1 GENES IN HODGKIN’S LYMPHOMA RISK


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 environmental exposure to cytotoxic and genotoxic agents may be associated with an increased risk of HL. The ability to metabolise carcinogens is variable in human beings. The enzymes of the glutathione S-transferase (GST) system catalyse the conjugation of electrophilic molecules of numerous carcinogenic chemicals, such as benzene and polycyclic aromatic hydrocarbons, to glutathione reducing them to less toxic levels. Gene coding for GST mu1 (GSTM1) and theta1 (GSTM1) proteins are polymorphic in humans and are absent or homozygous null in 12% of patients. A number of recent studies have reported that the GST mu1 and theta1 null genotypes are associated with increased risk of HL. For this purpose, genomic DNA from peripheral blood of 79 HL patients (40 male, 39 female; mean age±SD: 32.2±14.9 years) and peripheral blood of 367 controls (198 male, 169 female; mean age±SD: 53±6.6 years) was extracted using proteinase K and lithium chloride protocol. Statistical signficance 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. We have observed an increased risk of HL in patients with GSTM1 null genotypes (OR=3.54, p=0.0001), a lower lymphocyte count (p=0.005 for PFS). In univariate analysis gender, age at diagnosis or at ASCT, extranodal disease, bulk or bone marrow involvement at diagnosis, initial treatment type, response to first line chemotherapy, duration of first remission, time from diagnosis to ASCT, number of treatment lines before ASCT did not have an impact on OS. Three risk factors for worse OS were identified in multivariate analysis (mixed cellularity or lymphocyte-depleted histology, stage III or IV disease at diagnosis, and haemoglobin ≤ 10g/dl at ASCT). Patients with 0 (n=12), 1 (n=26), or 2-3 (n=29) of these three risk factors had 5 year OS probabilities of 100%, 75% and 52% respectively. Conclusions. We conclude that LACE followed by ASCT is an effective treatment for the majority of patients with chemo-sensitive relapsed Hodgkin lymphoma. A proportion of apparently chemo-refractory patients may also benefit.

0291

67GA-SPET HAS A ROLE IN PREDICTING DISEASE FREE SURVIVAL (DFS) AND OVERALL SURVIVAL (OS) IN PATIENTS WITH PRIMARY MEDASTINAL LYMPHOMA AND HODGKIN LYMPHOMA WITH MEDASTINAL INVOLVEMENT

A.M. Liberati,' I. Palumbo,' G. Desantis,' B. Palumbo,' L. Falchi,' P. Cerroni,' D. Siepi,' V. Capparella,' A. Lugini,' M. Cianciulli

‘Medicina Interna e Scienze Oncologiche, PERUGIA, Italy; ‘Section of Nuclear Medicine, PERUGIA, Italy; ‘Section of Clinical and Experimental Med, PERUGIA, Italy; ‘Pr General Hospital S. Camillo De Lellis, Rieti, Italy

Background. FDG-PET has a superior accuracy than gallium scan (Ga-SPET) in staging and post-therapy restaging of malignant lymphomas. However, in Hodgkin lymphoma (HL) with predominant mediastinal involvement and in primary mediastinal lymphoma (ML), this latter, less expensive, nuclear imaging technique might still have a clinical utility. Aims. The main objective of this prospective study was to assess the predictive value of Ga-SPET in terms of DFS and OS. Methods. Ga-SPET was performed 72 hours after intravenous injection of 370 MBq (8-10
mCi) of 67Ga citrate. SPET data acquisition included a 360° rotation, with 60 projections at a rate of 20s per projection. The matrix size was 64x64 and a Butterworth filter (0-4-0.6) was used. Survival curves were calculated by Kaplan-Meyer survival analysis and the comparison between groups was performed by the log-rank test.

Results. The actual final analysis includes 66 evaluable patients (mean age 28, range 12-80) of the 68 initially enrolled in this prospective study. The main disease features of the 66 (43/23 F/M) patients are: stage II/39, III/10, IV/17; histology HL/58 (SN/42, CM/11, DL/1, unclassified/5) and ML/8; bulky mediastinal disease yes/no 29/37; B symptoms yes/no, 41/25. Forty-two patients received conventional chemotherapy, 4 non-myeloablative and 20 myeloablative chemotherapy with peripheral blood stem cell support because of unfavourable disease or resistant or relapsing disease to primary treatment. Two patients were excluded because they did not have Ga-SPET at the end of treatment. A total of 109 Ga-SPET/CT restaging were obtained after chemotherapy and/or chemo-radiotherapy completion. Sensitivity, specificity and accuracy were 89%, 91% and 91%, respectively for the Ga-SPET, while they were 100%, 27% and 37% for the CT scan. After a median follow-up of 34 (4-141) months, DFS and OS for patients with a pathological Ga-uptake (Ga-SPET) at the end of the treatment program were 95% (2-60) and 62 (9-70) months, respectively. In contrast, the corresponding figures in patients with pathological Ga-uptake after treatment as compared with patients with a negative Ga-SPET were 100%, 27% and 37% for the CT scan indicating a complete disease remission after a median time of 37+ (5-139) months and 55+ (4-147) months respectively. DFS and OS differed significantly (p<0.0001) in patients with pathological Ga-uptake after treatment as compared with patients with a negative Ga-SPET. In contrast, DFS and OS were not significantly different between patients with post-therapy CT scan suggestive of persistent disease and patients with CT scan indicating a complete disease remission (Figure). Conclusions. Ga-SPET is still a useful, sensitive and not expensive method to determine the presence of eventual post-therapy active disease in the mediastinum.

0292
AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH REFRACTORY OR RELAPSED HODGKIN LYMPHOMA: CLINICAL OUTCOME OF 61 PATIENTS FROM A SINGLE INSTITUTION
C. Martínez, O. Salamero, L. Arenillas, M. Rovira, A. Urbano-Ispizua, F. Fernández-Avilés, E. Carreras, E. Montserrat
Institute of Hematology and Oncology, BARCELONA, Spain; Hematology Department, Hospital Clinic, Spain

Background. Patients with Hodgkin lymphoma (HL) who do not achieve complete remission (CR) with conventional chemotherapy have poor prognosis. The treatment of choice for these patients is high-dose chemotherapy and autologous stem cell transplantation (ASCT) that may result in prolonged progression-free survival as shown in many studies, particularly registry-based. Aim. To investigate the results of ASCT in patients from a single institution with refractory or relapsed HL at the time of the procedure. Patients and Methods. Sixty-one patients, 27 males and 34 females with a median age of 31 years (range 15-60) transplanted from 1988 to 2005 were analysed. All patients had active HL at the time of ASCT: 51 patients were in partial remission (50%), 18 had refractory disease (30%), and 12 had a non-treated relapse (20%). At transplantation, 26 patients (43%) had advance stage, 15 (25%) presented with B symptoms and 8 (13%) with bulky disease. Seventy percent of the patients had received two or more lines of therapy before ASCT. Results. Nine patients (15%) died during the first 5 months after ASCT due to transplant related mortality (TRM). Median follow-up of the surviving patients was 44 months (range 4-140). After haematological recovery, 16 patients received complementary radiotherapy on residual mass or involved radiotherapy because of unfavourable disease or resistant or relapsing disease to primary treatment. Two patients were excluded because they did not have Ga-SPET at the end of treatment. A total of 109 Ga-SPET/CT restaging were obtained after chemotherapy and/or chemo-radiotherapy completion. Sensitivity, specificity and accuracy were 89%, 91% and 91%, respectively for the Ga-SPET, while they were 100%, 27% and 37% for the CT scan. After a median follow-up of 34 (4-141) months, DFS and OS for patients with a pathological Ga-uptake (Ga-SPET) at the end of the treatment program were 95% (2-60) and 62 (9-70) months, respectively. In contrast, the corresponding figures in patients with pathological Ga-uptake after treatment as compared with patients with a negative Ga-SPET were 100%, 27% and 37% for the CT scan indicating a complete disease remission after a median time of 37+ (5-139) months and 55+ (4-147) months respectively. DFS and OS differed significantly (p<0.0001) in patients with pathological Ga-uptake after treatment as compared with patients with a negative Ga-SPET. In contrast, DFS and OS were not significantly different between patients with post-therapy CT scan suggestive of persistent disease and patients with CT scan indicating a complete disease remission (Figure). Conclusions. Ga-SPET is still a useful, sensitive and not expensive method to determine the presence of eventual post-therapy active disease in the mediastinum.

0293
COMPARISON BETWEEN FLUORODEOXYGLUCOSE POSTERIOR EMISION TOMOGRAPHY AND STANDARD RESTAGING IN AGGRESSIVE LYMPHOMA PATIENTS TREATED WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION
AOS S. Giovanni Battista, TORINO, Italy; SCDU Medicina Nuclear, TORINO, Italy; SCDO Ematologia, TORINO, Italy; SCDO Radiologia, TORINO, Italy

Background. Positron emission tomography (PET) using (18) Fluoro-deoxyglucose (18-FDG) is an important non invasive technique to assess response in lymphoma patients. Early response evaluation by 18-FDG-PET after few cycles of chemotherapy may predict chemosensitivity and the response, prognostic features and overall survival in this subset of patients. High dose chemotherapy (HDC) followed by autologous stem cells transplantation (ASCT) is reserved to aggressive disease or refractory-relapse disease after standard first line treatment. Chemosensitivity, remission duration and IPI at relapse are best prognostic factors to predict favourable outcome post HDC+ASCT. Early evaluation based on 18-FDG-PET response may be a surrogate of a chemosensitivity test. In this study we compared the use of 18-FDG-PET staging/restaging with standard staging/restaging before and after ASCT in these patients. Aims and Methods. From February 2004 to February 2006, 21 aggressive NHL or HD patients with a planned HDC+ASCT were included: 15 males and 6 females respectively with a median age of 38 yrs (range 19-63). We included 7 cases of aggressive NHL (5 DLCL and 2 mantle cell NHL at first diagnosis) and 14 cases of HD in relapse or refractory disease. All patients were referred to our Hematology Department for clinical management and were previously studied with conventional staging techniques: physical examination and contrast-enhanced CT of the neck, chest, abdomen and pelvis. Full laboratory tests were performed as well as bilateral posterior iliac crest biopsy for bone marrow evaluation. If necessary, NMR was planned. Before and after ASCT all patients underwent to conventional and 18-FDG-PET restaging. Results. Before the ASCT phase the comparison between standard and 18-FDG-PET restaging tests was as follow: CR in 18 pts vs 16 pts and PR in 3 pts vs 5 pts respectively. At that time the restaging by conventional procedure and 18-FDG-PET was concordant in 17 pts: 15 pts in CR and 2 pts in PR respectively. Discordant restaging was observed only in 4 pts. The final evaluation was concordant with both procedures in 20/21 pts: 19 CR and 1 PR. One patient was in PR at the 18-FDG-PET and in CR at the standard evaluation. Involved field radiotherapy was added in the 5 pts with discordant restaging before or after ASCT with achievement of CR. At a median FU of 18 months only 1/17 pts who achieved CR at the end of treatment was in PD. Conclusions. 18-FDG-PET is an important imaging technique for the end-treatment evaluation in lymphoma disease, because it may better define CR patients. However, 18-FDG-PET findings must be correlated with clinical data, others imaging analysis and, if necessary, biopic specimens. Indeed, more large studies are needed to determine the real impact of 18-FDG-PET on early evaluation before ASCT in aggressive NHL or HD patients.
Hodgkin Lymphoma · Clinical trials

0294

CLINICAL RESISTANCE TO PRETRANSPLANT IMATINIB THERAPY IS AN ADVERSE PROGNOSTIC FACTOR FOR THE OUTCOME OF ALLOGENIC STEM CELL TRANSPLANTATION IN CML

Klinikum Grosshadern, MUNICH, Germany; Stiftung Deutsche Klinik fuer Diagnostik, WIESBADEN, Germany; Munich Leukemia Laboratory, MUNICH, Germany

Background. The ABL tyrosine kinase inhibitor imatinib is highly effective in the treatment of chronic myelogenous leukemia (CML). However, due to short follow up the long term effects of imatinib are unknown. At present, allogeneic transplantation remains the only treatment with curative potential. Aims. In order to determine the effect of imatinib therapy before allogeneic transplantation we analyzed patients who had received imatinib as part of pretransplant therapy with respect to clinical response or resistance to imatinib therapy. Patients and Methods. Clinical resistance to imatinib was defined as i) primary cytogenetic unresponsiveness or ii) detection of increasing percentage of Ph+ metaphases and/or increasing BCR-ABL positive interphase nuclei in FISH analysis, and iii) hematological progress under ongoing imatinib therapy. Fifty eight patients from two centers were evaluable. The median age was 46 years (range 17-65). Twenty patients were transplanted in 1st chronic phase (CP), 38 in 2nd and higher CP. Seventeen patients had a sibling donor, 41 had an unrelated donor. The median follow up time after allogeneic transplantation was 360 days (range 24-1524). Results. Imatinib resistance, stage of disease, time from diagnosis to transplantation, and age were significant prognostic factors for overall survival (OS: p<0.001, p=0.001, p=0.027, p=0.016, respectively) and leukemia free survival (LFS: p<0.001, p=0.002, p=0.058, p=0.042, respectively) in univariate analysis. Multivariate analysis by Cox regression demonstrated that clinical resistance to imatinib was an independent adverse risk factor for OS (p=0.001) and LFS (p<0.001). Stage was the only other independent risk factor for LFS (p=0.045) and OS (p=0.057). Conclusion. Our data suggest that allogeneic HSCT should be planned as long as there is cytogenetic response to imatinib.

0295

IL-12, MYELOID DENDRITIC CELLS AND THE TH1 MODEL IN ACUTEGRAFT-VERSUS-HOST DISEASE AFTER REDUCED INTENSITY CONDITIONING ALLOGENIC STEM CELL TRANSPLANTATION

M. Mohy, B. Gaugler, C. Fauchier, S. Furst, D. Olive, D. Blaise
Institut Paoli-Calmettes, MARSEILLE, France

Inflammatory cytokines act as mediators of aGVHD. The use of RIC regimens has modified the natural history of transplant-related complications, especially aGVHD. Our current knowledge of the pathophysiology of aGVHD is based primarily on results obtained in the myeloablative setting. The aim of this study was to investigate the role of inflammatory cytokines on aGVHD incidence and severity in 113 patients who received a RIC allo-SCT from an HLA-identical sibling. Plasma levels of: IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-18, TNF-α, IFN-α, IFN-γ, and Fas-ligand, were measured by ELISA prior to allo-SCT, at day 0 prior to graft infusion, and at regular times within the first 3 months after allo-SCT. Except for IL-12p70, all measured cytokines showed little variations in the first three months. The incidence of grade II-IV aGVHD was 45% (95% CI, 36-54%; median onset, 32 days after allo-SCT). In the subgroup of patients for whom all tested cytokines could be measured accurately, but rigorously prior to aGVHD clinical onset, a high IL-12p70 level (p<0.04) measured around the first month after allo-SCT were significantly associated with the development of clinically significant grade II-IV aGVHD. IL-12p70 levels were significantly correlated to the severity of aGVHD: grade 0-I, median 486 pg/ml; grade II, median 2538 pg/ml; and grade III-IV, median 4615 pg/ml (p<0.0001). In patients experiencing grade II-IV aGVHD, IL-12p70 levels decreased after aGVHD therapy. Interestingly, we found a more rapid recovery of monocytes, that are the main pool of IL-12p70-secreting myeloid dendritic cells (DC), prior to aGVHD clinical onset in patients with grade II-IV aGVHD, as compared to patients with grade 0-I aGVHD (median, 829/L vs. 552/L; p=0.005). At the effector level, we observed a significantly more robust recovery of genuine naive CD3+CD4+CD45RA-CD27+ T cells prior to aGVHD clinical onset, in patients with grade II-IV aGVHD, as compared to patients with grade 0-I aGVHD (median, 50/L vs. 16/L; p=0.006).

0296

MOLECULAR REMISSION IN FOLLICULAR LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA AFTER REDUCED INTENSITY ALLOGENIC STEM CELL TRANSPLANTATION CORRELATES WITH A BETTER DISEASE FREE SURVIVAL

Istituto Nazionale dei Tumori, MILANO, Italy; Istituto Scientifico San Raffaele, MILANO, Italy; Ospedale Rovato, BERGAMO, Italy; Policlinico Universitario, UDINE, Italy; Ospedale San Camillo, ROMA, Italy; Ospedale Universitario Torrette, ANCONA, Italy; Ospedale Cervello, PALERMO, Italy

Background. Reduced intensity allogeneic stem cell transplantation (RIC allo-SCT) can be an effective salvage treatment for relapsed chronic lymphocytic leukemia (CLL) and follicular lymphoma (FLC). In our series, progression-free survival is 80% and 60% at 4 years for FCL and CLL patients (pts) respectively. In the autologous SCT setting, it has been shown that the attainment of clinical and molecular remissions can be predictive of a better disease-free survival (DFS). Aims. Aim of this study was to investigate whether RIC allo-SCT is able to induce durable clinical and molecular remissions (MR) in relapsed FCL and CLL pts and whether minimal residual disease (MRD) status correlates with a better survival. Methods. Thirty-four pts (16 FCL and 18 FCL), having a molecular marker, were in complete remission (CR) after a RIC transplantation (containing thiopeta, bucarabine and cyclophosphamide) from a HLA-identical sibling (n=32), unrelated (n=1) or haploidentical (n=1) donor. The median age was 52 years (range, 32-69 years). The median number of previous treatments was 2 (range, 1-5); 25% pts had failed a previous autologous SCT. Before transplant, 11 pts (52%) were in CR, 14 pts (41%) were in partial remission and 9 pts (27%) were progressive or stable disease. Bcl-2 (n=15) or immunoglobulin heavy chain gene rearrangements (IGH, n=19) were used as molecular markers. After allo-SCT, serial BM samples were analyzed for MRD by nested-PCR. The median molecular follow-up was 24 months (range, 6-64). Results. Overall, 24 of 34 pts (71%) pts attained MR, 7 pts (20%) were PCR-positive and 3 pts (9%) showed an intermediate pattern of PCR positivity and negativity. All but one of the PCR-negative pts achieved MR within the first year after allo-SCT. Sixteen of 18 FCL pts (89%) achieved MR, while only 8 of 16 CLL pts (50%) were MRD-negative at the last follow-up (p=0.002). FCL and CLL pts were not different for number of previous treatments, pre-transplant disease status and incidence of chronic and acute graft versus host disease (aGVHD). Among pts who were persistently PCR-negative, only one CLL pts relapsed at a nodal site that showed Richter transformation, while among pts who were PCR-positive 4 pts relapsed. The difference was statistical significant (p=0.0051) and translated into a better DFS for PCR-negative pts (94% vs 33%, p<0.0001). None of the pts who relapsed experienced GVHD, while all the pts who were persistently PCR-positive or had an intermittent pattern without relapsing showed acute or/and chronic GVHD. Eighty percent of PCR-negative patients developed GVHD that preceded or was concomitant with the achievement of MR. The overall incidence of chronic GVHD among PCR-negative pts was 54%, and among pts who were PCR-positive or had an intermediate pattern and did not relapse was 67% (p=0.67). Conclusions. i) MR can be attained after RIC allo-SCT in the large majority of FCL pts and in 50% of CLL pts; ii) the achievement of MR correlates with a lower relapse risk and a better DFS; iii) MRD monitoring can be used to tailor post-transplant immunotherapy.

0297

IMPACT OF T-CELL CHIMERISM AFTER REDUCED INTENSITY CONDITIONING ALLOGENIC STEM CELL TRANSPLANTATION

Institut Paoli-Calmettes, MARSEILLE, France; CHU de Montpellier, MONTPELLIER, France

Here, we investigated the impact of different factors on the establish-
The analysis has been performed on the apheretic products. More than 60% of allogeneic stem cells transplants are at the time of transplant (p=0.047) and with the number of CD20+ cells in the inoculum and higher was the probability of developing acute GVHD. The high number of CD20+ cells in the inoculum also correlated significantly with the TRM (p=0.02). Summary/Conclusions. The results of this analysis suggest that the concentration of B cells in the apheresic product may predict the incidence of acute GVHD and TRM in patients undergoing an allogeneic PBSCT transplantation, thus influencing the clinical outcome. This is in agreement with the recent evidences regarding the role played by B cells in the pathogenesis of GVHD. These findings suggest possible new preventive and therapeutic strategies in the clinical management of GVHD.

**0299**

**ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTS (HSCT) FOR PATIENTS WITH RELAPSED ACUTE LEUKEMIA : LONG TERM OUTCOME**


Ospedale San Martino, GENOVA, Italy

**Background.** Patients with acute leukemia may be referred for allogeneic hemopoietic stem cell transplantation (HSCT) at the time of relapse. The outcome of allogeneic HSCT for these patients is relevant when discussing treatment strategies and donor selection. **Aim of the study.** To assess the long term outcome of 152 patients with acute myeloid (AML) or acute lymphoid leukemia (ALL) undergoing an allogeneic HSCT in our Unit between 1977 and 2004. Patients have undergone 152 patients with relapsed ALL (n=43) or AML (n=109). The median blast count in the marrow was 30% (7-100), and the median blast count in the peripheral blood was 2 (0-99). Median age was 31 (11-62), and the median year of transplant 1995. Conditioning regimen included total body irradiation (TBI) (10-12 Gy) in 115 patients. The donor was a matched sibling donor (MDS) in 106 , a family mismatched donor (FMD) (n=20) or an unrelated donor (UD) in 26. The graft was T cell depleted (TCD) in 12 cases. Leukemia was diagnosed in first relapse in 42 and more advanced disease, or primary refractory in 110. **Results.** The overall actuarial survival at 20 years is 15%, the cumulative incidence of transplant mortality (TRM) is 42%, and the CI of relapse related death (RRD) is 48%. There was no impact of stem cell source and no improvement of results with time (<201995). In multivariate analysis on survival favorable predictors were the use of a donor other than family mismatched (RR 0.45); and bone marrow blast count less than 30% (RR 0.82). The actuarial 20 year survival for 65 patients with both favorable and unfavorable risk features is 15%. Actuarial survival at 100 days of patients surviving 100 days, the presence of chronic GVHD was the strongest favorable predictor (RR 0.88, p=0.0008) followed by donor other than family mismatched (RR 0.52, p=0.0008), donor age less than 34 years (RR 0.55, p=0.02), and blast count less than 30% (RR 0.58, p=0.07). For 18 patients with all 4 favorable predictors, the actuarial 20 year survival is 54%. This study confirms that 15% of relapsed leukemias can be cured with an allogeneic transplant. The use of young, HLA matched donors and a marrow blast count less than 30% significantly increases the likelihood of long term survival, which is further improved if chronic GVHD develops. This may be relevant when discussing transplant strategies in patients with relapsed leukemia.
This is a single Center study testing the efficacy of ECP in patients with steroid-resistant cGVHD. Patients. Twenty-six patients entered this study. Their median age was 40 (range, 5-61) years. The median interval from diagnosis of cGVHD to ECP treatment was 12 (range, 6-168) months. All patients received at least 2 lines of immunosuppression including cyclosporine (CyA) alone, CyA and steroids, CyA and mycophenolate mofetil (MMF), steroids and MMF, steroids and tacrolimus (Tac), and combination methods. Patients were treated on 2 consecutive days (once a cycle) at 1 week interval for the first month, at 2 weeks interval for the second month and at 4 weeks interval for the subsequent 4 months, for a total of 10 cycles. At 6 months a decision was made whether to continue the ECP treatment as monthly maintenance, depending upon the clinical response. ECP treatment continued to receive the baseline immunosuppressive therapy and were followed in the outpatient clinic. Results. After a median of 15 (range, 7-33) cycles, 11 (76%) of the 14 patients with skin involvement had a partial or complete response. In particular, 6 patients with severe scleroderma 2 had complete resolution of skin contraction, abrasion and thickening, 2 showed a significant improvement and 2 had no response. A return to normal values or reduction of abnormal liver function enzymes by at least 50% from baseline were observed in 9 of 12 patients (75%) with liver involvement. Of 9 patients with ocular symptoms due to sicca syndrome, 6 had a complete or partial response. A complete response was observed only in 1 of 3 patients with lung cGVHD. The first signs of response appeared at a median of 3 months. Throughout the ECP treatment course systemic immunosuppressive medication was increased in 4 patients, reduced in 8 and discontinued in 14. The steroid therapy was discontinued at a median of 4.5 months. At a median follow-up of 41 (range, 3-69) months 4 patients are alive, 3 patients died of cGVHD related infectious complications and 1 of leukemia relapse. Twelve patients (46%) discontinued ECP after a median of 18 (range, 6-24) cycles because of complete and sustained resolution of cGVHD and 5 patients (19%) because of minimal or inadequate response. The ECP treatment is ongoing in 5 patients. The procedures were well tolerated and completed in all cases and no relevant adverse events were observed. Conclusions. Our results confirm the role of ECP in controlling refractory cGVHD. The response of a single organ is independent from the others. The response of liver cGVHD is comparable to mucocutaneous response. New strategies are requested for lung cGVHD.

0301 IMPACT OF DISPARITIES AT SINGLE OR MULTIPLE HLA LOCI ON OUTCOME AFTER UNBILICAL CORD BLOOD TRANSPLANTATION FOR ADULTS WITH HEMATOLOGIC MALIGNANCIES
Hospital La Fe, VALENCIA, Spain

Background. The number of HLA disparities considering HLA-A, -B, -C, -DRB1 and -DQB1 and 'DPB1 is strongly related to engraftment, disease free survival (DFS), and overall survival (OS) in children undergoing UCBT. The influence of HLA mismatching seems not as important in adults as compared to children undergoing UCBT. These data show the high-resolution DNA typing for HLA-A, -B, -C, -DRB1, -DQB1 and 'DPB1 is not essential in UCB searches for adults.

0302 CORTICOSTEROIDS FOR PREVENTING GRAFT-VERSUS-HOST DISEASE AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION: A COMPREHENSIVE META-ANALYSIS
S. Quellmann, J. Bohlus, K. Hubeil, G. Schwarzer, A. Engert
University of Cologne, COLOGNE, Germany; University of Freiburg, FREIBURG, Germany

Background. Graft-versus-host disease (GvHD) remains a major complication in allogeneic myeloablative stem cell transplantation (SCT) and is considered as the main cause for transplantation related morbidity limiting its wider application. The current standard regimen for preventing GvHD combines cyclosporine (CSA) with a short-course of methotrexate (MTX). The question if the addition of steroids improves patients' outcomes has not been clarified yet as the results of single studies are ambiguous. Aims. To determine the effectiveness of corticosteroids used for the prevention of GvHD after myeloablative SCT in improving overall survival (OS), disease-free survival (DFS), relapse incidence (RI), non-relapse mortality (NRM), acute GvHD grade I-IV, II-IV and III-IV and chronic GvHD. Methods. We conducted a comprehensive literature search in Cochrane Library, EMBASE, MEDLINE, internet databases for ongoing trials, and conference proceedings (1975-2004). Randomised controlled trials evaluating GvHD prophylaxis regimens differing only in the use of corticosteroids were included. A minimum of 75% of the patients undergoing allogeneic myeloablative SCT had to be adults. All authors were asked to provide unpublished and/or missing data. Trial selection, quality assessment and data extraction were done independently by two reviewers. To analyse outcomes with time-to-event data hazard ratios (HR) were calculated on the basis of individual patient data or if not available extracted from the publication using well-established Methods. The weighting was done according to the method of Peto, which assumes a fixed effect model. Heterogeneity of treatment effects between the trials was assessed by using a Chi-squared test with a significance level of p<0.1. Results. 1,709 references were screened, of which 5 randomised controlled trials with 604 patients met the criteria and were finally included in the review. The addition of corticosteroids reduced statistically significant the incidence of acute GvHD grade I-IV (HR 0.58, 95% CI 0.45 to 0.76) as well as grade II-IV (HR 0.69, 95% CI 0.51 to 0.92). No significant differences seen for acute GvHD grade III-IV (HR 0.76, 95% CI 0.52 to 1.15) and chronic GvHD (HR 1.21; 95% CI 0.89 to 1.65) as well as no improvements were found for OS (HR 0.99, 95% CI 0.79 to 1.25), DFS (HR 0.95, 95% CI 0.74 to 1.12), RI (HR 0.82, 95% CI 0.57 to 1.18) or NRM (HR 0.88, 95% CI 0.61 to 1.26). Summary/Conclusions. The addition of corticosteroids to GvHD prophylaxis regimens reduces the risk for acute GvHD grade I-IV and II-IV. However, based on the randomised trials currently available there is no evidence that this benefit improves long-term outcomes such as OS, DFS, RI, NRM or chronic GvHD.

0303 REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: DOES THE CONDITIONING MATTER? A RETROSPECTIVE STUDY OF THE SFGM-TC ON 61 PATIENTS
Hopital Cl.Huriez, LILLE, France; Agence de la biomdecine, PARIS, France; Hopital St Louis, PARIS, France; Hotel Dieu, NANTES, France; Pessac, BORDEAUX, France; Hopital Pit-SalpTriere, PARIS, France; CHU, NICE, France; Hopital E Heriot, LYON, France; Hopital Cl.Huriez, LILLE, France;
Reduced-intensity allogeneic stem cell transplantation (RIA) has emerged as an alternative to myeloablative transplantation in patients with myelodysplastic syndrome (MDS). Given the uncertainty regarding the appropriate conditioning, SFGM-TC conducted a retrospective multicenter study with the attempt to evaluate the impact of conditioning on patients’ outcome. The record of 61 patients (57 males) with MDS who received a graft of cGVHD after RIA was evaluated. Twenty-one patients had RIA-T at diagnosis and 6 CML, of whom 8 progressed to AML before transplantation. The median time from diagnosis to RIA was 12 months (6-129). Conditioning regimen consisted of Fludarabine (Flu) plus busulfan (FB; n=29), Flu plus 2-Gy TBI (F-TBI; n=20) and idarubicin plus ara-cytine and Flu (Flaglda; n=12). Donors were HLA-identical siblings (n=52) and HLA-matched unrelated (n=9). All pts received peripheral blood stem cells. The median of CD34+ infused cell dose was 5×10^6/kg (0.5-17.3). At the reference date of analysis of 1 July 2005, median follow-up was 44.7 months (21-85). Estimated 3-year overall survival (OS), progression free survival (PFS), relapse and transplant-relapse mortality (TRM) were 85%, 75%, 69% and 50%, respectively. Neither of the 3 conditioning regimens used (FB, F-TBI and Flaglda) had impact on patients’ outcome. In multivariable analyses, while acute III/IV grade GVHD development was the only factor found to adversely influencing OS (HR=6.9; 95% CI: 1.1-12.5), chronic GVHD development was the only factor found to influence PFS and relapse rate from 0.1-0.7 and HR=0.2; 95% CI: 0.1-0.6, respectively. TRM was adversely influenced by male sex of patient (HR=9.2; 95% CI: 1.5-66.6). RIA seems to be an effective treatment in MDS patients irrespective of conditioning type. While acute III/IV grade GVHD appeared to be detrimental, the benefit effect of chronic GVHD was to be bound to GVL effect as demonstrated by the improvement of PFS and relapse rates in patients who developed chronic GVHD. New approaches with focus on immunosuppressive treatment are needed to enhance the GVL effect with an acceptable risk of GVHD.

**0304**

**PROGENITOR CELLS ARE TRAPPED IN FILTERS USED FOR MARROW HARVEST: RECOV-ERING CELLS FROM MARROW FILTERS REDUCES GRAFT VS HOST DISEASE AND TRANSPLANT MORTALITY**

D. Vicente, 1, M. Podest, 1 A. Pittro, 1 S. Pozzi, 1 S. Lucchetti, 1 T. Lamparelli, 2 E. Tedone, 3 I. Abatucci, 4 O. Fignani, 4 F. Frassoni, 4 M.T. Van Lint, 1 G. Piaggio, 5 N. Sacchi, 6 A. Bacigalupo 7

1 Ospedale San Martino, GENOVA, Italy; 2 The Italian Bone Marrow Donor Registry, OSPEDALE GALLIERA, GENOVA, Italy

**Background.** A bone marrow harvest is filtered either in the operating room, in the laboratory, or during infusion to the patient. Filters are usu-

ally discarded. Little is known of haemopoietic progenitor cells trapped in the filters. **Aim of the study.** To evaluate haemopoietic progenitor cell content in the filters and to assess the outcome of transplants with filter-dis-
ered or filter-recovered cells. **Patients and Methods.** Haemopoietic progenitors were grown from filters of 19 marrow transplants. We then compared the outcome of 59 filter-recovered transplants from HLA identical siblings (years 2001-2004) with a matched cohort of 43 filter-dcorded marrow grafts (years 1997-2000). Haemopoietic progenitors. Filters contained on average 21% LTC-IC and 15% CFU-F of the total progenitor cell content. Patients outcome. Filter-discarded transplants had significantly more grade II-IV GVHD (40% vs 15%, p=0.006) as compared to filter-recovered transpl-

ants, and more TRM (20% vs 3%, p=0.04). The actuarial survival at 5 years was 69% vs 87% respectively (p=0.15). Conclusions. This study suggests that (1) a significant proportion of LTC-IC are lost in the filters together with CFU-F; (2) recovery and add back of progenitors trapped in the filters may reduce GVHD and transplant related mortality.

**0305**

**EXTRACORPOREAL PHOTOPHERESIS FOR TREATMENT OF FASCITIS IN CHRONIC GRAFT-VERSUS-HOST DISEASE: A PILOT STUDY**

M. Stadler, 1 A. Achenbach, 4 J. Funke 5 S. Buchholz, 6 H. Kamal, 1

M. Eder, 8 H. Bertenstein 1 A. Ganser, 4 R. Stadler 2

1 Hannover Medical School, HANNOVER, Germany; 2 Dermatological Hospit-
al, MINDEN, Germany; 3 Central Hospital, BREMEN, Germany

**Background.** Fasciitis is a rare, late onset manifestation of chronic Graft-

versus-Host Disease (cGvHD) after allogeneic hematopoetic cell transplan-

tation. It is characterized by condensed and hardened subcutaneous and fascial tissues, as opposed to cutaneous sclerosis. On biopsy, edema and fibrosis of the fasciae and intermediate septa with pericapillary lymphocytic infiltrates are found, without involvement of the muscle itself. Typically, conventional immunosuppressive therapies fail or yield incom-

plete responses. Extracorporeal photopheresis (or photochemotherapy, ECP) is an attractive alternative for patients with fasciitis of cGvHD because of its systemic action and steroid-sparing effects. Although still not fully understood, tolerance induction through ev evi poral-sen-

sitive and UVA-irradiated T-cells is now believed to be the central mechanism of action of ECP. **Patients and Methods.** Here we report our seven years experience (2/1999-2/2006) of ECP in 16 consecutive patients with fasciitis of cGvHD: 4 females and 12 males; age 24-63 (median: 44) years; 5 AML, 4 ALL, 3 CML, 1 MDS and 3 lymphoma patients; 13 with matched related and 3 with matched unrelated donors; 4 patients received RIA between 1998 and 2003, from 22 French transplan-

tation centres, were reviewed. Participating centres were asked to veri-

fy data referred to French registry and provide additional information on each patient. According to the FAB classification, 11 patients had RA at
diagnosis, of whom 1 had progressed to REAB and one to AML before transplantation. Thirty two patients had RAB at diagnosis, of whom 2 had progressed to REAB-T and 7 to AML before transplantation. Twelve patients had RAB-T at diagnosis and 6 CML, of whom 8 progressed to AML before transplantation. The median time from diagnosis to RIA was 12 months (6-129). Conditioning regimen consisted of Fludarabine (Flu) plus busulfan (FB; n=29), Flu plus 2-Gy TBI (F-TBI; n=20) and idar-

ubicin plus ara-cytine and Flu (Flaglda; n=12). Donors were HLA-identical siblings (n=52) and HLA-matched unrelated (n=9). All pts received peripheral blood stem cells. The median of CD34+ infused cell dose was 5×10^6/kg (0.5-17.3). At the reference date of analysis of 1 July 2005, median follow-up was 44.7 months (21-85). Estimated 3-year overall survival (OS), progression free survival (PFS), relapse and transplant-relapse mortality (TRM) were 85%, 75%, 69% and 50%, respectively. Neither of the 3 conditioning regimens used (FB, F-TBI and Flaglda) had impact on patients’ outcome. In multivariable analyses, while acute III/IV grade GVHD development was the only factor found to adversely influencing OS (HR=6.9; 95% CI: 1.1-12.5), chronic GVHD development was the only factor found to influence PFS and relapse rate from 0.1-0.7 and HR=0.2; 95% CI: 0.1-0.6, respectively. TRM was adversely influenced by male sex of patient (HR=9.2; 95% CI: 1.5-66.6). RIA seems to be an effective treatment in MDS patients irrespective of conditioning type. While acute III/IV grade GVHD appeared to be detrimental, the benefit effect of chronic GVHD was to be bound to GVL effect as demonstrated by the improvement of PFS and relapse rates in patients who developed chronic GVHD. New approaches with focus on immunosuppressive treatment are needed to enhance the GVL effect with an acceptable risk of GVHD.

**0306**

**REDUCED INTENSITY CONDITIONING (RIC) IN ALLO-BMT FOR RESISTANT-RELAPSED LYMPHOMAS**

P. Mazza, 1 M. Palazzo, 2 M.R. Specchia, 3 G. Consolo, 3 A.M. Carella, 1 G. Pispisa, 1 P. Iacopino 2

1 Ospedale S.G. Moscati, TARANTO, Italy; 2 Ematologica Ospedale S.G. Moscati, TARANTO, Italy; Centro Trapianti Midollo Osseo OSPEDALE REG-

GIO CALABRIA, TARANTO, Italy; 3 Divisione Ematologia IRCCS, S.GIOVANNI ROTONDO, ITALY

**Background.** The use of RIC conditioning regimen is now largely applied in conditioning regimen for TMO in haematological malignan-

ties however no definite indications exist on which patients may be

benefited by this procedure although most of experiences are focused on patients with lymphomas resistant or relapsed to other therapies. **Aims.** To disclose if RIC may be a therapeutic option as salvage treatment with acceptable results in a population of relapsed-resistant patients with lymphomas, mostly already treated by high-dose therapy and PBSCT. The inclusion in the RIC procedure of those patients with age over 60 years leads to valuate if the toxicity, namely related to TRM, is acceptable. Methods. We present a cooperative experience on RIC focused on 42 advanced, relapsed and resistant lymphomas to several therapies includ-
ing high dose and autologous PBSCT. The mean age of patients was 49 (22-78) years. RIC consisted in the conditioning with Fludarabine 50 mg/m2 and CTX 300 mg/m2 for 27 patients (46%) and oth-
er similar combinations for the other patients. Diseases included 13 HD resistant, 9 HGBL, 3 FL, 6 HGTL, 4 CLL, 2 ALC and 3 LPL relapsed and
resistant to a series of therapies. Thirty-one patients have been already treated by high-dose therapy and PBSCT. Results: Thirty-one patients (86%) are evaluable for response, 6 patients died within 3 months from RIC for causes not related to disease progression (TRM=14%). The response valuated within 6 months from RIC showed 26 pts in CR (72%) and 10 patients with persistence of disease (28%), 3 relapses (14%), were registered following 12, 18 and 24 months from RIC. Eight patients who did not show disease progression among evaluable patients at 6 months, after death was registered for chronic GVHD 18 months following RIC accounting at 15 the total number of deaths including TRM (36%). Five patients are alive with lymphoma and 22 alive in CR (52%) at a mean follow-up of 25 months (7-59 months). The incidence of GVHD was regis-tered in 16 patients (55%) and 6 of them had grade 3-4. This was not corre-lated to previous therapy or to type of RIC conditioning regimen or to the age of patients. Deaths accounted 11 on 31 (35%) patients who received autologous PBSCT and 4 on 11 (36%) on those who did not.

Conclusions. RIC transplantation provides a high rate of remissions in patients with advanced lymphoma and an acceptable TRM. The results are improved by the disease-free survival of the patients transplanted with the incidence of GVHD and relapses are not correlated to different condition-ing regimen. Future prospective trials including RIC transplantation are planned.

0307
FLUDARABINE BASED REDUCED INTENSITY CONDITIONING FOR ALLOGENIC TRANSPLANTATION IN PATIENTS WITH NON-MALIGNANT HEMATOLOGICAL DISORDERS
B. George, V. Mathews, A. Viswambady, A. Srivastava, M. Chandy
Christian Medical College, Vellore, VELLORE, TAMILNADU, India

Patients who are multiply transplanted or septic have a poor outcome after allogeneic stem cell transplantation. Seventy patients (53 males and 17 females) with non-malignant disorders underwent allogeneic BMT using a fludarabine based conditioning regimen between 1998 and 2005. The median age was 20 years (range, 4-38) and consisted of 25 children and 45 adults. Indications for BMT included severe aplastic anemia (SAA) in 54, Myelodysplastic syndromes (MDS) in 8, Fanconi’s anemia (FA) in 6 and Thalassaemia in 2 patients. All had 6 antigen matched sib-bing or family donors. Multiple transfusions (>20), sepsis or previous immunosuppressive therapy were considered high risk (HR) and 51 patients (72.8%) were considered high risk patients. The median time from diagnosis to transplant was 16 months (range: 2-108) and the medi-an transplants prior to BMT was 35 (range: 2-880). Conditioning ther-apy included Fludarabine (Flu) 180 mg/m2 over 6 days, Busulfan (Bu) 8 mg/kg over 2 days and ATG 40 mg/kg/day over 4 days (24), Flu 180 mg/m2 over 6 days, Cyclophosphamide (Cy) 120 mg/kg over 2 days + ATG 40 mg/kg/day over 4 days (35), Flu 180 mg/m2 over 6 days, Cy 20 mg/kg over 2 days + ATG 40 mg/kg/day over 4 days (6), Flu/TBI/OKT3 in 4, Flu/Mel in 1. Graft versus host disease (GVHD) prophylaxis con-sisted of Cyclosporine alone or in combination with mini methotrexate. Graft source was peripheral blood stem cells in 56 patients and G-CSF stimulated bone marrow in 14. The median cell dose was 5.4×108 MNC/kg (range: 2.1-13.6) for PBSCT and 6.2×108 TNC/kg (range: 2.1-16) for bone marrow. Nine patients expired within the first 10 days due to sepsis, 59 (96.7%) patients engrafted with a mean time to ANC > 500 of 12.2 days (range: 5-29), and platelet count > 20,000 of 14.2 days (range: 9-58), 12/18 patients underwent primary graft failure and expired. Acute GVHD was seen in 18 patients (30.5%) with Grade III-IV GVHD in 6 (10.1%). Chronic GVHD was seen in 14 patients (29.1%) with 9 having limited and 5 with extensive GVHD. Bacterial infections were seen in 16 patients, fungal infections in 19 and CMV in 8 patients. Veno-occlusive disease was seen in 5 patients (7.1%) while hemorrhagic cystitis was seen in 3 (4.2%). Four patients (2 with aplastic anemia and 2 with thalassaemia) had secondary graft rejection. Day 100 mortality was 28% and was related mainly to sepsis. At a median follow up of 20 months (range: 2-84), 46 patients (65.7%) are alive with 44 patients (62.8%) being free of disease. Among patients who were low risk, 17/19 (89.4%) are alive and free of disease. The disease free survival was 66.6% in SAA, 62.5% with MDS, 50% with FA and 0% with Thalassaemia. In conclusion fludarabine based conditioning regimen ensure adequate engraftment with reduced toxicity in high risk patients who are infect-ed or multiply transplanted at the time of BMT. Its role even in good risk patients needs to be further explored.

0308
SAFETY AND EFFICACY OF BORTEZOMIB IN RELAPSED MULTIPLE MYELOMA FOLLOWING REDUCED INTENSITY/NONMYELOABLATIVE CONDITIONINGS AND ALLOGENIC HAEMATOPOIETIC CELL TRANSPLANTATION
1. University Terni, TORINO, Italy; 2. University Udine, UDINE, Italy; 3. OSPEDALE SANTA Croce, CUNEO, Italy; 4. Istituto Nazionale Tumor, MILANO, Italy; 5. IRCCS Istituto Scientifico HS Rafieffe, MILANO, Italy; 6. IRC, CAN-DIOL, Italy; 7. Università di Firenze, FIRENZE, Italy; 8. Università La Sapienza, ROMA, Italy; 9. OSPEDALE Ferrarotto, CATANIA, Italy

Background. Despite the promising results obtained in patients with multiple myeloma (MM) undergoing allo-HCT, the role of the conditioning regimen and allogeneic haematopoietic cell transplantation (HCT), relapse remains an issue. Several clinical trials showed the effi-cacy of bortezomib in the treatment of refractory/relapsed MM, by inhibi-tion of NF-κb. These findings and the demonstration of the role of NF-κb in the pathophysiology of graft-versus-host disease (GVHD) provide the rationale for using bortezomib in patients with MM relapsed after allogeneic HCT. Aims. We evaluated safety and efficacy of bortezomib after reduced intensity/nonmyeloablative conditioning regimen and allo-grafting. Methods. We retrospectively evaluated 24 myeloma patients relapsed after allografting. Conditioning regimens were 2 Gy total body irradiation (TBI) followed in 19 patients with a combination of thiotepa, cyclofos-mide, and melphalan in 5. Donors were HLA identical siblings in 22 patients, and unrelated in 2. All patients received cyclosporine as part of post-grafting immunosuppression. Bortezomib was administered after a median of 20 months from RIC (range 5-54) and 34 months from diag-nosis (range 19-164): 6 patients were in first relapse after HCT, 10 patients in second and 8 beyond the third relapse. Patients received bortezomib 1.0 (n=8) or 1.3 mg/m2 (n=16) on day 1, 4, 8, 11 every 3 weeks for a median of 3 courses (1-7), alone (n=5) or in combination with dexamethasone 20 (n=13) or 40 mg (n=5) on days 1-4 and 15-18 or daily prednisone 75 mg (n=1). No patient was on cyclosporin or thalido-mide, and none had active GVHD at the time of administration. Results. Adverse effects were reported in 75% (18/24) of patients: thromboci-topenia was observed in 33% (8/24); >grade 3/5, peripheral neuropa-thy in 18% (4/24); >grade 3/5, 14/5. Three additional patients experienced grade 2 urticaria, grade 2 liver toxicity and grade 3 neutropenia, respectively. Bortezomib was discontinued after the first cycle in 4 patients due to neurological toxicity, and in 2 patients for disease pro-gression. A dose reduction was required in 3 patients due to neurolog-i cal toxicity. Flaring of prior chronic limited GVHD was observed in one patient who developed milder liver GVHD. After a median follow up of 136 days (range 42-503), 21/24 patients are alive. Two non-responsive patients were the only patients that flared from previous GVHD. Among patients who completed at least 2 courses, overall response was 67% (12/18) including 5 immunofixation-negative complete remissions. No significant differences in toxicity and response rates were seen between bortezomib plus steroids and bortezomib alone. Conclusions. Bortezomib is capable of inducing disease remissions in patients with MM relapsed after allogeneic transplant. No significant effect on GVHD was noted. Interestingly, in this subset we observed a higher incidence of peripheral neuropathy compare to the non-transplant population, which may be related to previous prolonged treatment with high dose cyclosporine. Longer follow up will demonstrate whether remissions will be durable.

0309
CYTOKINES AND T-CELL SUBSETS CHANGES IN PATIENTS HAVING GRAFT-VERSUS-HOST DISEASE
S.P. Yeh, C.F. Chiu, W.J. Lo, C.L. Lin, Y.M. Liao, C.C. Lee
China Medical University Hospital, TAICHUNG, Taiwan

Background. GVHD is the consequence of the activation of donor T-lymphocytes attack the tissue of host. Animal studies strongly suggest that T-cell activation in patients with acute GVHD have a CD4 subset imbalanced favoring T helper 1 (Th1), which secrete type 1 cytokines interleukin (IL)-2, IL-12, interferon (INF)-γ, and TNF-α. On the other hand, the polarization toward Th2, which secrete type 2 cytokines IL-4 and IL-10, and subsequent Th2 humoral immune response may be responsible for the development of chronic GVHD. Both Th1 and Th2 are derived from naive T cells and both cytokine production. Th1 inducers are themselves cytokines: INF-γ and IL-12 for Th1, and IL-4
and IL-10 for Th2. Understanding the cytokines and T cell subsets change in patients with GVHD will theoretically of great help in elucidating the pathophysiology of GVHD. Aim. To see the cytokines and T cell subset changes in patients having acute and/or chronic GVHD. Methods. Consecutive 23 patients received allogeneic hematopoietic stem cell transplantation at China Medical University Hospital were enrolled in this study. 10 mL peripheral blood was collected every 7 days from Day 7 after transplantation till Day 200 (or Day 300 for patients with chronic GVHD). Plasma level of INF-γ, IL-4, IL-10, and IL-12 were determined by ELISA (R&D, Minneapolis, MN, US). Flow cytometric analysis of intracellular INF-γ and IL-4 in mononuclear cells with or without phorbol 12-myristate 13-acetate (PMA) + ionomycin (I) stimulation was used to determine the relative fraction of Th1/Th2 subset. The serial plasma level of each cytokine and relative fraction of Th1/Th2 were then compared to the clinical events in each patient. Results. Plasma IL-10 level increased markedly during period of both acute and chronic GVHD. Plasma INF-γ level also increased in most events of acute and chronic GVHD. With effective immunosuppressive therapy, plasma IL-10 and INF-γ level decreased rapidly. Plasma IL-4 and IL-12 were below the detectable level (0.13 pg/mL and 0.5 pg/mL respectively) in most patients, even during period of severe GVHD. Figure 1 demonstrates the correlation between plasma level of each cytokine (INF-γ, IL-4, IL-10, and IL-12) and clinical course of a patient with both acute and chronic GVHD involving liver. Flow cytometric analysis showed that Th1 (CD4+INF-γ+) fraction increased markedly within the CD4+ T cell population during period of both acute and chronic GVHD. Of great interesting is that the CD4+INF-γ+ T cells can be detected in the blood of many patients of GVHD without adding PMA+I to stimulate T cells. Conclusion. Immune reactions in patients of GVHD are much more complicated than in animal models. Type 1 cytokine (INF-γ) and type 2 cytokine (IL-10) may be increased in the blood at the same time during acute and chronic GVHD. Increased Th1 fraction could also be found during both acute and chronic GVHD. Th1 as well as IL-10 and INF-γ may therefore play an important role in the pathogenesis of both acute and chronic GVHD. Besides, they may also be served as good biomarkers in monitoring the clinical course of GVHD.

Figure 1. Neighbour-joining tree of Polish and other populs.

0310
increased incidence of cytomegalovirus retinitis after allogeneic hematopoietic stem cell transplantation

Hôpital Saint-Louis, PARIS, France

Cytomegalovirus (CMV) has been recognized as a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). CMV retinitis (CMVR), frequent in patients with acquired immunodeficiency syndrome but rarely involved in HSCT recipients, can lead to retinal destruction and blindness if untreated. We observed 6 cases from 2002 to 2005 in our center whereas only one case was diagnosed from 1985 to 2001. We described clinical and biological features of patients who developed a CMVR from 2002 to 2005 and determined incidence and risk factors of CMVR. Among 312 patients, who received HSCT in our center from 2002 to 2005, 117 had CMV reactivation and 24 had at least one episode of CMV disease. Of the 24 patients with CMV disease, 6 had CMV retinitis. Cumulative incidences were determined with death as competing event and risk factors (SPLUS 2000 Software). The Cox proportional hazard regression model was used to test significance of covariate. Of the six patients with CMVR, five received an HLA-identical bone marrow transplant from a related (n=1) or an unrelated (n=4) donor. The other patient received an unrelated HLA mismatched cord blood (UCB). CMVR was diagnosed in median 152 days after HSCT either on visual symptoms (n=5) or on a systematic ophthalmologic examination (n=3). All patients experienced at least 2 CMV reactivations prior to retinitis diagnosis and one patient had a previous CMV disease. The median lymphocyte count at diagnosis was 0.5×10⁹/L (range: 0.32 to 1.21×10⁹/L), the CD4 count was lower than 0.2×10⁹/L in all patients and lower than 0.05×10⁹/L in all but one. Retinitis resolved with a systemic intravenous antiviral treatment (foscarnet or gancyclovir) in all treated patient. One patient remained with sequelar visual trouble. Three patients relapsed from retinitis and were successfully treated again by intravenous antiviral treatment. Three-year cumulative incidence of CMVR was 2.2% among all transplanted patients, 3.5% in CMV-seropositive recipients and 6.5% in CMV-seropositive recipients transplanted with a CMV-seronegative donor. The combination of a CMV-seropositive recipient and CMV-seronegative donor was the only risk factor found in our study. The source of stem cell, conditioning regimen, the use of antithymoglobulin, age and GVHD were not related to an increased risk of CMV retinitis but small number of CMVR limited power of the analysis. We observed an increased incidence of CMVR compared with incidence before 2002 in our center and published incidence < 0.5% (Crippa E CID 2001). This increase could be explained by a change in HSCT recipient management: improvement in supportive care, antiCMV pre-emptive therapy and increase in proportion of unrelated donor as well as cord blood. Finally, in patients with multiple CMV reactivations, we suggest to practice regular ophthalmologic examination in order to diagnose retinitis before visual trouble.

Figure 1. Cytokines’ change and clinical course of GVHD
Background. Availability of matched stem cell donor is limiting factor for transplantations to patients who might benefit from this therapy. At most, 25% of patients may be supported by family donors and 50-50% of them receive stem cells from unrelated or other alternative donors. For the remaining patients no suitable donor is available because of unacceptable HLA mismatches. Several approaches are undertaken to increase HLA polymorphism of unrelated donor registries. Preferential typing of ethnic minorities or further typing of donors with rare phenotypes were used optionally. Aims. The aim of this study was to show the structure and genetic differences between urban and rural Polish subpopulations and to check the utility of dispersed rural population for increasing of unrelated donor registry HLA polymorphism. Methods. Five HLA loci (A, Cw, B, DRB1 and DQB1) were DNA typed at allele (4 digit) level in Polish population. The analysis comprised 200 unbiased, healthy individuals living in cities with >100 000 citizens (urban, N=106) and those living out of big cities (rural, N=94). The genetic structure measures of these two Polish subpopulations and five locus phylogenies along with other European, Mediterranean and Far-Eastern populations were analysed. Results. All loci were in Hardy-Weinberg equilibrium in both subpopulations (p>0.05). A significant heterozygote excess was confirmed for DQB1 (p=0.014, SE=0.001) and globally (p=0.028, SE=0.009) in rural sample and on the contrary, global heterozygote deficit was found in urban population (p=0.039, SE=0.012). As could be expected estimates of Nm, the number of migrants exchanged per generation, appeared to be high (Nm=19.60) after correction for sample size (mean sample size, N=100), suggesting high gene flow between two populations. Genic and genotypic differentiation tests revealed that none of five loci differentiations caused predominantly by country-to-city migration. Genic and sample size, N=100, suggesting high gene flow between two populations. Genic and genotypic differentiation tests revealed that none of five loci differentiations caused predominantly by country-to-city migration. Genic and sample size, N=100, suggesting high gene flow between two populations. Genic and genotypic differentiation tests revealed that none of five loci differentiations caused predominantly by country-to-city migration. Genic and sample size, N=100, suggesting high gene flow between two populations. Genic and genotypic differentiation tests revealed that none of five loci differentiations caused predominantly by country-to-city migration. Genic and sample size, N=100, suggesting high gene flow between two populations. 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0313
Hematopoietic Stem Cell (HSC) Recruitment has an Influence on Transplant Outcome after Reduced Intensity Allogeneic Peripheral Blood Stem Cell Transplantation (PBSCT): A Study of the Societe Francaise de Greffe de Moelle Osseeuse et de Therapeutique Cellulaire (SGFM-TC) Registry

T. Prebet; Q.H. Lê; J.M. Boiron; D. Blaise; A. Huyhn; I. Yokoub Agha; H. Esperou; M. Michallet

Purpose

Impact of graft product on transplant outcome after PBSCT is actually demonstrated. We investigated retrospectively the potential impact of HSC recruitment procedure (i.e. G-CSF stimulation schedule and apheresis number) and graft composition (CD34+ and CD3+ cell number) on transplant outcome (GVHD, OS, EFS).

Methods

Our analysis concerned 488 HLA matched sibling allogeneic reduced intensity conditioning (RIC) PBSCT for haematological malignancies (116 MM, 110 AML, 109 NHL, 41 CML, 41 MDS, 24 CML, 19 HD, 17 ALL and 11 MPS) reported on the SGFM-TC registry between 1998 and 2004. RIC-PBSCT was performed during first line treatment in 225 (49%) patients and a previous HSCT was recorded in 55% of the cases. Before RIC, 161 patients were in Complete or Partial Remission, 34 in Stable Disease and 132 in Progressive Disease. Follow-up was updated in April 2005. G-CSF median duration was 5 days (3-7 days) at a median dose of 10µg/kg/day (4.6-16). G-CSF was given in 40% of the stimulations. Filgrastim was used in 59% of the donors (Lenograstim: 41%). Only 107 donors (22%) had a single apheresis. The median number of CD34+ cells infused was 5.6±1.6x10^6 CD34/kg (1-26) and the median CD3+ cells was 302±10^6 CD3+ (63-996).

Results

Conditioning regimen was most frequently an association of Fludarabine Busulfan and Anti Thymocyte Globuline (246 cases, duration of ATG 1 day: 18%, 2 days: 20%, 3 days: 20%, 4 days: 8%, 5 days: 88%); or Fludarabine + TBI 2 Gy (123 patients). GVHD prophylaxis was a cyclosporine based treatment in 472 (85%) patients. Median follow-up after transplantation was 35 months (range: 0.36). Acute GVHD (grade II-IV) and cGVHD incidences were 85% (n=163) and 50% (n=217 for 480 patients) respectively. The 3-year OS was 40% and the 3-year EFS was 34%. Treatment related mortality was 15% at 3 years. In multivariate analysis studying pre and post transplant factors a significant impact was shown of G-CSF duration (HR: 0.79 (0.62-1.0) p=0.05), G-CSF daily dose (HR: 1.13 (1.12-1.28) p=0.04) on OS and a trend for G-CSF dose on EFS (HR: 1.1 (0.97-1.25) p=0.12). Other variables also influenced OS (NHV vs AML, aGVHD grade II vs 0-I and IV-III vs 0-I and cGVHD: yes vs no) and on EFS (Sex mismatch, ABO Incompatibility, NHL vs AML, RIC ATG duration: 5 days vs 2 days, aGVHD grade II vs 0-I and IV-III vs 0-I and cGVHD: yes vs no). No influence of graft composition or stem cell recruitment was demonstrated on incidence and severity of aGVHD and cGVHD although we found a significant impact of conditioning (FBs ATG 1 day vs 2 days and Fluda-TBI vs FBs ATG 2 days). In conclusion, our demonstration, grasping the complexity, of graft composition has no impact on transplant outcome. Prolonged administration of moderate dose of G-CSF seems to be the best schedule for PBSC recruitment.

0315
Comparative Outcomes of Fludarabine-Based Nonablative and Ablative Conditioning for Patients with Advanced Hematological Malignancies

I. Kim; B. Kim; S. Kim; Y. Kim; S. Bang; S. Yoon; J.S. Lee; S. Park; B.K. Kim

Seoul National University Hospital, SEOUL, South-Korea; Seoul Cancer Research Institute, SEOUL, South-Korea; Clinical Research Institute, SEOUL, South-Korea

Background
The role of nonablative allogeneic transplantation is not defined for advanced hematologic malignancies. Aims. We have conducted a comparison of the outcomes of nonablative and ablative conditioning directly for the treatment of patients suffering from advanced hematological malignancies. Methods. Adult patients with advanced hematological malignancies (n=137; acute leukemia, 56; chronic myeloid leukemia beyond 1st chronic phase, 6; refractory Non-Hodgkin's lymphoma, 10; refractory multiple myeloma, 3) received transplants from human leukocyte antigen-matched donors, either related or unrelated, coupled with either nonablative (n=40; fludarabine/melphalan, 25; fludarabine/cyclophosphamide, 12) or ablative conditioning (n=55, busulfan/cyclophosphamide). The patients receiving nonablative conditioning were elderly, or exhibited contraindications for ablative conditioning. Results. Neutrophil engraftment (i.e., time to ANC>0.5x10^9/L) occurred more rapidly in the nonablative group (median, 9 days; range, 0-19 days) than in the ablative group (median, 18 days; range, 11-38 days)(p<0.0001). The time required to achieve a platelet count in excess of 20x10^9/L was 12 days (median, 7-28 days) in the nonablative group, and 22 days (median, range, 9-64 days) in the ablative group (p=0.0001). Acute graft-versus-host disease (grade II) occurred at comparable frequencies in the nonablative and ablative groups (25% vs 26%). Hepatic veno-occlusive disease developed in 1 patient (3%) in the nonablative group, and 7 patients (20%) in the ablative group (p=0.02) Day-100 and 1-year NRMs were 33% and 47% in the nonablative group patients, as compared with 38% and 56% in the ablative group patients (p=0.68). The overall 1-year survival rates of the nonablative and ablative group patients were 44% and 15%, respectively (p=0.0175). Conclusions. We noted a clear trend towards a more favorable overall survival rate in the nonablative group patients. The results of this study indicate that patients suffering from advanced hematologic malignancies might benefit from treatment via nonablative transplantation.
G. Lucarelli studies
We used pulse Cy in the treatment
W. Leti, B. Erer, Twenty days post transplant, an
Corticosteroid-refractory GvHD is difficult to manage,
There were 55% CR (11/20), 10%
This is a ret-

M.C. Sirianni, C. Gramiccioni, A. Isgrò, LOIDENTICAL STEM CELL TRANSPLANTATION FOR THALASSEMIA
IMMUNO HAEMATOLOGICAL RECONSTITUTION AFTER T-CELL-DEPLETED HLA-HAP-
Background. We evaluated haematological and immunological characteristics of four thalassemia patients after T-cell-depleted HLA-haploidentical stem cell transplantation. Methods. We evaluated the clono-
ogenic capability by the colony forming cell assay (CFC) and the long term culture-initiating cell (LTC-IC) assay at baseline and 20 days after transplant. Stromal cells were obtained from long term culture of bone mar-
row mononuclear cells (BMMCs) and analysed by immunohistochem-
istry. Lymphocyte subsets were studied by flow cytometry; and stromal IL-7 production by BMMCs was analysed by ELISA. Results. At baseline, no significant differences were observed in haematological and in immunological parameters in thalassemia patients when compared with a group of normal subjects. Day + 20 after transplant, a reduced clono-
genic capability was observed (4±2 vs. 41±40 CFU-E, 17±9 vs. 109±22
BFU-E, 3±1 vs 9±6 CFU-GE-MM and 16±10 vs. 66±23 CFU-GM). The number of primitive bone marrow (BM) progenitor cells was also decreased (1.8±1.4 vs. 15.4±3.6 LTC-CFC/106 BMMCs). In addition, stromal cells secreted lower IL-7 levels (0.3 ± 0.1 pg/mL vs. 0.8 ± 0.1
pg/mL, in controls) and displayed by immunohistochemistry an altered phenotype. Upon light microscopy examination, the majority (75%) of these cells appeared as moderately large cells, frequently rounded, with abundant cytoplasm, whereas in control subjects about 90% of the stroma-
mal cells exhibited a different morphology characterized by irregular or
spindle shape and branching cytoplasmatic processes (fibroblast-like).
Compared with normal subjects, thalassemia patients showed: reduc-
tion of naïve CD4+ T-cells (2±0.5% vs 50±10%), reduction of thymic
 naïve CD4+ T-cells (1±0.2% vs 40±12%), and a significant increase of
CD4+ cells activation markers (CD95, HLA-DR and CCR5). IL-7 recep-
tor (CD127) expression was also significantly decreased on CD4+ T-
cells and on naïve CD4+ T-cells (CD4+/CD45RA+CD62L+/CD127+) .
NK cells were among the first lymphocytes to repopulate the peripher-
al blood, and up to 70% of these cells were CD56 bright whereas CD16+
NK cells were decreased. Conclusions. Twenty days post transplant, an
impaired growth and differentiation capacity of stem/progenitor cells
were observed in thalassemia patients, in parallel with an altered home-
ostasis of T-cells and a reduction of T-cell naive compartment. We
hypothesize that the damage of T cell compartment may be at least par-
tially due to an altered production of new T cells starting from the
haematopoietic stem/progenitor cells. CD56+ NK cells develop more
rapidly than other lymphocytes, but CD16+ NK cells (with cytokotic
potential) require more prolonged exposure to maturation factors (IL-2)
in the bone marrow. An IL7/IL7R pathway dysregulation has been also
observed, possibly involving bone marrow stromal cells. In vitro studies
are ongoing about the use of cytokines (IL-2, IL-7, IL-2 plus IL-7) sup-
porting T cell development.

M. Krejci, J. Mayer, M. Doubek, Y. Brychtova, J. Kamelander, J. Vorlicek
Faculty Hospital Bnso, BRNO, Czech Republic

Background. Corticosteroid-refractory GvHD is difficult to manage, and is associated with high morbidity and mortality. Cyclophosphamide (Cy) is an established immunosuppressive and cytotoxic drug widely
used as a part of conditioning regimens. Pulse Cy in the GvHD treatment
is based on the Cy efficiency for the treatment of many autoimmune dis-
orders and the autoimmune nature of GvHD. In our previous work, we
showed that intestinal GvHD responded poorly to pulse Cy, whilst liv-
er, skin and oral GvHD responded well. The liver GvHD is more fre-
quent than other GvHD forms. Aims. We used pulse Cy in the treatment
of corticosteroid-refractory liver GvHD with aims to evaluate efficacy,
toxicity and influence of Cy to some clinically significant parameters. We
analyzed our new data concerning liver GvHD. Methods. This is a ret-
rospective study of 20 patients (pts) with hematological malignancies
after allogeneic peripheral blood stem cell transplantation: 12 pts had
acute GvHD (2 pts grade I, 3 pts grade II, 7 pts grade III), 4 pts had chron-

ical extensive GvHD and 4 pts developed liver GvHD upon DLI. Three pts
had only liver GvHD, 17 pts had GvHD with involvement of liver and/or
oral mucosa, skin, gut. Nine patients had hepatic variant of liver GvHD
(sero-inmano transferase ALT or AST elevation above 10 times the upper
normal limit). All patients were treated by cyclosporine A and steroids
in dose 2 mg/kg before pulse Cy; six patients had another previous ther-
apy (mycophenolate mofetil, tacrolimus, ATG, alemtuzumab). Steroid-
refractory GvHD was defined as lack or response to steroids adminis-
terd for at least 5 consecutive days. Twenty pts with corticosteroid-
refractory liver GvHD were treated by Cy at median dose of 1g/m2
(range 460 mg/m2-1800 mg/m2). Sixteen patients received one pulse Cy,
4 patients two pulses of Cy. Results. There were 55% CR (11/20), 10%
PR (2/20) and 35% NR (7/20). However, in 3 pts with NR their clinical
status stabilized and they responded to another treatment. Eight pts
(89%) from nine pts with hepatic variant of liver GvHD reached CR.
Five pts died, 3 from intractable liver and intestinal GvHD, 1 from intest-

tal GvHD with liver GvHD in PR, and 1 from relaps of leukemia. No
influence of pulse Cy to chimerism and disease status was observed.
Leukopenia and/or thrombocytopenia WHO grade 4 developed in 5
patients. When myelosuppression appeared, it was usually short-lived
(1-4 days). Twelve infectious complications occurred in 8 of 20 pts (pneu-
monia 2x, febrile neutropenia 1x, CMV positivity 6x, BKV positivity
3x), all of them resolved after antimicrobial therapy. No other signifi-
cance toxicity after Cy pulse was observed. Overall survival is 75%,
with median and maximum follow-up of 12 and 58 months, respectiv-
ely. Conclusions. Pulse Cy has a good toxicity profile and the cost of the
drug is negligible. According to our results, pulse Cy is very effective
therapy of steroid-refractory liver GvHD.
Apoptosis / Transcriptional control / Signalling

0318
HALOFUGINONE, INHIBITOR OF TRANSFORMING GROWTH FACTOR (TGF)B, INDUCES APOPTOSIS AND CELL CYCLE ARREST OF MULTIPLE MYELOMA CELLS IN VITRO AND IMPROVES HIND LIMB PARALYSIS IN THE ST2 MM MOUSE MODEL IN VIVO

J. Medvedev1, K. Halevi-Tobias1, M. Leiba1, M. Ohana1, L. Drucker1, M. Lisher1, S. Yarkoni1, A. Nagler1
1. Collgard Biopharmaceuticals Ltd, PETACH TIKVA, Israel; 2. Chaim Sheba Medical Center, TEL HASHOMER, Israel; 3. Sapir Medical Center, KFAR-SABA, Israel

Multiple myeloma (MM) is a devastating malignancy which remains incurable despite recent new novel therapeutic compounds. It was previously shown that Activin A, a member of the transforming growth factor (TGF)B superfamily, is a potent inhibitor of myeloma cells that act by blocking the cell cycle and inducing apoptosis. Halofuginone is a new novel inhibitor of TGFβ signaling that works by inhibiting Smad3 phosphorylation. The purpose of this study was therefore to assess the effect of Halofuginone on MM cell lines in vitro and to evaluate its putative therapeutic potential using the mouse ST2 MM tumor model that mimics human MM including bone lesions. The sensitivity of the MM cell lines to Halofuginone was monitored by the WST-1 viability assay, as well as by DNA fragmentation analysis, Annexin staining and cell cycle analysis. Halofuginone suppressed proliferation and induced apoptotic cell death of the MM cell lines in a dose dependent manner (IC50 varied between 15-200 nM). Incubation with Halofuginone resulted in cell shrinkage, chromatin condensation, nuclear DNA fragmentation and Annexin staining. Cell cycle analysis showed induction of cell cycle arrest and cell death in Halofuginone treated MM cells. Finally, Halofuginone administration to the ST2 MM mouse model resulted in reduction in hind limb paralysis and extended survival. In summary, Halofuginone suppressed proliferation and induced apoptotic cell death of MM cell lines and demonstrated an anti MM effect in the ST2 mouse model in vivo. Therefore, Halofuginone may indeed have a therapeutic potential for MM.

0319
DOWNREGULATION OF RXRα EXPRESSION IS ESSENTIAL FOR THE DIFFERENTIATION AND PROLIFERATION OF NEUTROPHIL GRANULOCYTES

S. Taschner1, C. Kösters2, W. Ellmeier1, H. Strobl1
1. Medical University Vienna, VIENNA, Austria; 2. Charitee, BERLIN, Germany

Neutrophil granulocytes are short-lived leukocytes that have to be constantly regenerated from myeloid progenitors. Retinoid-X-receptor-α (RXRα) is the predominant RXR protein in myeloid cells. RXRα is able to heterodimerize with other nuclear receptor (NR) family members or form signal-competent homodimers. RXRα partner availability regul-ated by intracellular RXRα abundance is thought to determine NR signaling. However its regulation in primary neutrophil versus monocyte differ-entiation remained uncharacterized. Here we show that myeloid pro-genitors express RXRα protein at sustained high levels during M-CSF-induced monopoiesis. In sharp contrast, RXRα is downregulated during G-CSF-induced late-stage neutrophil differentiation. Ectopic RXRα inhibited G-CSF-dependent cell proliferation of granulocyte progenitors as well as their differentiation to late stage LF+ neutrophils in a serum-free culture model of CD34+ human progenitors. Furthermore, ectopic RXRα was sufficient to redirect G-CSF stimulated progenitors to monocytes. In line with its elevation in monocytes, RXRα failed to inhibit, but rather augmented M-CSF-dependent monocyte generation. Functional genetic interference with RXRα signaling in hematopoietic progenitor/stem cells using a dominant-negative RXRα promoted the generation of late stage granulocytes in vivo and in vitro. Therefore, downregulation of RXRα protein expression is determined by granulocyte versus monocyte cytokine signals.

0320
TRAIL-R3 EXPRESSION ON MYELOID LEUKEMIC BLASTS IS RELATED TO SHORTENED OVERALL SURVIVAL

M.E.D. Chamuleau, L. van Dreunen, A. Zevenbergen, G.J. Ossenkoppele, A.A. van de Loosdrecht
VUMC Medical Center, AMSTERDAM, Netherlands

Background. Since chemotherapy and transplantation can cure only around 35% of patients with acute myeloid leukemia (AML), there is still need for complementary and targeted treatment modalities. One of them could be the use of TNF-related apoptosis-inducing ligand (TRAIL). TRAIL is expressed by effector T cells and induces apoptosis via the death receptor intrinsic pathway. Activation of this pathway in addi-tion to the mitochondrial pathway (by chemotherapy) has synergistic effects in vitro. In human, 4 membrane bound receptors have been identified: two of them (TRAIL-R1 (R1) and TRAIL-R2 (R2)) contain a func-tional death domain and are capable of starting the apoptotic cascade, and two others (TRAIL-R3 (R3) and TRAIL-R4 (R4)) lack a functional death domain and function as decoy receptors. Most normal cells express R3 and R4, where many tumor cells express R1 and R2. This makes soluble recombinant TRAIL an attractive candidate for targeted therapy; phase 1 clinical studies for solid tumors are launched. Until now, sparse data on TRAIL sensitivity of myeloid leukemic cells have demonstrated low TRAIL sensitivity. Aims. Investigation of possible role for TRAIL treatment in AML patients. Methods. We investigated blood and bone marrow samples of 113 patients with AML for TRAIL receptor expression by flow-cytometry. Results were correlated to clinical data. Four myeloid leukemic cell lines with different expression levels of TRAIL receptors were tested for TRAIL sensitivity by treatment with soluble TRAIL. Downregulation of R3 expression was performed by treatment PI-PLC and cyclohexamide. Results. In contrast with published data, we found presumably (pro-apoptotic) R1 and R2 expression (mean percent-age positive cells 16% and 54%, range 0-79% and 0-97% respectively) versus R3 and R4 expression (mean 9% and 10%, range 0-71% an 0- 45%) indicating a TRAIL sensitive profile for myeloid blasts. Surprising-ly, the expression of the anti-apoptotic R3 strongly correlated to sur-vival. Expression of > 25% blasts positive for R3 resulted in shortened overall survival (p=0.0051), see figure 1. In multivariate analysis R3 expression remained a significant prognostic factor next to cytogenetics (p=0.05 and p=0.018 respectively). In vitro studies on myeloid leukemic cell lines confirmed TRAIL sensitivity in cell lines that expressed R1 and R2. Furthermore, simultaneous expression of R3 clearly reduced the amount of apoptosis, suggesting that TRAIL effects are inhibited by binding to R3. Removal of R3 by treatment with PI-PLC resulted in 50% reduction of R3 expression and partially restored TRAIL sensitivity in vitro. Conclusions. Our data suggest that, in contrast to earlier reports, there might be a role for TRAIL in apoptosis induction of AML blasts. R3 expression is a strong predictor for overall survival. In AML cell lines R3 expression resulted in less TRAIL sensitivity and removal of R3 partially restored TRAIL sensitivity. Modulation of R3 might yield additional new therapeutic options for AML patients.

Figure 1.
**0321**

HTLV-1 PROPELS UNTRANSFORMED CD4+ LYMPHOCYTES INTO THE CELL CYCLE WHILE PROTECTING CD8+ CELLS FROM DEATH, AND ESTABLISHES A CD4+ RESTRICTED PRELEUKEMIC PHENOTYPIC

D. Sibon,1 A.S. Gabet,2 M. Zandecki,2 C. Pinatel,2 J. Thete,1 M.H. Delfau-Larue,1 S. Rabaaoui,3 A. Gessain,3 O. Gout,3 S. Jacobson,3 F. Mortreux,1 E. Wattel1

1Centre Lorrain Brard, LYON, France; 2Larc, LYON, France; 3Chu D’Angers, ANGERS, France; 4Chu Henri Mondor, CRETEIL, France; 5Institut Pasteur, PARIS, France; 6National Institutes of Health, BETHESDA, USA

**Background.** HTLV-1 is the etiologic agent of adult T-cell leukemia/lymphoma (ATLL). In vivo, HTLV-1 infects both CD4+ and CD8+ lymphocytes, yet induces ATLL that is regularly of the CD4+ phenotype. **Aims.** To compare infection of CD4+ and CD8+ T-cells by HTLV-1 in vivo and ex vivo in carriers without malignancy, and its implication in genesis of ATLL. **Methods.** In vivo: comparative analysis of proviral loads (real-time quantitative PCR) and clonality pattern (inverse PCR) of highly purified CD4+ and CD8+ infected cells from 10 patients without malignancy. **ex vivo:** comparative analysis of 66 clones (infected versus uninfected / CD4+ versus CD8+) generated by limiting dilution from 4 infected patients. Monoconality was confirmed by analysis of TCR-γ chain gene rearrangements of each clone (multiplex PCR-γ denaturing gradient gel electrophoresis analysis). Studied parameters: cell proliferation (cell count and 3H-thymidine incorporation, with and without interleukine-1), cell cycle (measurement of DNA content by flow cytometry after propidium iodide (PI) staining), apoptosis (flow cytometry after annexin V and PI staining), viral expression (ELISA and real-time quantitative RT-PCR) and cytology.

**Results.** Here we show that, in vivo, infected CD4+ and CD8+ T-cells display similar patterns of clonal expansion in carriers without malignancy. Cloned infected cells from individuals without malignancy had a dramatic increase in spontaneous (without interleukine-2) proliferation, which was achieved by CD8+ lymphocytes and depended on the amount of viral-encoded tax mRNA. In fact, the cloning dependent expansion of HTLV-1 positive CD8+ and CD4+ lymphocytes relied on two distinct mechanisms: proliferation of CD4+ and accumulation of CD8+. This proliferation depended on the level of tax expression. Moreover, infected tax-expressing CD4+ lymphocytes cumulated cellular defects characteristic of genetic instability, multidirectional differentiation, multinucleated and chromatin bridges [image]. **Summary/Conclusions.** HTLV-1 infection establishes a preleukemic phenotype that is restricted to CD4+ infected clones. Finally, our results support that targeting CD4+ cell cycling is of interest in the prevention or treatment of ATLL.

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el of Spred1 in 80 samples collected from CML patients at diagnosis (15 PB and 65 BM), and 9 BM samples from patients in blastic phase (BC). Furthermore, 12 CP patients were evaluated also at the time of the achievement of complete cytogenetic remission. Finally, 36 normal controls (20 PB and 16 BM) were studied. The protein level was analyzed by western blot and immunofluorescence assay. Sequence analysis of the coding and promoter regions was performed. In order to establish the effects induced by the absence of Spred1 on proliferation, we transfected K562 cells with Spred1 plasmid. After transfection colony growth was evaluated in semisolid medium, the proliferation rate was estimated by MTT assay and by the incorporation of 3H thymidine. Results. We found that Spred1 transcript amount is significant reduced in CP CML samples (mean value of 2-ΔΔ Ct = 0,02; range 0,1-0,0002) when compared to normal controls (mean 2,4) with a p value of 0,000002. This difference is even more sound in BC CML cells where Spred1 transcript is 4 logs lower compared to normal controls (2-ΔΔ Ct = 0,9; p=0,00007 compared to diagnosti-
sis) reaching values similar to normal controls (p=0,09). Western blot demonstrated the reduction or the absence of Spred1 protein in CML cells in CP and BC. By contrast, the protein reappeared after the achievement of cytogenetic remission. Sequence analysis allowed to exclude the presence of mutations in Spred1 coding and promoter regions. In order to better understand the mechanism leading to the abrogation of Spred1 we analyzed the factors responsible for Spred1 transcription. We demonstrated that the transcription factor WT1 binds to and activates the promoter region of Spred1. Moreover, we demonstrated a defective transcription activity of WT1 in CML patients due to the absence of one of the isoforms responsible for transcription. K562 cells transfected with Spred1 (K562+) showed a 55% reduction of the proliferation rate com-
pared to untransfected K562 cells (K562-). Moreover a significant reduc-
tion of colony growth was observed in K562+ when compared to K562-
(mean value of 25±7 vs 180±12). Conclusions. This study clearly demon-
strates that the absence of Spred1 protein, a physiological inhibitor of RTK mediated signalling, is a common finding in CML cells and this may support the abnormal proliferation in Bcr-Abl positive cells.

0324 ROLE OF ID AND HES PROTEINS IN ACUTE PROMYELOCYTIC LEUKEMIA
Radboud University Medical Centre, Nijmegen, Netherlands

Acute promyelocytic leukemia (APL) is uniquely sensitive to treatment with all-trans retinoic acid (ATRA), which overcomes the differentia-
tion arrest and induces terminal granulocytic differentiation of the leukemia blasts. In 98% of the cases of APL, the leukemic cells express a promyelocytic leukemia (PML)-retinoic acid receptor (RARα) fusion protein as a result of a t(15;17) chromosome translocation. Previously, we have identified ID1 and ID2 as direct retinoic acid target genes. These proteins act as antagonists of basic helix-loop-helix (bHLH) transcription factors. ATRA induced a rapid, transient increase in ID1 and a sustained upregulation of ID2 both in the APL cell line NB4 as well as in primary leukemia cells from APL patients. To assess the relevance of this upreg-
ulation, ID1 and ID2 were overexpressed in NB4 cells. Overexpression inhibited proliferation and induced a G1/G1 accumulation. These results indicate that ID1 and ID2 are important retinoic acid responsive genes in APL. In addition, we studied another group of antagonists of bHLH transcription factors, the Hairy and Enhancer of split (HES) genes. We identified HES1, which is involved in Notch signalling, and has a very similar biochemical function as the ID-proteins, as a direct ATRA-respon-
sive gene. In NB4 cells and in APL patient cells, ATRA induced a rapid but transient increase in HES1 followed by a sustained downregulation of HES1 expression. In the 5’ upstream promoter we identified a retinoic-
acid response element. Chromatin-immunoprecipitation assays revealed an interaction of PML-RARA with the HES1 promoter, suggesting a role for HES1 during ATRA-induced differentiation of APL cells. Overexpres-
sion of HES1 in APL cells will provide insight into the function of HES1 during APL cell proliferation and differentiation, and apoptosis.

0325 REGULATION OF AUTOPHAGIC PROGRAMMED CELL DEATH BY THE BALANCE BETWEEN CERAMIDE AND SPHINGOSINE-1-PHOSPHATE THROUGH MAMMALIAN TARGET OF RAPAMYCIN (MTOR) IN HUMAN LEUKEMIA HL-60 CELLS
N.D. Domaee
Osaka Dental University, KIRAKATA-CITY, Japan

Background and Aim. The balance between ceramide and sphingosine-
1-phosphate has been suggested to be critical to cell death and survival in the fate of leukemia cells. Autophagy is recognized as one of the important mechanisms in the metabolism of cellular components, and has recently emerged as a caspase-independent programmed cell death (PCD) system different from classic apoptosis. Unlike apoptotic PCD, the role of ceramide and sphingosine-1-phosphate (SIP) in amino acids deprivation (AA(-))-induced autophagic PCD remains unclear. So, in this study, we examined the role of ceramide and SIP on the induc-
tion of autophagy and autophagic PCD. Methods. Human leukemia HL-60 cells were cultured in RPMI 1640 medium containing heat-inacti-
vat ed 10% fetal bovine serum and transferred to AA(-) medium for induc-
tion of autophagy. Autophagy was assessed by the autofluorescent drug monodansylcaldavermine (MDC), electronmicroscopy and cleavage of MAP-LC3 from 18 to 16kD. Apoptosis was judged by nuclear DNA(4',6-
diamidino-2-phenylindole) staining and subsequent cleavage of the apoptosis executioner caspase-3. Auto- and caspase-dependent PCD was assessed by in vitro kinase assay based on the levels of phosphory-
lization of 4E-BP1 and p70S6 with immunoprecipitated mTOR protein. The expression plasmid constructs used were the constructs for constit-
titively activated mTOR kinase and kinase-dead mTOR kinase, which have been previously described. HL-60 cells were transiently transfected by the electroporation method using NucleofectorTM kit (Ama 

0326 THE MECHANISMS UNDERLYING THE CYTOTOXIC EFFECT OF CDK INHIBITOR (ROSCOVITINE) ON LEUKEMIC CELL LINES
H. Song, Z. Hassan
Karolinska Institute, STOCKHOLM, Sweden

Background. Roscovitine is a 2,6,9-trisubstituted aminopurine ana-
logue that compete with ATP for binding to the active site on Cyclin-
dependent kinases (CDKs). It inhibits CDK2/cyclinE, CDK7/cyclinH and CDK9/cyclinT. The cytotoxic effect of roscovitine and its analogues has been reported in several cancer cell lines in vitro and in animal models of cancer xenografts in vivo. The phase II clinical trials in lung and breast can-
cer and phase I trial in glomerulonephritis are currently ongoing. Aim. We have studied the mechanisms of roscovitine-induced cytotoxicity and cell death in leukemic cell lines HL60 (myeloid), Jurkat and K562 cells cultured in RPMI1640 supplemented with 10% FBS. Cells were treated with roscovitine in concentrations of 5 µM, 25 µM and 50 µM up to 48 hours. The cells were examined for viability using trypan blue exclusion assay, proliferation using 3H-thymidine incorporation assay, apoptosis using morphological criteria in Giemsa staining, cell cycle using propidium iodide and flow cytometry. Specific proteins were detected by Western blotting. Results. Cytotoxic effect of roscovitine expressed as decrease in viability and proliferation was concentration- and time dependent in HL60 and Jurkat cells. In contrast, no remarkable effect on K562 cells was observed. Apoptotic morphology was firstly observed 8h after the treat-
mant with roscovitine and markedly increased 6h in HL60 and Jurkat cells, but not in K562 cells. In HL60 and Jurkat cells, the cell cycle analys-
is has shown an increase in sub-G1 cells at 6 hours with maximum at 24h without preceding cell cycle arrest. In K562 cells sub-G1 peak increased subsequently to G2/M arrest. In HL60 cells, cleaved fragment of caspase 2 was found from 6 hours of incubation with Roscovitine.
Activated fragments of caspases 3, 7 and 9 were observed at the same time point. Poly ADP-ribose polymerase (PARP) was cleaved to 89kDa, confirming caspase-3 activation. In the mitochondrial pathway, Bcl-2 was cleaved to 23kDa and release of cytochrome c and AIF were observed. Activated fragment of caspase 8 was observed at 24 hours in Roscovitine 50μM. In Jurkat cells, caspase 2 was cleaved later than in HL60 (24 hours), while caspase 8 at 6 hours. Caspase 3, 7, and 9 were cleaved similarly in HL60 cells. Release of cytochrome c and AIF from mitochondria at 6 hours was detected. However, Bcl-2 was not activated. In K562, no caspase activation was detected at studied time points.

Conclusion. Roscovitine has shown a potent cytotoxic effect in both HL60 and Jurkat cells, whereas K562 has been resistant. Caspase 2 is involved in DNA damage and cytochrome c release in HL60. Apoptosis is induced by caspase 8 activation and mediated by mitochondrial pathway in Jurkat cell line. K562 cells are resistant to Roscovitine that maybe due to Bcr/Abl gene and loss of p53 function.

0327

THE BIOLOGIC SIGNIFICANCE OF CD40/CD40-LIGAND AND FAS/FAS LIGAND INTERACTIONS ON HAEMOPOETIC PROGENITOR CELLS

I. Mavroudi, K. Pyrrovolaki, G. Eliopoulos, H.A. Papadaki
University of Crete School of Medicine, HERAKLION, Greece

Background. Members of the tumor necrosis factor α (TNFα) family and their receptors (TNF- Receptors, TNFR), such as Fas and Fas-Ligand (Fasl), have been implicated in the apoptotic depletion of the CD34+ haemopoietic progenitor cells. The molecules CD40/CD40L also belong to the TNF/TNF family, however, their role in phenotype and/or the pathophysiology of haemopoiesis is entirely under investigation. Aims. To investigate the expression of CD40/CD40L molecules and their biologic significance on the haemopoietic progenitor cells. Methods. The human-derived CD34 positive myelogenous leukemia cell-line KG-1 has been used for this study. The expression of CD40 and Fas molecules on the KG-1 cells was evaluated using flow-cytometry under steady state conditions and following 72-hour incubation with different concentrations of recombinant human TNFα (rhTNFα). To probe the function of CD40 and Fas on the KG-1 cells, we investigated the expression of Fas and CD40, respectively, as well as the FRI and TNFR2 following 72-hour incubation with a combination of rhTNFα and rhCD40L or Fasl, using flow-cytometry and semi-quantitative RT-PCR. The proportion of the apoptotic cells in the above conditions with or without the addition of rhFasL or rhCD40L respectively was studied, by flow-cytometry and the use of 7-aminoactinomycin-D (7-AAD) stain. Results. The KG-1 cells do not express CD40 and Fas under steady state conditions. However, the incubation of these cells with rhTNFα upregulates the expression of the above molecules, in a dose-dependent manner (p<0.05 and p<0.005, respectively). The induction of CD40 on KG-1 cells, following incubation with rhTNFα, and its activation with rhCD40L, upregulates Fas (p<0.05) and TNFR1 (p<0.05) expression, while downregulates the expression of TNFR2 (p>0.05) mRNA as well as protein level. Similarly, the induction of Fas on KG-1 cells, following incubation with rhTNFα, and its activation with rhFasl, induces TNFR(L) (p<0.05) expression, while downregulates the expression of TNFR2 (p<0.05) and CD40 (p<0.05) mRNA as well as protein level. Furthermore, the above induction and activation of CD40 on KG-1 cells, results in a significant increase in the proportion of apoptotic cells (64.2% ± 9.9%) compared to the proportion of apoptotic cells in the presence of rhTNFα alone (29.5% ± 9.9%; p<0.05). The presence of rhFasL increases further the proportion of apoptotic cells (62.4% ± 25.1%, p<0.05). Summary-Conclusions. The TNFR family member CD40 is not expressed under normal conditions on the CD34+ KG-1 cells. Its expression, however, is remarkably induced by rhTNFα. The activation of CD40 induces apoptosis of the cells and this effect is mainly mediated indirectly by up-regulating Fas and TNFR1 and reinforcing therefore the apoptotic effect of the Fas/FasL and TNFα/TNFRI system on KG-1 cells. The interaction of CD40/CD40L with other TNF/TNFR family members may represent a contributing mechanism for the apoptotic depletion of CD34+ haemopoietic progenitor cells characterizing certain TNFα-associated bone marrow failure syndromes.

0328

METHYLATION-ASSOCIATED TRANSCRIPTIONAL SILENCING OF THE C/EBPα GENE IN ACUTE MYELOGENOUS LEUKEMIA

E.J. Jost, n. do O, S. Wilop, J.G. Herman, R. Osieka, O. Galm
‘UK Aachen, AACHEN, Germany; ‘Sidney Kimmel Comprehensive Canc, BALTIMORE, USA

Background. A regulatory network including various transcription factors controls the differentiation of hematopoietic stem cells and progenitor cells. The CCAAT/enhancer binding protein α (C/EBPα) is a transcription factor implicated in the regulation of myelopoiesis that plays an important role in the coordination of cellular differentiation with proliferation. Specific point mutations of C/EBPα have been reported in acute myelogenous leukemia (AML). Mutated forms of C/EBPα may impair granulocytic differentiation and thus contribute to leukemogenesis. Aims. Aberrant CpG island methylation in association with transcriptional silencing has been recognized to act as an alternative to mutations and deletions to disrupt tumor suppressor gene function. A large number of genes involved in fundamental cellular pathways have been shown to be affected by this epigenetic phenomenon. In this study, we investigated the possible role of CpG island hypermethylation in the transcriptional regulation of the C/EBPα gene in AML. Methods. Aberrant methylation of C/EBPα in hematopoietic cell lines and patient samples was assessed by methylation specific PCR (MSP). Methylation patterns in cell lines were further analyzed in detail by bisulfite sequencing. Expression of C/EBPα was determined by real time reverse transcription PCR. Results. In hematopoietic tumor cell lines, aberrant methylation of the C/EBPα promoter region was associated with transcriptional silencing. Treatment of cell lines, which carry a hypermethylated C/EBPα gene, with the demethylating agent 5-aza-2′-deoxycytidine resulted in C/EBPα reexpression. In the cell lines L540 and Raji, bisulfite sequencing of individual alleles revealed dense methylation throughout the region around the transcription start site, while HL-60 cells were almost completely unmethylated. The analysis of diagnostic bone marrow and blood specimens from adult patients with AML by MSP showed aberrant methylation of the C/EBPα promoter region in 12/69 (17.4%) samples. Hypermethylation of C/EBPα in AML could be detect ed in all cytogenetic risk groups, but was restricted to the French-American-British (FAB) subtypes M1, M2, M4 and M5. There was a trend towards a better overall survival in AML cases with C/EBPα hypermethylation. Summary. These data indicate that hypermethylation of the transcription factor C/EBPα is a common epigenetic event in adult AML. Hypermethylation-associated silencing of C/EBPα may, in addition to genetic aberrations, interfere with the cellular differentiation process and thus contribute to the malignant phenotype. The exploration of the growing knowledge about epigenetic aberrations in leukemogenesis may help develop novel strategies in diagnosis and treatment of AML for the future.

0329

ROLE OF SIGNAL TRANSDUCTION PATHWAYS AND THE MICROENVIRONMENT IN THE EMERGENCE OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOGENOUS LEUKEMIA

M.A. van der Pol, W.J. Scholten, B. Moschaver, R. de Boer, N. Feller, G.J. Ossenkoppele, S. Zweegman, G.J. Schuurhuis
VU University Medical Center, AMSTERDAM, Netherlands

Background. Relapse is common in patients with acute myeloid leukemia (AML) due to the emergence and outgrowth of minimal residual disease (MRD). High frequency of flow cytometric (FACS) detected MRD identifies patients with high risk of relapse (Feller et al., Leukemia 22, 3662-3668, 2008). New treatments that can specifically and effectively eradicate these MRD cells in order to improve survival. Aims. Our research focuses on how aberrant signal transduction, e.g. constitutive phospho-AKT (pAKT) and phospho-ERK (pERK) expression and Nuclear Factor kappa B (NFκB) activity, all in interaction with the bone marrow microenvironment (BM-ME) contribute to the emergence, persistence and outgrowth of MRD and thereby effect prognosis of the patient. Methods. A sensitive and reproducible FACS assay was developed for the quantification of phosphorylated protein expression in AML. Results. Good correlations were found between the FACS assay and Western blot and ELISA techniques in both cell lines and patient samples. Specificity of the signals was proven using the inhibitors LY294002, for PISK-dependent AKT phosphorylation, U0126 for MAPK dependent ERK phosphorylation, and MG132 a proteasome inhibitor for NFκB activity. Using a NFκB activity ELISA both a cell line (HL60) and patient samples (n=5) showed NFκB activity that was upregulated by adherence to
fibronectin to stimulate the BM-ME factor 1.6 in HU60 and 1.5 in 3/4 patient samples, with no or less upregulation in non-adherent cells. Response of pAKT, pERK and pNFκB in reaction to fibronectin binding is under investigation using firstly Western Blot but eventually using our FACS assay after optimisation for the BM-ME conditions. Subsequently, the FACS assay was adapted to study AML subsets, in particular stem cells (CD34+CD38- and MRD cells). pAKT, pERK and pNFκB expression could be shown in the CD34+CD38- stem cells. Also the expression of pAKT, pERK and pNFκB in subpopulations with aberrant immunophenotypes (e.g. CD34+CD7+, CD34+CD56+) enables the comparison with signal transduction in MRD cells identified by these aberrancies. Summary/Conclusions: Akt, Erk and NFκB signaling can now be studied in subpopulations highly relevant for clinical outcome, i.e. stem cells, MRD cells and MRD stem cells. We have shown how to detect stem cells under MRD conditions (van Rhenen et al. Blood 2005; 106: 4, abstract van Rhenen et al. this conference). In particular changes of signaling in the course of disease, as may be inferred from reported FLT3 ITD changes from diagnosis to relapse, can suggest the possibility to study signal transduction under MRD conditions. This and the study of the interactions between leukemic cells and the microenvironment contributing to persistence and outgrowth of MRD might ultimately guide the development of new treatment strategies, directed at the MRD (stem) cell and/or the microenvironment.

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**3330**

**TRANSIENT POST-TRANSLATIONAL UPREGULATION OF TELOMERIC ACTIVITY DURING MEGAKARYOCYTIC DIFFERENTIATION**

M. Nakadake
Tokyo Womens Medical University, TOKYO, Japan

**Background.** Telomerase is a ribonucleoprotein reverse transcriptase that adds hexameric repetitive sequences (TTAGGG) to the ends of chromosomes. Telomerase plays a key role in maintaining telomere length and in replicative senescence. Telomerase is active in immature somatic cells and is suppressed in differentiated cells, but the mechanism by which telomerase activity is regulated in relation to cell differentiation remains unclear. Several regulatory mechanisms for telomerase have been reported: (1) transcriptional regulation and (2) translational mechanisms, suggesting that the regulation of telomerase activity is a complex process. Aims. To determine the mechanisms modulating telomerase activity during differentiation in various lineages of hematopoietic cells. Methods. A human chronic myelogenous leukemia cell line (K562) was induced to differentiate into megakaryocytes by exposure to TPA, and into erythroid cells by exposure to STEL1. A human acute myeloblastic leukemia cell line (HL60) was induced to differentiate into monocytes by exposure to TPA. To assess the effect of PKC inhibitors during megakaryocytic differentiation, K562 cells were preincubated with Bisindolylmaleimide or Rottlerin, and then TPA was added. Telomerase activity, the expression of human telomerase reverse transcriptase (hTERT) protein, mRNA, and functional binding transcription factors within the telomerase promoter region were examined. Cells were separated into cytoplasmic and nuclear fractions to examine the localization of telomerase. Results. TPA induced a transient increase of telomerase activity during the megakaryocytic differentiation of K562 cells, while expression of hTERT decreased gradually throughout differentiation. The transient increase of telomerase was mainly observed in the nuclear fraction rather than the cytoplasmic fraction. Pretreatment of K562 cells with a PKC inhibitor blocked both megakaryocytic differentiation and the transient increase of telomerase activity, while a dose-dependent increase of telomerase activity after exposure to recombinant PKC was observed. To further assess the transcriptional control mechanism of telomerase, a chromatin immunoprecipitation (ChIP) assay was performed. STAT3 (which was bound to the hTERT promoter) became dissociated from the promoter during megakaryocytic differentiation, while Sp1 remained stable during differentiation. Conclusions. A transient increase of nuclear telomerase activity was detected during megakaryocytic differentiation stimulated by TPA, and this increase was suppressed by PKC inhibitors. In addition, telomerase activity was dose-dependently increased by recombinant PKC. These results suggest that PKC is one of the post-translational regulators of telomerase activity during the megakaryocytic differentiation of K562 cells. Megakaryocytes are unique hematopoietic cells that undergo DNA replication during differentiation into mature polyploid cells. This may mean that post-translational activation of telomerase is necessary for the immediate stabilization of replicated chromosomes before the formation of de novo synthesized telomeres commences. On the other hand, STAT3 was suggested to be one of the transcription factors regulating telomerase activity during megakaryocytic differentiation. These results indicate that telomerase activity during megakaryocytic differentiation stimulated by TPA is regulated at least by two mechanisms, with one being transcriptional and the other being post-translational.

**3331**

**PAX5/TEL CAUSES DOWN MODULATION OF CD19 IN PRE B CELLS**

G. Fazio, A. Biondi, G. Cazzaniga, A. Rolink
Centro Ricerca Tettamanti, MONZA (MI), Italy; 1University of Basel, BASEL, Switzerland

Background. We previously cloned the PAX5/TEL chimeric gene, originated from the translocation t(9;12)(q11;p13) in an ALL patient. Recent data indicate that PAX5/TEL fusion defines the cytogenetic entity dic(9;12)(p13;13), a recurrent chromosome abnormality that accounts for about 1% of childhood ALL, almost exclusively B-progenitor ALL. PAX5/TEL is likely to be an aberrant transcription factor, resulting from joining the 3′ region of PAX5 (a transcription factor essential for B cell development) to the 3′ region of TEL/ETV6 (Ets-family DNA binding domain). Aim of the study was to investigate the functions of the PAX5/TEL chimeric protein in preB cells. Methods. We have cloned the whole length chimeric PAX5/TEL cDNA in the retroviral vector pMSCV-IRES-GFP (MigR1). Murine PAX5+/– preB cells and wild type preB cells were transduced with the retroviral construct to analyze cell proliferation, differentiation and growth-dependence on IL-7. Both PAX5+/– preB cells and wild type preB cells were cultured on OP9 and DL1-OP9 stroma cells. Results. Wildtype preB cells, transduced with pMSCV- PAX5/TEL-IRES-GFP vector, showed down modulation of CD19 when cultured on OP9 stroma in the presence of IL-7. Semiquantitative RT-PCR didn’t show any difference in transcription of PAX5 target genes such as BLNK, MB-1, M-CSFR. PAX5/TEL-preB cells cultured on DL1-OP9 showed a different phenotype, with up-regulation of c-KIT and down-regulation of CD44, PAX5+/– preB cells infected with PAX5/TEL and grown on OP9 were CD19 negative even in the presence of PAX5/TEL, in absence of IL-7 they died following the same kinetic of the control cells. By semiquantitative RT-PCR, we didn’t detect mRNAs of CD19 and no difference in BLNK, MB-1 and M-CSFR mRNA level was found. On DL1-OP9, PAX5/TEL cells were able to differentiate maintaining the developmental plasticity of the PAX5+/– preB cells. Conclusions. Preliminary results showed a role of PAX5/TEL as a transcriptional suppressor, down regulating CD19 expression, thus suggesting a function on B cell differentiation. PAX5/TEL cannot replace PAX5 functions in PAX5+/– cells. Further analysis are needed to better evaluate the role of the PAX5/TEL protein, both in vivo and in vitro models.

**3332**

**OVEREXPRESSION OF 14-3-3 SIGMA IS ASSOCIATED WITH TYROSINE KINASE ACTIVITY OF P210 BCR-ABL FUSION PROTEIN OF CHRONIC MYELOID LEUKEMIA**

M. Mancini, E. Zuffa, G. Brusa, P. Corrado, E. Barbieri, M.A. Santucci
Istituto di Ematologia Seràgnoli, Bologna, Italy; 1Istituto di Radioterapia L. Galvani, BOLOGNA, Italy

The 14-3-3 proteins are a family of phosphoserine/threonine-binding molecules and critical mediators of intracellular signaling pathways, including those controlling proliferation, cell cycle checkpoints, activation and survival. In particular, upon c-Jun NH2-terminal kinase (JNK)-mediated phosphorylation in response to stress they release c-abl tyrosine kinase and let its nuclear import, the prerequisite for its pro-apoptotic and growth arrest function. Here we show that constitutive tyrosine kinase activity of P210 bcr-abl influences the expression of 14-3-3 sigma (14-3-3σ, one being transcriptional and the other being post-translational). The results indicate that telomerase activity during megakaryocytic differentiation stimulated by TPA is regulated at least by two mechanisms, with one being transcriptional and the other being post-translational.

**Summary/Conclusions.** Akt, Erk and NFκB signaling can now be studied in subpopulations highly relevant for clinical outcome, i.e. stem cells, MRD cells and MRD stem cells. We have shown how to detect stem cells under MRD conditions. This and the study of the interactions between leukemic cells and the microenvironment contributing to persistence and outgrowth of MRD might ultimately guide the development of new treatment strategies, directed at the MRD (stem) cell and/or the microenvironment.

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towards drug resistance. Further studies are presently in progress to eluci-
date the reason for this overexpression and enhanced binding properties, whether they may be targeted by drug combinations that have been advanced for clinical trials.

**0333**

THE INVOLVEMENT OF C:18 CERAMIDE AND HUMAN LONGEVITY ASSURANCE GENES IN IMATINIB INDUCED APOPTOSIS

Y.B. Baran,1 J.B. Bielawski,1 C.E.S. Senkal,1 B.O. Ogretmen,1 U.G. Gunduz1

1Middle east Technical University, ANKARA, Turkey; 2Medical University of South Carolina, CHARLESTON, USA

Background. Ceramides have essential roles in many aspects of cell metabolism, from inflammatory responses through the regulation of cancer-cell growth, cell proliferation, apoptosis, cell migration and senescence. Many cytokines, anticancer drugs and other stress-causing ago-
nists result in increases in endogenous ceramide levels through de novo synthesis and/or the hydrolysis of sphingomyelin. Since human longevity assurance genes (LASS) are responsible for the de novo synthesis of ceramides, the expression levels of LASS genes are important in stress induced apoptosis. Aims. Ceramide metabolism in imatinib induced apoptosis in hematological malignancies was examined in this study. Sensitive and resistant chronic myeloid leukemia (CML) cells, K562 were used as a model system to investigate the changes in ceramide metabol-
ism upon imatinib treatment. Methods. The Ph1 human K562 cells were exposed to step-wise increasing concentrations of imatinib. Subpopula-
tions those were able to grow in the presence of 0.2 and 1 μM imatinib, were then selected, and referred to as K562/IMA-0.2 and K562/IMA1 respectively. Caspase-3 activity was determined using the caspaCELL kit. The mitochondrial membrane potential (MMP) was measured using a JC-1 MMP detection kit. The cellular levels of endogenous ceramides were measured using high performance liq-
uid chromatography/mass spectrometry (LC/MS). Plasmid and siRNA transfection of K562 cells were conducted using an Effectine and Dhar-
maFECTTM siRNA transfection reagent, respectively. Results. Measurement of endogenous ceramide levels by LC/MS showed that treatment with imatinib increased the generation of ceramide, particularly C:18-
ceramide, significantly in a time-dependent manner in parental sensitive cells, whereas in resistant cells, there was no significant changes in its levels in response to imatinib at 48 hr. Partial inhibition of human longevity assurance gene 1 (hLASS1) by small interfering RNA (siRNA), which blocks the generation of partially inhibited imatinib induced cell death, as detected by activation of pro-caspase-3, and loss of mitochondrial membrane potential, in sensitive K562 cells. In reci-
procal experiments, overexpression of hLASS1 caused a marked increase in imatinib-induced C:18-ceramide generation and apoptosis in resistant K562/IMA-0.2 and K562/IMA1 cells. Interestingly, analysis of mRNA levels of hLASS1, for the generation of C:18-ceramide did not show any significant differences in these resistant cells when compared to controls, suggesting that accumulation and/or metabolism, but not rate of synthesis, might be altered in imatinib-resistant cells. Summary/Conclusions. These data suggest that increased ceramide gen-
eration and/or accumulation might be involved in mediating imatinib-
induced apoptosis, and that defects in C:18-ceramide accumulation and/or metabolism might play a role in a decrease in imatinib-induced apoptosis, thus results in resistance to therapy.

**0334**

SURVIVIN AND BCL2 EXPRESSION IN CD30-POSITIVE LYMPHOPROLIFERATIVE DISORDERS OF THE SKIN COMPARED TO SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMAS: AN IMMUNOHISTOCHEMICAL STUDY OF 28 CASES

G. Goteni,1 S. Kupoli,1 D. Stramazzotti,1 F. Fazioli,1 A. Tassetti,1 S. Pulini,1 D. Monchetti,1 P. Leoni,1 L. Lomuzio,1 G. Fabris1

1Institute of Pathology, TORRETTI DI ANCONA, Italy; 2Clinic of Hematol-
ogy, TORRETTI DI ANCONA, Italy; 3Molecular Pathology, TORRETTI DI ANCONA, Italy; 4Polytechnic University of Marche, ANCONA, Italy

Backgrounds. Cutaneous CD30-positive lymphoproliferative disorders (LPDs) are a spectrum of indolent diseases ranging from lymphomatoid papulosis (lyP) to primary cutaneous anaplastic large lymphoma (C-
ALCL). Apoptosis has been investigated in systemic anaplastic large lymphomas (ALCL), which are potentially aggressive, but has not been elucidated in cutaneous CD30-positive LPDs. Aims. We investigated the expression of two inhibitors of apoptosis, survivin and BCL-2 protein, in a series of cutaneous primitive CD30-positive LPDs and systemic ALCL. Results. bcl-2 and survivin expression were evaluated in a series of cutaneous LPDs diagnosed between 1995 and 2003 with the clinical history: 10 cutaneous CD30-positive LPDs (5 lyP, 5 C-ALCL) and 18 system-
ic ALCL. Immunohistochemical analysis was performed with anti-
bodies against ALK1 protein, survivin and BCL-2 protein, on tissue sec-
tions with the DAKO Envision system. RT-PCR studies for ALK and ALK/NPM were performed on RNA extracted from paraffin blocks of all 28 cases. Results. All the cutaneous CD30+ LPDs were negative for ALK by immunostaining and RT-PCR. Among systemic ALCL cases, 7 were ALK negative and 11 ALK positive. The positive cases showed a 366 bp ALK transcript by RT-PCR and the specific NPM/ALK fusion transcript of 98 bp, ruling out the presence of a different rearrangement. All the 28 cases examined showed a clear cytoplasmic positivity for sur-
vivin, independently from their clinicopathological group. Five cases of systemic ALCL, which were all ALK negative, showed in addition a nuclear dot-like immunoreactivity for survivin. Nuclear expression of survivin was not observed in the other groups (chi2: p<0.045). Protein BCL-2 cytoplasmic expression was found in 10 cases; systemic ALK-
positive ALCL show a lower frequency of BCL-2 expression (chi2: p=0.045). Conclusions. Our result showed that lyP and C-ALCL share a heterogeneous expression of cytoplasmic survivin and BCL-2, similarly to systemic ALK-negative cases. Our results suggest that survivin might be expressed also in indolent and potentially regressing lesions and is not an absolute marker of malignancy. Survivin has been indeed demonstrated in many nonneoplastic cells of non-lymphoid nature. BCL-2 was also expressed in half of our cases of lyP and PC-
ALCL, similarly to systemically ALCL, suggesting that either BCL-2 nor cytoplasmic survivin expression does not help in distinguishing in the spectrum of cutaneous CD30-positive LPDs nor between cutaneous and systemic diseases. It might be postulated that apoptosis is still potentially inducible in these BCL-2 and survivin-expressing cells because these lesions have the potential to undergo spontaneous regression. Alterna-
tive mechanisms including the immune control mediated by activated cytotoxic lymphocytes (CTL) can play a major role in these indolent diseases as postulated in systemic disorders. Our data confirm that BCL-
2 is less frequently expressed in ALK-positive than in ALK-negative sys-
temic ALCL cases. The most interesting and unexpected feature was the observation that 45% of our systemic ALK-negative ALCL cases showed nuclear survivin immunostaining, in contrast with others who have found survivin exclusively located in the cytoplasm by immunohisto-
chemistry and by Western blotting.

**0335**

TARGETING IAPS OVERCOMES APOPTOSIS RESISTANCE OF PANCREATIC CARCINOMA CELLS AND SUPPRESSES TUMOR GROWTH AND INVASION IN VIVO

S. Fulda, M. Vogler, K. Duerr, K.M. Debatin

University Children’s Hospital, ULM, Germany

Pancreatic cancer is one of the leading causes of cancer-related death due to its resistance towards conventional therapies. To improve cancer therapy, it is crucial to better understand the molecular mechanisms underlying apoptosis resistance of pancreatic cancer. Here, we identify X-linked inhibitor of apoptosis (XIAP) as a key determinant of apopto-
sis resistance of pancreatic carcinoma cells. XIAP was expressed at high levels in the majority of pancreatic carcinoma cell lines and primary tumor samples. Stable downregulation of XIAP by RNA interference significantly reduced viability and enhanced TRAIL-induced apoptosis in pancreatic carcinoma cells. Importantly, knockdown of XIAP also strongly inhibited clonogenicity of pancreatic cancer cells treated with TRAIL, indicating that XIAP represents a clinically relevant druggable target. Down-regulation of XIAP significantly increased CD95- or γ-irradiation-induced apoptosis, whereas it had no effect on 5-fluorouracil, etoposide or gemcitabine-induced apoptosis. Analysis of apoptosis signaling pathways revealed that knockdown of XIAP resulted in enhanced activation and enzymatic activity of caspase-3, -9, -2 and -8. Similarly, downregulation of XIAP also led to enhanced drop of mitochondrial membrane potential and increased cytochrome c release after stimulation with TRAIL, indicating that XIAP functions upstream of mitochondria in TRAIL-induced apoptosis. In support of this notion, inhibition of caspase-3 completely inhibited drop of mitochondrial membrane potential in TRAIL-treated pancreatic carcinoma cells. XIAP knockdown was knocked down. Most importantly, knockdown of XIAP profoundly inhibited tumor growth and invasion of pancreatic carcinoma cells in vivo. Similarly, inhibition of XIAP by small molecule antagonists sensi-
ized pancreatic cancer cells to TRAIL-, CD95- or γ-irradiation-induced apoptosis. By demonstrating that targeting IAPs significantly enhanced the effects of apoptosis in human K562 and Meg-01 cells. The effects of GCS on the intracellular activation of caspases and the colorimetric caspase activation assay kits. C2-ceramide treatment showed fluorescence and colorimetric caspases activation of 5-FU marrow cells, which increased in intensity within one hour. However, selective inhibitors of caspase 8 and 9, -3, and -6, respectively, in that order. Upstream of the caspases, C2-ceramide activated p58 and the selective p8 inhibitor SB203580, thus reversed the activation of these caspases that had been induced by C2-ceramide, resulting in a significant recovery from apoptosis. On the other hand, IETD-cleaving caspase such as caspase-8 was not activated by C2-ceramide. These results suggest that C2-ceramide initiates apoptosis in B-CLL via activation of the caspase-9-dependent caspase cascade mediated by p58.

0338
CERAMIDE GLYCOSYLATION BY GLUCOSYL CERAMIDE SYNTHASE INHIBITS THE APOTOTIC EFFECT OF IMATINIB ON HUMAN K562 AND MEG-01 CELLS
Y.B. Baran, 1 J.B. Bielawski, 2 C.E.S. Senkal, 3 B.O. Ogerehin, 4 U.G. Gunduz 5
1 Middle east Technical University, ANKARA, Turkey; 2 Medical University of South Carolina, CHARLESTON, USA; 3 Middle East Technical University, ANKARA, Turkey.

Background: Glucosyl ceramide (Glc-Cer) has recently been shown to be associated with resistance to chemotherapy. Activation of glucosylceramide synthase (GCS), the enzyme responsible for the conversion of ceramide to Glc-Cer, inactivates the p38 MAP kinase pathway, thereby contributing to drug resistance. Accumulation of glucosylceramide in tumor cells, in which glucosylceramide synthase is activated, has been shown to correlate with clinical response to chemotherapy.

Summary/Conclusions. The findings indicate that targeting IAPs represents a novel, promising strategy to overcome apoptosis resistance of pancreatic cancer, which has important clinical implications.

0339
P38-MEDIATED ACTIVATION OF CAPSASES IN C2-CERAMIDE-INDUCED APOTOPSIS OF MOUSE HEMATOPOIETIC CELLS
S. Ota, 1 M. Musashi, 1 N. Toyoshima, 1 K. Kondo, 1 J. Sugita, 1 T. Toubai, 1 N. Kato, 1 J. Tanaka, 1 K. Kimura, 1 M. Imamura, 1 M. Asaka 1
1 Hokkaido University, SAPPORO, Japan; 2 Akiu Hospital, SAPPORO, Japan.

Cell-permeable C2-ceramide induces apoptosis in various types of cells. Here, we have studied the effects of C2-ceramide on mouse bone marrow cells containing primitive hematopoietic progenitors (PHPs) and primitive hematopoietic progenitors (PHPs) little is known about the signaling pathways in the apoptosis induced by C2-ceramide. The C2-ceramide was found to induce apoptosis in a time dependent manner, as defined morphologically by nuclear condensation and fragmentation visualized with propidium iodide staining and by a positive annexin V (AV) and by a reduced colony forming ability of the bone marrow cells of mice. C2-ceramide suppressed colony growth derived from mouse Day-2 post 5-FU marrow cells (5-FU marrow cells) in a dose dependent manner. Incubation of 5-FU marrow cells with 15 µM of C2-ceramide for three hours gave 41.7±17.2% of mean % control of colonies. Further, an extended study using more PHP-enriched lineage marker-negative cells (Lin- cells), which were isolated from mononuclear 5-FU marrow cells, revealed that C2-ceramide completely suppressed colony formation. To obtain direct evidence of induction of apoptosis in Lin- cells, we detected that about 90% of AV- cells were changed into AV+ cells in Lin- cells by incubation with C2-ceramide, suggesting PHP activation. Furthermore, next, we studied the effects of C2-ceramide on the intracellular activation of caspases in PHP, using the cell-permeable fluorescence-labeled substrates of several caspases and the colorimetric caspase assay kits. C2-ceramide treatment showed fluorescence and colorimetric caspases activation of 5-FU marrow cells, which increased in intensity within one hour. However, selective inhibitors of caspase 8 and 9, -3, and -6, respectively, in that order. Upstream of the caspases, C2-ceramide activated p58 and the selective p58 inhibitor SB203580, thus reversed the activation of these caspases that had been induced by C2-ceramide, resulting in a significant recovery from apoptosis. On the other hand, IETD-cleaving caspase such as caspase-8 was not activated by C2-ceramide. These results suggest that C2-ceramide initiates apoptosis in B-CLL via activation of the caspase-9-dependent caspase cascade mediated by p58.
cification of PDMP and/or C9DGJ increased the sensitivity of CML cells to imatinib.

0339
NOSCAPINE INDUCES APOPTOSIS THROUGH ACTIVATION OF CASPASES AND MITOCHONDRIAL EVENTS IN P53-NULL MYELOBLASTIC LEUKEMIA CELL LINE K562
B.G. Goliaeii, 1 N.H. Heidari, 1 M.M. Mahmoudian, 1 P.R. Rahimi 2
1 University of Tehran, TEHRAN, Iran; 2 Iran University of Medical Sciences, TEHRAN, Iran

Monitoring apoptosis is becoming increasingly important in finding new chemotherapeutic drug and their mechanism. Previously, the microtubule opium alkaloid noscapine was discovered as a microtubule destabilizing agent that arrests mammalian cells at mitosis and induces apoptosis. Because noscapine is water-soluble and absorbed after oral administration, and has little toxicity to normal tissue and no inhibition of immune responses, its chemotherapeutic potential in human cancer merits through evaluation. We selected drug resistant, P53-null myelogenous leukemia cells to monitor apoptosis and study of noscapine's mechanism. K562 cells showed delayed but effective response to noscapine treatment, and we could monitor apoptosis by the DNA fragmentation, PARP cleavage and increasing activity of caspase 2,3,6,9 with 20 µM noscapine after 24-48hr treatment. The increased Bax/Bcl-2 ratio more than three times with 20 µM noscapine in time-dependent manner from 3-48 hr can prove some mitochondrial event in response to this drug. These results help to elucidate some critical points in noscapine mechanism as a good candidate for preventive and therapeutic application in chronic myeloid leukemia.

0340
ERYTHROID-SPECIFIC TRANSCRIPTIONAL REGULATION OF THE HUMAN PROTOPHRYMINOGEN OXIDASE GENE IS MEDITATED BY TWO GATA-1 SITES IN EXON 1
University Medical Center Utrecht, UTRECHT, Netherlands

Background. Protoporphyrinogen oxidase (PPOX) catalyzes the six-electron oxidation of protoporphyrin IX to protoporphyrin IX. Like other heme biosynthetic proteins, PPOX is involved in synthesizing heme for red cells (erythroid-specific expression) and heme as a cofactor for the respiratory cytochromes (housekeeping expression). Whereas tissue-specific regulation of other heme biosynthetic enzymes is extensively studied, there is little knowledge concerning transcriptional regulation of PPOX. Aims. The aim of this study was to investigate molecular mechanisms involved in the erythroid-specific regulation of PPOX. Methods. Functional studies were performed using transient transfection of PPOX promoter constructs in human K562 erythroleukemia cells. DNA-protein interaction at the GATA-1 sites in exon 1 of PPOX was studied using Electrophoretic Mobility Shift Assay's (EMSA) with K562 cells. Results. In vitro transfections studies revealed that reporter constructs containing exon 1 showed a 300% increase in promoter activity compared to constructs lacking this exon. Transfection experiments of wild-type and mutant reporter plasmids in K562 cells demonstrated that erythroid-specific transcriptional regulation of PPOX was mediated by two GATA-1 sites in exon 1. The highest level of transcription depended on the integrity of both sites. Electrophoretic mobility shift assay and supershift experiments using K562 nuclear extracts demonstrated that both GATA sites were able to bind GATA-1 in vitro. Exon 1 did not have any effect on PPOX promoter activity in human hepatoma HepG2 cells. In HeLa human cervical carcinoma cells, however, the presence of exon 1 decreased promoter activity. Summary/Conclusions. Exon 1 of the human PPOX gene contains two GATA-1 binding motifs, which both are required for erythroid-specific expression of PPOX and, in addition, bind GATA-1 in vitro. These results contribute to a better understanding of the molecular mechanisms involved in differential regulation of the human PPOX promoter in erythroid and non-erythroid cells.
CLINICAL AND PROGNOSTIC SIGNIFICANCE OF PS3 GENE MUTATION IN ACUTE LEUKEMIA

M. Awad, Y. Al-Tonbary, O. El-Agrody, S. El-Sharawy, D. El-Ghanam

'Mansoura University Faculty of Medicine, MANSOURA, Egypt; Pediatric Hospital, MANSOURA, Egypt; Mansoura Faculty of Medicine, MANSOURA, Egypt

Back ground and aim of the work: PS3 is a tumor suppressor gene, located at the chromosomal region 17p 13, consisting of 10 introns and 11 exons, of which exons 2 to 11 are transcribed. 3- Wild type (wt) PS3 acts as tumor suppressor protein whereas mutant (mut) PS3 may exhibit gain of function properties such as immortalization of primary tumor cells. The PS3 protein plays a crucial role in maintaining genetic stability at the cellular level. This work was planned aiming to recognize the potential role of PS3 in leukemogenesis and explain the correlation between mutations of PS3 in acute leukemic patients and clinical subtypes, clinical behavior and prognosis of the disease. Subject and methods: This study was carried on 38 patients with acute leukemia, twenty eight of them were newly diagnosed patients and ten patients were at relapse. Accordingly they were categorized into 3 groups: Group I (at diagnosis): this group included 26 newly diagnosed patients with acute leukemia. They were classified into 15 cases with ALL and 13 cases with AML. Group II (after induction): this group included 28 patients who were followed up after induction for 1, 6 and 12 months. Group III: This group included 10 relapsed patients with acute leukemia (sampling taken once at relapse). They were classified into 5 cases with ALL and 5 cases with AML. In additions 10 subject were selected as control group. Patients and controls were subjected to the following laboratory investigation: Complete blood picture, bone marrow aspiration smears, liver function tests and s. creatinine, s. uric acid and serum L.D.H. Bone marrow aspiration smears, cytochemistry stains on blood or bone marrow smears were helpful in distinguishing AML from ALL and in subclassifying AML. assay of mutant PS3 protein by immunophenotyping techniques. Detection of p53 gene mutation by PCR-SSCP and Sequencing techniques (ABI 310 genetic analyzer, Perkin Elmer). assay of mutant PS3 protein using FTC-conjugated monoclonal mouse antibody (human p53 protein clone DO-7 Code No. 7054 Lot 050. Edition 15.06.00).

RESULTS: In this study we found that the incidence of p53 mutations were higher in ALL patients (13.3%) than AML patients (7.6%). Cases who showed p53 mutations at diagnosis were among cases who resisted chemotherapy (3 cases out of cases), they had exon 5 and 6 mutations. PS3 mutations showed higher incidence in relapsed AML and ALL patients (20% and 60% respectively). Exon 8 mutations were the most frequent type of mutation, affecting mainly relapsed AML and ALL. Followed by exon 6 and exon 7 mutations, that were restricted to relapsed AML cases only. Conclusion: PS3 mutations were present in de novo acute leukemia. Leukemia higher incidence of p53 mutations were in aggressive and relapsed leukemia ALL was associated with higher incidence of p53 mutations than AML. The p53 mutations-bearing patients did not differ in their clinical and laboratory data from those without mutations. Mutations of exon 8 were found in high frequency in relapsed acute leukemia (ALL and AML). Mutations of exon 5 were found in acute leukemia patients with poor response to chemotherapy. Mutations of exon 6 were found in patients with poor response to chemotherapy and in relapsed ALL. Mutations of exon 7 were found in relapsed ALL. Mutations of p53 usually affect both alleles with positive LOH.

MATURATION OF NK CELL PHENOTYPE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION


'Silesian Medical University, KATOWICE, Poland; Ludwik Hirszfeld Institute of Immunology, WROCŁAW, Poland

Background: NK cells play a role in anti-infectious and anti-leukemic reactions after allogeneic hematopoietic cell transplantation (alloHCT), which is related to the expression of various stimulatory and inhibitory receptors. Although quantitative reconstitution of NK cells after alloHCT is fast, their phenotype pattern is a cause of non-relapse mortality (0% vs. 28%, respectively). In a univariate analysis the effect was independent for both above factors. No other factor including the genotype of donors and recipients was studied before transplantation. Methods: In the present study we used antibodies specific for KIR2DL1/S1, KIR2DL2/3/5/52, KIR3DL1, and NKp2A. Individual pattern was created for each patient and his donor based on frequencies of NK cells expressing respective receptors together with median fluorescence intensity (MFI). To quantify the differences between donor and recipient NK cell phenotype pattern at various time-points after alloHCT, we developed a new discrepancy index based on sum of differences defined as distances in coordinate system constructed by frequencies and MFI axes normalized by standard deviations. Results: Discrepancy index equaled 8.6 (±2.5) on day +28 and dropped to 8.2 (±2.7) (p=NS) on day +56, 7.0 (±3.0) (p=0.03, vs. baseline) on day +100, and 5.9 (±2.1) (p=0.0002, vs. baseline) on day +180. On days +28, +56, and +100 the index was higher for patients given transplant from unrelated donors compared to sibling alloHCT (p=0.03, p=0.05, and p=0.02, respectively). On day +100, values of the discrepancy index were increased in case of HLA-C incompatibility (p=0.005) as well as for patients who had experienced acute GVHD grade II-IV (p=0.02). In a multivariate analysis the effect was independent for both above factors. No other factor including the genotype of KIRs and KIR ligands, nor other patient-, donor-, and procedure-related variables was found to influence significantly NK cell phenotype pattern after alloHCT. The probability of the overall survival at 10 months was higher for patients with the discrepancy index ≤8.27 (median) vs. >8.27 on day +56 (100% vs. 63%, p=0.02). The discrepancy resulted mainly from the incidence of fatal infections, which occurred after engraftment and were a cause of non-relapse mortality (0% vs. 28%, respectively, p=0.05). Conclusions: The pattern of NK cell phenotypic reconstitution after alloHCT generally tends to recapitulate the donor-type, but is negatively affected by the incidence of acute GVHD and HLA-C incompatibility. In turn, delayed maturation seems to be associated with impaired anti-infectious defense translating into decreased survival. The proposed discrepancy index appears feasible to quantify the differences between the donor and recipient NK cell phenotype pattern.
published results. Results. Acute lethal X-GVHD generally occurs with-1
in 3 weeks after iv transfer of fresh huPBMC (15x106 CD3+ cells) into RAC2-/-γc-/- mice. X-GVHD occurred in 99% of 68 mice, including 87% acute lethal X-GVHD and 12% chronic X-GVHD. For 14 different donors, no significant difference between donors was observed with regard to development of acute X-GVHD. Acute X-GVHD could be effectively prevented by FK506 sc at day 0 resulting in 100% survival of mice. FK506 administered later time points after treatment with prednisolone iv or OKT3 sc did not abrogate acute X-GVHD nor did IL-2 ip exacerbate acute X-GVHD. Further analysis showed the overall impact of ex vivo culture on development of X-GVHD was significant for fresh huPBMC in dose-1-dependent of 86 mice (p<0.8, p<0.008) and for cultured huPBMC in 130 mice (p=0.8, p<0.007). In contrast to fresh huPBMC, only 44% of mice developed X-GVHD after injection of huPBMC (15x106 CD3+ cells) that were cultured and stimulated with OKT3, including 25% acute lethal X-GVHD and 19% chronic X-GVHD. Strik-ingly, this was different for CD3/28 costimulated huPBMC, of which 58% developed X-GVHD, including only 10% acute lethal X-GVHD and 48% chronic X-GVHD. These results suggest that CD3/28 costim-ulation of huPBMC stimulates the development of chronic X-GVHD. We speculate that a more efficient activation by CD28-ligation leads to an increase in in vivo survival and proliferation of human T cells that permits the development of chronic X-GVHD. Conclusion. The huPBMC-RA(C) xenogeneic transplant model can be considered a xenogeneic T-cell model that shows a high sensitivity to model to date for evaluation of human T cells in vivo and will be a valuable addition to current allogeneic murine T cell models. Future studies will involve further exploration of the influence of CD3/28 cos-timulation on development of chronic X-GVHD.

0346
IMATINIB IMPAIRS PROLIFERATION AND FUNCTION OF CD8+ T LYMPHOCYTES SPECIFICALLY DIRECTED AGAINST THE LEUKEMIA-ASSOCIATED ANTIGEN RHAMM/CD168
J. Chen,1 A. Schmitt,2 B. Chen,3 M. Ringhofer,1 S. Von Harold,4 J. Greiner,1 H. Döhner,2 D. Bunjes,3 M. Schmitt
1University of Ulm, ULM, Germany; 2Medical School of Southeast University, NONJING, China
Background. The competitive Bcl-Ab1 tyrosine kinase inhibitor imatinib (STI571, Gleevec) is highly effective in the treatment of patients with chronic myelogenous leukemia (CML), and is increasingly used in patients with residual disease or relapse after allogeneic stem cell transplantation (allo-SCT) setting. Aims. Since the graft-versus-leukemia (GVL) effect after allo-SCT depends on T-lymphocytes and an impairment of anti-viral CD8+ T lymphocyte function has been described, we ques-tioned whether imatinib also affects anti-leukemic CD8+ T lymphocytes. Methods. After eight days of mixed lymphocyte peptide culture (MLPC), we assessed CD8+ T cells from the peripheral blood of healthy volunteers and patients with CML by tetramer staining, multi-color flow cytometry and enzyme linked absorbent spot (ELISPOT) assays. Results. The release of interferon-γ and granzyme B by CD8+ T lymphocytes specific for the RHAMM/CD168 was inhibited by imatinib in a dose-dependent fashion. This inhibitory effect could not be ascribed to an increased rate of apoptosis. The inhibition of CD8+ T lymphocyte func-tion was reversible after removal of imatinib from the MLPC after day four. Moreover, administration of imatinib to patients with CML decreased the functional activation of CD8+ T lymphocytes in vivo compared with the T cells of the same patients after cessation of imatinib. Conclusion. Due to the light of these findings, administration of high-dose imat-inib might result in the reduction of efficacy of GVL or T cell based ther-apies.

0347
BORTEZOMIB INDUCES APOPTOSIS AND MODIFIES THE MATURATION PATTERN OF DENDRITIC CELLS: ROLE IN THE INDUCTION OF IMMUNOTOLERANCE AFTER ALLOGENEIC TRANSPLANTATION
J.A. Pérez-Simón,1 I. Sánchez-Abarca Bernal,1 B. Blanco Durango,1 S. Tavares,1 M. Diez-Campello,2 M. Alberca,3 M. Sánchez-Guijo,1 N. López-Holgado,1 E. Villarín,1 C. del Cañizo,1 J. San Miguel1
1Hospital Universitario, SALAMANCA, Spain; 2Centro de Investigación del Cáncer, SALAMANCA, Spain
Background. The NF-kB family has emerged as a key transducer of inflammatory signals involved in dendritic cell (DC) maturation. Accord-ingly, stimulation of Toll-like receptors (TLR) by ligands such as lipopolysaccharide (LPS) or CpG-containing DNA induce DCs matura-tion through NF-kB activation. Thus, the proinflammatory milieu gener-ated in the context of transplantation favours DCs maturation and increases their T cell priming ability, so that DCs play a key role in the development of GVHD. Bortezomib is a potent, selective and reversible inhibitor of proteasome that blocks the nuclear translocation and tran-scription of NF-kB. Aims. To determine whether imatinib also affects anti-leukemic CD8+ T-lymphocytes and on the cytokine pattern of DCs. Methods. In the present study we have ana-lyzed the effect of bortezomib in both DCs' viability and maturation as well as the ability of bortezomib-treated DCs to generate a tolerogene-ic T-cell response. Results. Bortezomib showed a detrimental effect on DCs viability at 80nM and it was specially evident in cases cultured in the presence of TNFa and LPS while cell viability was not significantly affected in cultures performed without TNFa and LPS, indicating that bortezomib induced apoptosis of DCs in conditions which induce fully DCs activation. We next tested the effect of bortezomib on the expression of costimulatory molecules and on the cytokine pattern of DCs. Interestingly, the addition of bortezomib decreased the expres-sion of CD86 both in un-stimulated or fully activated (TNFa and LPS treated) DCs while the expression of CD80, CD40 and HLA-DR was also modified at 10nM of the drug. Concerning the cytokine pattern, the intracellular expression of IL-12 significantly decreased at a concentration of 10nM of the drug. In addition, we evaluated the effect of the DCs
cured with or without bortezomib at 10 nM in the activation pattern and cytokine profile of T-cells after mixed lymphocyte cultures (MLRs) and we found that, among MLRs performed using un-stimulated T lymphocytes were activated as compared to 43% (95% CI = 11.5-88) among T cells co-cultured with DCs fully activated with TNFα and LPS (p=0.02); by contrast, the percentage of activated DCs were 21% (95% CI = 6-42) vs 82% (95% CI = 16-48) (p=0.04) when they were co-cultured with bortezomib-treated DCs non-activated vs fully activated with TNFα and LPS, respectively. This results indicated that bortezomib-treated DCs were unable to properly stimulate T cells even after exposure to a proinflammatory milieu. Moreover, T lymphocytes previously cultured with bortezomib-treated DCs were unable to become activated when they were further stimulated with fully activated untreated DCs from the same donor, indicating that T lymphocytes exposed to bortezomib-treat ed DCs become tolerant to the antigens presented by DCs. Conclusion. Bortezomib affects viability and modifies the maturation and cytokine pattern of DCs. The latter effect results in an impaired capability to induce allogeneic T cell stimulation and generates a tolerogenic response of T cells cultured with bortezomib-treated DCs. These results suggest a potential role for the in vivo use of the drug prior to allogeneic transplantation through its effect on host DCs and/or for the in vitro generation of tolerogenic DCs.

### 0349 STEM CELL FACTOR ENHANCES T-CELL RECOVERY AND THYMOPYEOSIS FOLLOWING EXPERIMENTAL BMT

E.J. Wils, 1 J.C. Rombouts, 2 E. de Haas, 1 H. Spits, 1 B. Löwenberg, 1 E. Braakman, 1 J.J. Cornelissen 1
1ErasmusMC, ROTTERDAM, Netherlands; 2Academic Medical Center, AMSTERDAM, Netherlands

Deficient thymopoiesis and retarded recovery of newly developed naïve CD4+ T-cells is one of the most important barriers of impaired immune competence following hematopoietic stem cell transplantation (HSCT). Recently, we showed that Fms-like tyrosine kinase-ligand 3 (FL) accelerates T-cell recovery following experimental bone marrow transplantation (BMT) via expansion of bone marrow (BM) lymphoid progenitors prior to recovery of thymopoiesis. Several studies have suggested an important role for stem cell factor (SCF)-β in interactions in T-cell development, but is unclear at which level SCF primarily affects T-cell development. Here we evaluated whether SCF would affect T-cell recovery, thymopoiesis and BM lymphoid progenitor cell numbers following experimental BMT. Three C57BL/6 Rag-1−/− mice (C57Bl/6 Ly5.2 background) were used as recipients of T-cell depleted congenic bone marrow cells (4×105 C57Bl/6 Ly5.1 origin). Mice were treated with PBS, SCF (100 µg/kg s.c.), FL (800 µg/kg) or SCF combined with FL 3 times weekly for 4 weeks following BMT (n=6/group). Peripheral blood (PB), splenic and thymic lymphocyte subsets and BM lymphoid progenitors (LSKflt3−, LSKflt3+, common lymphoid progenitors (CLP)) were quantified using FACS-analysis at day 28 post-BMT. SCF- or FL-treated mice showed higher numbers of both PB and splenic T-cells as compared to PBS-treated control mice (PBS vs. SCF (mean absolute T-cell numbers ×103/spleen±SEM): 0.3±0.1 vs. 4.2±2.8; p=0.02). No additive or synergistic effect was observed in mice treated with both SCF and FL. In contrast to FL, SCF did not increase peripheral B-, NK and dendritic cell numbers. SCF- or FL-treated mice showed an increase in thymic cellularity (PBS vs. SCF (mean absolute cell numbers ×103/thymus±SEM): 6.2±0.2 vs. 11.4±4.1; p=0.46), numbers of donor-derived thymocytes (0.13±0.08 ×106 vs. 7.2±4.5; p=0.06) and numbers of all thymocyte subsets, including DN (0.5 ×104/thymus±1.04±z; p=0.07), DP (10.7±7 vs. 4731±316; p=0.01), CD4SP (1.5±0.7 vs. 16±8; p=0.08) and CD8SP (0.5±0.1 vs. 69±32; p=0.04). In addition a trend towards increased percentages and absolute numbers of BM LSKflt3+ was observed in SCF-treated mice (PBS vs. SCF (mean absolute BM LSKflt3+ cell numbers ×103/106mum±SEM): 8±4 vs. 35±24; p=0.12). These data show that SCF or FL may enhance T-cell recovery and thymopoiesis. However, in contrast to FL, SCF did not increase other peripheral lymphoid cell lineages, but selectively promoted T-cell recovery. Collectively these data suggest, that SCF may enhance T-cell recovery by improvement of thymopoiesis and possibly also by expansion of lymphoid progenitors after BMT. These results may provide a rationale for clinical application in recipients of HSCT with a retarded T-cell recovery mainly due to transplantation of limited numbers of progenitor cells such as may occur in cord blood transplantation.

### 0349 IS BODY-WEIGHT-BASED CALCULATION OF IV BUSULFAN FIXED DOSE THE APPROPRIATE DOSAGE OF BUSULFAN IN CHILDREN UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

C. Vassal, 1 H. Esprou, 2 J.C. Gentet, 2 F. Doz, 1 D. Valteau-Couanet, 1 B. Neven, 1 C. Galambrun, 1 F. Mchnaude 2, C. Paillard, 3 H. Zouabi, 1 L. Nguyen, 1 G. Michel 1
1Institut Gustave Roussy; VILLEJUIF, France; 2Hopital Saint Louis, PARIS, France; 3Hopital La Timone, MARSEILLE, France; 2Institut Curie, PARIS, France; 1Hopital Necker-Enfants Malades, PARIS, France; 3Hopital Debroux, LON, France; 2Hotel Dieu, NANTES, France; 1Institut de Recherche Pierre Fabre, BOULOGNE BILLANCOURT, France

Background. High-dose oral busulfan (Bu) is often included in conditioning/preparative regimens prior to autologous (auto-) or allogeneic (allo-) transplantation (T). Studies have reported that pharmacokinetics (PKs) of Bu in HSCT are age-dependent with underexposition of children received the usual dosage 16 mg/kg over 4 days. Bu clearance (Cl) is highly variable in children and increased in the youngest. Thus age-based dosing and therapeutic drug monitoring (TDM) with dose adjustment are needed to target an area-under-the-curve-plasma-concentrations (AUC) equivalent to adults. An IVBu form was developed. In a first study (US trial), 24 children received an IVBu age-based dosing with TDM equivalent to the oral (1.0 mg/kg ≤4y and 0.8 mg/kg >4y) (Wall D., ASH 2000, #206). A retrospective analysis suggested that the dose of Bu should rather be calculated on the basis of the body-weight (BW) (Nguyen L et al BMT 2004). To validate the new dose-regimen a prospective study was conducted. Aims. To prospectively validate a new body-weight-based fixed dose of IVBu in children in its ability to target an AUC within a predefined therapeutic window for more than 75% of patients without any therapeutic drug monitoring. Methods. PKs of IVBU administered at the defined dosage were studied in children who received either IVBu/Melphalan or IVBu/Cyclophosphamide prior to auto- or allo-T, respectively. IV Bu (16 doses) were administered over 2 h at 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg, and 0.8 mg/kg for patients (pts) with <9 kg, 9 to <16 kg, 16-23 kg, >23-34 kg, and >34 kg strata of body weight, respectively. PK was performed at doses 1, 9 and 13 but no dose adjustment was allowed. Bayesian Bu AUCs were calculated. Results. Preliminary results are available in 55 pts, median age 6y (0.3-17.2), including 20 pts ≤4 y. A significantly better AUC targeting (900-1350 µM.min) was achieved with the new fixed dose as compared to the usual age-based dosing (76% vs. 54%, p=0.01). Moreover, there is no longer a significant difference in systemic exposure (mean ±sd.: 1248±205 µM.min) between children treated with this new dosage and adults given 12.8 mg/kg of IV Bu, although significant differences (p=0.001) on Bu Cl were observed among weight groups. AUC inter-patient variability was 2.5 fold reduced (CV ≤20%). AUC intra-patient variability (CV ≤10%) enabled reproducible exposure without the use of TDM in children. All AUCs were > 847 µM.min, and 84% < 1500 µM.min which may explain the high rate of engraftment (no graft rejection), and the low incidence of veino-occlusive disease of the liver (4/27 auto-T, 2/28 allo-T) that previously correlated with low and high Bu exposure, respectively. Conclusions. We conclude that BW-based IVBu dosage regimen enabled reproducible AUCs (without TDM) throughout the treatment period as compared to age-based dosing and oral Bu with PK monitoring. Based on this prospective study, the defined IVBUs doses according to BW are recommended in children.

### 0350 TRANSPLANTATION OF HUMAN PERIPHERAL BLOOD CD34-POSITIVE CELLS IN COMBINATION WITH EX VIVO EXPANDED MEGAKARYOCYTES IN NOD/SCID MICE

M.R. Tijsjen, 1 P.B. van Hennik, 1 F. di Summa, 1 P.L. Hordijk, 1 J.J. Zwaginga, 1 C.E. van der Schoot, 1 C. Voermans 1
1Sanquin Research, AMSTERDAM, Netherlands; 2Sanquin Research / AMC, AMSTERDAM / LEIDEN, Netherlands; 3Sanquin Research / LUMC, AMSTERDAM, Netherlands

The period of severe thrombocytopenia after high dose chemotherapy may be shortened by increasing the number of megakaryocytes (Mks), CD34+ cells in the recipient. One limitation of ex vivo expanded Mks to the graft might suit this purpose. Our aim is to study the fate, engraftment ability and platelet production capacity of Mks expanded from mobilized peripheral blood (MPB) stem cells in a murine xenotransplantation model for human hematopoiesis, the NOD/SCID mouse. In pilot experiments, we established that the detection threshold
of human platelets in mouse blood is 1×10^9/mL and that the injection of 0.5 pg (human) Tpo right after transplantation of unmanipulated MPB stem cells does not affect the numbers of human blood platelets or the percentage of human hematopoietic cells in the mouse bone marrow (BM). Next, MPB CD34+ cells were cultured for 7 days in the presence of Tpo (100 ng/mL) and IL-1B (10 ng/mL). An expansion of approximately 6-fold was observed after 7 days of culture. Over 50% of the expanded cells expressed CD41, but the numbers of CD34 expressing cells were detected. After sublethal irradiation, NOD/SCID mice were transplanted with unmanipulated CD34+ cells (group A), unmanipulated cells combined with ex vivo generated MKs (group B, C, D), or ex vivo generated MKs only (group E)(see Table 1 for the dosing scheme). As control, the mice of group F did not receive any cells after irradiation. Blood was collected at day 3, 7, 10, 14, 21 and 28 after transplantation. Already after three days human platelets could be detected in the blood of the mice that received the highest number of cultured cells (group C and E). After 7 days, human platelets were detected in the blood of the mice from all groups, except the mice of group A, which received only uncultured cells. In the mice of the groups A, B, C, and D platelet numbers increased till day 14 (to an average of 6.9×10^7/mLblood) with a small decrease towards day 21 (5.9×10^7/mL) and day 28 (4.5×10^7/mL). The mice of group E reached a maximum of 3.4×10^7 human platelets per mL blood at day 10 and numbers declined from thereon. At day 21 human platelets in the mice of group E were hardly detectable. The experiment will be terminated at day 55 and chimerism will be determined in the blood, BM and spleen. In summary, expanded MKs may significantly contribute to thrombopoiesis during the first days after transplantation. This indicates that the period of thrombopoietenia after intensive chemotherapy can be overcome by the co-transplantation of ex vivo expanded human MPB MKs. Since previously published clinical trials showed only a small effect of co-transplanted MKs it may be interesting to extend our protocol to a clinical setting.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells</th>
<th>Cells transplanted</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MPB CD34</td>
<td>4.5×10^6 NC/mouse</td>
<td>100% 4</td>
</tr>
<tr>
<td>B</td>
<td>MPB CD34: expanded CD34</td>
<td>4.05×10^6 NC</td>
<td>90%–100% 4</td>
</tr>
<tr>
<td>C</td>
<td>MPB CD34: expanded CD34</td>
<td>4.5×10^6 NC × 0.45×10^6 NC input culture/mouse</td>
<td>100%–100% 4</td>
</tr>
<tr>
<td>D</td>
<td>MPB CD34: expanded CD34</td>
<td>2.25×10^6 NC × 2.25×10^6 NC input culture/mouse</td>
<td>50%–50% 4</td>
</tr>
</tbody>
</table>

0350
THE GRAFT VERSUS HOST DISEASE AND SURVIVAL MIGHT BE DIFFERENT ACCORDING TO ADMINISTRATION ROUTE AND DOSE OF MESCENHYAL STEM CELL LINE IN MHC MISMATCHED MURINE HEMATOPOIETIC STEM CELL TRANSPLANTATION

P. Jeong,1 D. Lee,1 B. Cho,1 N. Chung,1 H. Kim,1 C. Han1
1The Catholic University of Korea, INCHON, South-Korea; 2St. Mary’s Hospital, SEOUL, South-Korea

Background. The stable engraftment and graft versus host disease (GVHD) should be overcome in allogeneic hematopoietic stem cell transplantation (HSCT). Mesenchymal stem cells (MSC) contributed to sustain early engraftment and lesser GVHD. In conventional HSCT, a lot of cells were sequestrated into liver or lung during intravenous infusion. Aims. We evaluated whether survival and GVHD in HSCT would be different according to administration route, and dose of MSC. Methods. We retrieved MSC through 5 consecutive subculture of C3H/10T1/2. All lethally irradiated 6 weeks-old female Balb/c mice received 1×10^7 bone marrow cells and 5×10^6 splenic cells of female C3H/He mice according to route and dose. The study groups were divided into the intravenous (IV) and intra-marrow injection (IBM) according to route, and also dose of MSC. In co-administration of MSC, mice were designed in HSCT with 1×10^6 MSC and 1×10^5 MSC, and some mice received with addition of MSC on post-HSCT 48 hours. All mice were observed daily for survival and GVHD clinical status. Results. All mice without MSC died with no different GVHD pattern in post-HSCT 8 day in spite of route. In HSCT with MSC, there were no difference of survival rate and GVHD score in mice co-transplantation with 1×10^6 MSC, and also with addition of MSC on post-HSCT 2 day in both IV and IBM group. However, mice received with 1×10^6 MSC were significantly better survival and lower GVHD score than others in both groups, although mice in IV group were longer survival than in IBM group. Conclusions. Our data suggested that the administration route of cells would not affect survival and GVHD pattern, and co-transplantation with high dose of MSC might prevent lethal GVHD in MHC mismatched allogeneic murine HSCT. We concluded that HSCT with IV infusion of high dose of MSC might prevent lethal GVHD and have survival benefit.

0351
THE T CELL RECEPTOR REPETITION USAGE DIFFERS BETWEEN CD4+CD25+ REGULATORY T CELLS AND THEIR CD4+CD25- COUNTERPART AFTER ALLOGENIC STEM CELL TRANSPLANTATION

C. Fozza,1 E. Nadal,1 M. Longinotti,1 F. Dazzi2
1 Imperial College, LONDON, United Kingdom; 2 Institute of Hematology, SAS-SAR, Italy

Background. After allogeneic haematopoietic stem cell transplantation (SCT) the overall T cell receptor (TCR) repertoire is characterized by a lower diversity and a markedly skewed pattern. Its normalization may start at about 6 months after transplant but most patients continue to show an abnormal profile until 2 or 3 years. Naturally occurring CD25+ regulatory T (Treg) cells also develop in the thymus and play a crucial role in the maintenance of peripheral tolerance. Although it is known that the administration of Treg cells has a protective effect in murine models of allograft rejection, their role after SCT has been only partially elucidated. Aims. We assessed whether naturally occurring Treg lymphocytes exhibit an impairment in their TCR repertoire after SCT. We analyzed the TCR Vβ repertoire of CD4+CD25+ Treg cells after allogeneic SCT, focusing on its overall complexity and on the degree of similarity to the CD4+CD25- conventional T (Tconv) cells. Methods. Two researchers analyzed 10 patients who had received SCT for chronic myeloid leukemia. After CD4+CD25+ and CD4+CD25- cell isolation, RNA extraction and reverse transcriptase PCR, CDR3 region fragment analysis was performed through capillary electrophoresis. Conventional spectratyping evaluation was carried out by calculating an overall complexity score and by determining the percentage of skewed and oligoclonal VB profiles. Moreover, we developed a new analysis method to quantify the proportion of VB subfamilies with similar profile between the Treg subset and its Tconv counterpart. Results. Although we observed a significantly higher percentage of skewed and oligoclonal VB subfamilies in both cell subpopulations and in patients less than 3 years after SCT, the conventional analysis systems showed essentially similar TCR patterns between Treg and Tconv cells. We then compared the spectratyping profiles of the 2 cell subsets within each VB subfamily in each subject. As a tool we developed a new similarity score, expressing the proportion of VB subfamilies with similar profile between Treg and Tconv subsets. We detected a positive correlation between similarity score and time after SCT (Pearson correlation coefficient = 0.65). A higher score was observed in patients more than 3 years after allografting (mean 0.90 vs. 0.61, p = 0.01). Noticeably, in patients less than 3 years after SCT the differences were very often ascribable to the detection in the same VB subfamily of an oligoclonal profile in the Tconv but not in the Treg subpopulation. This specific pattern was almost exclusively confined to this group of patients (mean 52% vs. 5%, p = 0.002). Conclusions. Our data show that the repertoires of Treg and Tconv cells exhibit significant differences early after SCT, while they tend to become identical with full reconstitution. These differences are mainly ascribable to VB subfamilies expressing an oligoclonal profile in the Tconv but not in the Treg subset. A VB subfamily either reflect a discrepancy in the in vivo reactivity against common antigenic stimulations or be the result of a different post-transplant ontogeny.

0352
TWO ISOFORMS OF HUMAN FOXP3 POSSESS SIMILAR CAPACITIES TO INDUCE DIFFERENTIATION OF REGULATORY T CELLS FROM CD4+CD25- T CELLS

T. Mutis, L.F. Verdonck, M.I. Aarts-Riems, M.E. Emmelot
University Medical Center Utrecht, UTRECHT, Netherlands

Background. Naturally occurring CD4+CD25+ regulatory T cells (Treg) are considered to play important roles in the clinical outcome of stem cell transplantation (SCT). The forkhead/winged helix transcription factor, Foxp3 is the key factor for the differentiation of Treg. While in rodents the Foxp3 gene is expressed as a single transcript, in humans it
We investigated the cellular distribution of human foxp3 isoforms using quantitative PCR (QPCR) primer sets that amplify either the Foxp3FL or the Foxp3'E2 gene. The role Foxp3 isoforms in Treg-differentiation was studied by retroviral transduction of foxp3FL and Foxp3'E2 genes separately into highly purified CD4+CD25- Foxp3- cells. Foxp3 transduced T cells were cultured briefly in 95% puromycin before phenotypical and functional characterization. Results. In PBMC of healthy individuals, both Foxp3 isoforms were preferentially expressed in CD4+CD25high cells. However, there was no quantitative relation between the gene expression levels of these isoforms. In Treg clones, generated by limiting dilution of CD4+CD25hi cells Foxp3 isoforms were expressed simultaneously; but there was no quantitative correlation between their expression levels. Phenotypic and functional analyses of foxp3 transduced T cells revealed that T cells transduced either with Foxp3FL or with Foxp3'E2 genes expressed high levels of CD25, CTLA-4 and GITR; were anergic to stimulation via CD3 and up-regulated the CD3 induced proliferation of autologous and allogeneic CD4+CD25- cells in a dose dependent manner. Summary and Conclusions. Our results reveal that the two isoforms of human FOXP3 possess similar capacities to induce differentiation of Treg from CD4+CD25- T cells. Since they can be quantitatively expressed independently from each other, studies aiming at correlating Treg cell cloning with the clinical outcome of SCT will benefit from quantitative determination of both foxp3 isoforms.


1University of Turin, TORINO, Italy; 2Istituto Nazionale Tumor, MILANO, Italy

Background. In human somatic cells, telomere length decreases at each mitotic division and progressively shortens with age. Due to this peculiarity, telomere restriction fragment (TRF) length has been used as a marker of cell aging. The systematic analysis of TRF has suggested that haematopoietic stem cells (HSCs) may undergo early cell aging if exposed to non-physiological proliferative stress, as it occurs during haematopoietic reconstitution following high dose (hd) chemotherapy and stem cell transplantation (SCT). Information is still lacking about the influence of telomere length of grafted cells on the degree of telomere erosion following SCT. AIM OF THE STUDY. To correlate TRF length in grafted peripheral blood stem cells (PBSC) and in haematopoietic cells taken at marrow reconstitution following SCT. PATIENTS AND METHODS. TRF length was monitored in a series of lymphoma patients undergoing an intensive high-dose sequential (HDTEM) program, including two consecutive mobilization procedures, with hd-cyclophosphamide (CY) and hd-Ara-C, and then PBSC injection. In a previous report (Ricca I et al. Leukemia 2005), we showed that TRF length is markedly shortened in PBSC collected at the second mobilization course compared to those collected at the previous one. Thus, TRF was assessed in 10 patients autografted with post-CY PBSC and 13 receiving post-Ara-C PBSC; in addition, TRF was assessed in 8 patients receiving allogeneic SCT. TRF was assessed both on grafted material and on bone marrow (BM) samples taken at a median time of 24 months after SCT. All patients were in complete remission of their underlying disease at the time of analysis. Results. As shown in the Figure (panels A and B), TRF length was markedly shortened in post-Ara-C PBSC compared to post-CY, with median TRF length of 13100 bp (range 11056-13204) and 15464 bp (range 13561-8906), following hd-CY and hd-Ara-C, respectively (p<0.0001). At post-SCT follow-up, median TRF length in patients autografted with post-Ara-C PBSC (panel A) was 7092 bp (range 5851-8227), thus analogous to pre-transplant value; by contrast, patients autografted with post-CY PBSC (panel B) had a significantly longer TRF length (7474 bp, range 6707-8808) compared to pre-transplant value (p<0.005). Panel C shows TRF values in the eight allografted patients: again, post-SCT TRF length was similar to the graft (p=NS) and markedly longer if compared to patient pre-SCT value (p<0.01). Conclusions. In the SCT setting, postgraft TRF length strictly correlates to TRF of grafted cells.
resulting in abnormal telomere loss at least in the early period after autol-
ogous bone marrow transplantation. Aims: to evaluate both TL and bone
S.
L), and 8/14 (57%) achieved a normal CD16/56 count (median 0.06, and
0.03). Their median age at the time of transplant was 44 yrs. (range 18-65). TL was determined by Southern Blot analysis on mononuclear cells (MNC) from PB and BM samples and on PB granulocytes (GN). MNC were separated through a ficoll den-
sity gradient, while GN were obtained from PB using a double step sepa-
ration: RBC sedimentation in the presence of 33% Emagel and then ficoll separation to obtain granulocytes. BM progenitors were investigat-
ed by the in vitro culture assays, and both committed (CFU-GEMM, CFU-GM, BFU-E) and immature (LTC-IC) progenitors were evaluated. Results. TL of separated PB granulocytes was significantly shorter in autografted subjects compared to age-matched healthy subjects. A sim-
ilar reduction was observed on BM cells, while no significant differences were observed on whole PB leukocytes. TL loss appeared to be stable, since the median difference in granulocyte TL between autografted sub-
jects and age-matched controls (TelIoss) was virtually the same in sub-
jects at different time-periods since autograft (see Figure). Both immatu-
ture and committed BM progenitors were found to be markedly reduced compared to normal controls (data not shown). Again, no correlation between degree of progenitor reduction and time-interval since autograft was observed. Conclusions. 1. telomere length is reduced in myeloid cells from subjects surviving up to 10 years following autograft; ii. TL reduc-
tion is long lasting suggesting that telomere-elongating enzymes are unable to reconstitute a normal telomere length even after a prolonged period following autograft; iii. a marked reduction of BM immature and committed progenitors is also maintained at long-term in spite of the large amounts of transplanted CD34+ve cells. Thus high-dose chemotherapy and PBSC autograft may result in myelopoietic cell abnormalities that appear to be irreversible. This observation is of both biological and clinical relevance.

**0357**

**HIGH DOSE CHEMOTHERAPY+AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE SCLEROSIS PATIENTS: TREATMENT OUTCOMES AT LONG-TERM FOLLOW-UP**

Y. Shevchenko, A. Novik, B. Afanasev, I. Lisukov, O. Ryvkavich, S. Shamsaniski, T. Ionova, A. Kulagin, O. Malyshova, N. Bazi, V. Mel-
nichenko, D. Fedorenko, I. Vereschagina, A. Kishtovich, Y. Fedotov, G. Gorodkin

'Russian Coop, MOSCOW, Russian Federation; 111 Cent. for Qol. and Health Outcome Res., NEW JERSEY, United States of America; Russian Cooper. Group for Cell. Therapy, Moscow, Russian Federation

**Background.** During the last several years HDCT+ASCT is more often used as a therapeutic option for MS patients. The major treatment out-
comes for MS patients are a period free of disease progression and improvement of a patient’s quality of life (QoL). Aims. We aimed to study treatment outcomes in MS patients at long-term follow-up after HDCT+ASCT in Russia. Twenty-five patients at 1 th MS (sex: male - 8, female - 17; Mean EDSS at base-line was 6.0 (range 2.0 -
8.0). The median follow-up duration was 15 months (range 6-72 months). All of the patients had previously undergone conventional treatment. Neu-
rological and QoL status were monitored using Integral QoL index. Results. Twenty patients with the follow-
up longer than 1 year were included in the analysis. 19 patients (95%) experienced a clinical stabilization or improvement. Three patients showed significant improvement in EDSS (by more than 1.0 point), 4 patients improved by 1.0 point, and 4 patients - by 0.5 points on EDSS. Eight cases remained stable. All of the patients with clinical stabilization and improvement exhibited negative MRI scans. One patient deteriorat-
ed to a worse EDSS score after 10 months of stabilization and died of acute leukemia 3 years post transplant. Two patients after 12 months of improvement progressed to a worse EDSS score.

**0358**

**DAY 15 NATURAL KILLER (NK) CELL RECOVERY PREDICTS PROGRESSION-FREE SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN’S LYMPHOMA**


Mayo Clinic Rochester, ROCHESTER, USA

**Background.** The peripheral blood absolute lymphocyte count (ALC) on day 15 after autologous stem cell transplantation (ASCT) has been shown to be an independent predictor for overall survival (OS) for many malignancies including acute myelogenous leukemia (AML), breast can-
cer, multiple myeloma (MM), prostate cancer, and Hodgkin’s and non-Hodgkin’s lymphoma (NHL). However, due to the retrospective nature of previous studies, the peripheral blood lympho-
cyte subpopulations that predict survival are unknown. Aims. To prospectively correlate peripheral blood lymphocyte subpopulations on day 15 after ASCT and progression free survival in NHL. Methods. Peripheral blood lymphocytes collected from 41 patients day 15 after ASCT were analyzed by four color flow cytometry for CD3, CD4, CD8, CD16, CD19, and CD56. Patients were then dichotomized into two groups: patients achieving normal numbers of lymphocyte subset (i.e. CD4, CD8, CD19, CD16/56) count versus those who did not. Progression free survival was then analyzed by the Kaplan-Meier method. Results. In our cohort of 14 patients, 9 were male and 5 were female. Nine patients were diagnosed with diffuse large cell B cell lym-
phoma, two with mantle cell, two with follicular, and one with periph-
eral T cell lymphoma. On presentation, one patient had stage I, four patients had stage II, four had stage III, and five patients had stage IV dis-
case. The median age at ASCT was 53 years (range: 26-70). The prelimi-
ary data from this ongoing prospective study of 14 patients shows that on day 15 after ASCT, 4/14 (29%) of patients achieved a normal CD8 count (median 521, range: 69-2069 cells/µl) 3/14 (21%) achieved a normal CD4 count (median 206, range: 81-1091 cells/µl), 6/14 (43%) achieved a normal CD 3 count (median 88.5, range: 13-813 cells/µl) 1/14 (7%) achieved a normal CD19 count (median 2, range: 0-227 cells/µl), and 8/14 (57%) achieved a normal CD16/56 count (median 85.5, range: 10-744 cells/µl). The median follow-up was 12 months (range: 3-45 months). On univariate analysis, patients achieving an absolute NK cell count of ≥ 80 cells/µl on day 15 had significantly improved progression-free survival compared to those who did not (not
reached vs 3 months, p<0.006, respectively). Similar analysis evaluating the absolute CD3, CD4, CD8, and CD 19 count was not significant (p=0.1, p=0.258, p=0.06, and p=0.55, respectively). Conclusion. To our knowledge, this is the first report detailing the critical role of NK cell immune reconstitution after ASCT in progression free survival.
AM3 THERAPY PREVENTS MUCOSITIS IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELLS TRANSPLANTATION

V. Recasens, A. Rubio-Martínez, D. Rubio-Feliz, P. Giraldo
Miguel Servei University Hospital, ZARAGOZA, Spain

Introduction. AM3(Inmunoferon®), is a glycoconjugate of natural origin with immunomodulatory properties indicated in secondary immunodeficiencies as well as coadjuvant treatment in neoplastic diseases with cellular immunity deficiency. Aim: To evaluate the influence of AM3 in the biological recovery of immune system in patients undergoing hematopoietic stem cells transplantation (HSCT)(autologous/allogeneic). As well as to register the clinical incidences as undercurrent infections and mucositis from day of infusion hematopoietic cells to 90 days post-transplantation in patients with and without AM3. Patients and Methods. Group A (cases): Inclusion of 19 consecutive patients undergoing HSCT. April 2004 - June 2005. Inclusion criteria: age > 18 years, first HSCT and signed consent form. AM3 dose: 20mg/8h orally since infusion day to 90 days posttransplantation. Group B (controls): 19 patients consecutive-ly undergoing HSCT from February 2003- March 2004. Biological evaluation of the immune system recovery: BCC, immunomodulants, chitotriosidase, CCL18/PARC) on days 74. p<0.05. Clinical evaluation and comparing -somes of incidence of mucositis severity evaluated according to WHO classification and microbiological documented infectious diseases. Results. The addition of AM3 as a coadjuvant therapy in patients under- going HSCT reduces significantly the percentage of oral mucositis (65.1% vs 89.5%) (p=0.025) being those mucositis of less complexity in the 1st 2 weeks posttransplantation. The number of infectious diseases was lower in patients under AM3 therapy (31.5% vs 42%) (p=0.025) (Detailed in Table). The recovery of the immune system evaluated by blood cells count and immunobiomarkers showed a initial recuperation at day +14 in autologous HSCT and at day 21 for allogeneic HSCT. Tolerance was satisfactory and only two patients needed discontinue therapy because of digestive intolerance. Wide studies must be performed in order to evaluate the benefit of this coadjuvant treatment. This work has been partially sponsored by a grant from FEHHA.

Table 1.

<table>
<thead>
<tr>
<th>Mean age (range)</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>44 (18-67)</td>
<td>48 (18-68)</td>
</tr>
<tr>
<td>AutoLOGous HSCT (M/F)</td>
<td>6 (31%)</td>
<td>7 (36.8%)</td>
</tr>
<tr>
<td>Autologus HSCT (M/F)</td>
<td>4 (21%)</td>
<td>6 (31.5%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Acute leukeimia: 7 (37%), MM 7 (37%), MDS 2 (10%), Hodgkin disease 2 2nd remission 2 (11%) lymphoblastic lymphoma 1 (5%)</td>
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</tr>
<tr>
<td>Mucositis</td>
<td>12 (63%); grade I: patient (9%); grade II: 4 (23%); grade III: 4 (33%); grade IV: 3 (15%)</td>
<td></td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>6 (31.5%); S. Epidemids 4 (21%); St. homonis 1 (5%); CMV 2 (10.5%); C. Albicans 1 (5%)</td>
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</table>

| Acute leukeimia: 4 (21%); MM 9 (47%); Hodgkin disease 2 2nd remission 2 (11%); NHL 4 (21%); |
| 17 (80.5%); grade II: 1 patient (9%); grade III: 6 (35%); grade IV 9 (53%) |
| 8 (42%) St coagualse negative 7 (36.8%); Sr viridans 1 (5.2%); S. epidemids 1 (5.2%); Ochrobactrum anthophila 1 (5.2%); P. amgenius 1 (5.2%); C. difficile 1 (5.2%); ADN Herpes virus 6: 2 (10.5%); CMN 3 (15.7%); C. albicans 2 (10.5%); C. kessel 1 (5.2%); P. carini 1 (5.2%) |

GVHD is a limiting factor. Several studies have shown that cells that are involved in initiation and promoting of GVHD are dendritic and T regulatory cells. It has been shown that GVHD is mainly due to the imbalance (activation or suppression) between these two groups of cells. This disparity somehow relates to the intensity of different chemo-radio-therapy conditioning on the recipients and cytokine storm preceding transplantation. Our aim was to study the influence of the admistration order of busulphan (Bu) and cyclophosphamide (Cy) on the chimerism and engraftment of the dendritic and regulatory T-cells. Methods. Sixty female Balb/c mice were divided in two groups. Group I (Cy-Bu) received Cy (100 mg/kg/day; two days) followed by liposomal Bu (15 mg/kg/day; four days) and group II (Bu-Cy) received the same dose of the drugs but in reverse order. Twenty of the recipients were transplant- ed using Sca-1 from Balb/c males. The chimerism and engraftment of dendritic and T-regulatory cells was studied at different time point by EACS and FISH analysis. Weight was followed as an indicator of the mice health status. The spleen weight and cellularity were followed as a sign of cytotoxicity and immune suppression. Results. In both groups, mice weight decreased dramatically on day 0, however the mice gained weight rapidly in group Cy-Bu compared to that seen in group Bu-Cy. The spleen weight and cellularity in group Cy-Bu reached the level of control mice faster (on day +3) compared to that found in group Bu-Cy (on day +6) indicating that the repopulation of lymphoid 1 and T cells in group Cy-Bu is faster than in group Bu-Cy. The level of chimerism was similar (30% vs 31%) and 50% of donor cells in spleen following the Y-chromosome at day 30 and 40, respectively, in Cy-Bu group compared to 10 and 20% in Bu-Cy group. The levels of IL2 and TNF-alfa were lower at day 0 and day +1 in group Cy-Bu compared to control group while in group Bu-Cy the levels were 2-fold higher in Cy-Bu compared to control group. Conclusion. We conclude that the use of Cy-Bu compared to the traditional Bu-Cy conditioning may be beneficial for the patients since it allow faster engraftment of the stem cells. These also may help in decreasing the side effect due to the lower levels of cytokines during transplan- tation period.

HIGH PROPORTIONS OF CD4+CD25+ CELLS IN BLOOD LYMPHOCYTES DETECTED EARLY POST HSCT ASSOCIATE WITH AGVHD AND HERALD ITS SEVERITY

A. Lange, D. Dlubek, D. Drabczak-Skrzypek, A. Młynarczewska
Institute of Immunology, WROCLAW, Poland

Background. CD4+CD25+ cells have been already described as regulatory cells exerting immunosuppression. Unexpectedly, we found that these cells are rather elevated at the beginning of aGVHD prior to steroid therapy. Aim. In the present study we investigated whether CD4+CD25+ cells proportions correlated with the severity of aGVHD and influenced chimerism post HSCT in patients receiving non-myoeloaablate conditioning regimen. Methods. Forty four cases (40 hematological disease and 4 immune deficiencies) transplanted from matched sibling (19) and unrelenlazed donors were studied. Thirty three patients were on non-myoeloaablate and eleven on myoeolaablate conditioning regimen. The presence of CD4+CD25+ cells in addition to routine lymphocyte profiling was investigated in three time intervals post HSCT (until +30 days, 30-60 days and 60-100 days). Post transplant chimerism was detected with the use of informative genes STR alleles determination between 12/30 days post HSCT. aGVHD were diagnosed clinically and usually the diagnosis was supported by target organ histopathology including immunos- taining. Results. Thirty four patients were investigated by one month post HSCT. Mean values±SD of CD4+CD25+ cells equaled 9.9%±7.6 with a bimodal distribution of individual results allowing dividing the entire group of patients into two subgroups: those lacking aGvHD and investigated at the similar time post trans-plantion (11,0±1,5 vs 3,7±0.7 respectively, p=0.00007). In addition we found that the level of CD4+CD25+ detected at the beginning of aGVHD correlated with the severity of this complication being full blown at some time post CD4+CD25+ cells measurements. The proportions of CD4+CD25+ cells were 8.4%±1.2 and 12.5%±2.5 in pts having grade 1 and more severe aGVHD, respectively. The highest proportions of CD4+CD25+ cells in blood lymphocytes were found in patients with

INFLUENCE OF THE ADMINISTRATION ORDER OF BUSULFAN AND CYCLOPHOSPHAMIDE ON THE ENGRAFTMENT AND CHIMERISM IN SYNGENIC STEM CELL TRANSPLANTATION MOUSE MODEL

B. Sadeghi, M. Jansson, Z. Hassan, C. Nilsson, M. Abedi-Valugerdi, M. Hassan
Karolinska Institute, STOCKHOLM, Sweden; Stockholm University, STOCKHOLM, Sweden

Background. Although stem cell transplantation (SCT) is considered to be a curative therapy for malignant and non-malignant diseases, still

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grade IV aGVHD and these values were significantly higher as compared to grade I aGVHD cases (15.2%±3.7 vs 8.4%±2.0, p<0.05). Summary. It appears that higher proportions of CD4+ CD25+ in blood lymphocytes measured soon after HSCT tended to be associated with mixed chimerism but importantly associated with early manifestation of aGVHD and heralded a severe course of this complication.

**0362**
SHORTENING OF NEUTROPENIA IN LYMPHOMA PATIENTS AFTER TRANSPLANTATION OF LIN ENRICHED CELLS EXPANDED **EX VIVO**
M. Klabusay, Z. Koristek, J. Vinklarkova, J. Mayer
University Hospital Brno, BRNO, Czech Republic

**Background.** Hematopoietic stem cells are able to regenerate hematopoiesis in all lineages. They are clinically used in transplantation of bone marrow or peripheral blood stem cells (PBSC) after myeloablative regimens of chemotherapy in the patients with diagnosis of leukemia or lymphoma. **Aims.** The methods of enrichment, isolation, cultivation and expansion of hematopoietic stem cells open the way for specific cellular therapy. In this study, the influence of **ex vivo** expanded Lin' enriched stem cells on the speed of engraftment was evaluated. **Methods.** Authors analyzed expansion of hematopoietic stem cells (HSC) selected by immunomagnetic separation of Lin' cells in the culture of serum-free medium in vitro with combination of 5 cytokines (SCF, Flt-3-L, IL-3, IL-6, G-CSF). Cell counts, morphology, immunophenotyping, S-phase, electron microscopy, and biological tests of LTC-IC, CFU-GM and CFU-Meg were analyzed. Clinical protocol was designed based upon the results obtained. Hematopoietic stem cells were enriched from apheresis products collected from patients undergoing mobilization chemotherapy by Lin' separation and expanded in vitro. Clinical transplantation protocol based on these results was developed. 10 patients with diagnosis of Hodgkin's or non-Hodgkin's lymphoma indicated for high-dose chemotherapy and autologous PBSC transplantation were enrolled to the protocol. All patients underwent standard PBSC collection, BEAM-chemotherapy regimen from day -7 and autologous transplantation at day 0. Besides that, an extra PBSC graft was collected, hematopoietic stem cells were enriched by Lin' procedure and cells were frozen. At day 14, enriched cells were thawed and cultured in the presence of 5 cytokines in serum-free medium. Expanded cells were infused at day 0 to the patients at the escalating dose from 5.10^5 to 3.10^6 cells. Patients were closely monitored, side effects and time to engraftment in leukocytes and platelets was observed. The results were compared to historical controls of 143 patients with diagnosis of lymphoma transplanted with identical BEAM regimen and PBSC grafts. **Results.** Isolated Lin' cells in culture differentiate, the relative proportion of CD34+ cells decreases below 5% at day +14. Growing number of granulocytic progenitor cells correlates with number of CFU-GM colonies. The highest number of CFU-GM colonies and total cell expansion was observed at day +14 in cytokine combination SCF+IL-3+FLT-3-L and IL-6, which was used in the clinical protocol. The procedure of Lin' cells transplantation was free of side effects in all patients. Engraftment in leukocytes occurred from day +6 to day +9 in the study group. Compared to historical controls, there was a significant shortening of neutropenia to 5.6 days in average and to 5.0 days in patients who received doses over 1.10^6 cells. There was no significant change in the engraftment in platelets (day +10 versus day +11). **Conclusions.** Hematopoietic stem cells can be enriched from PBSC grafts, cultured and expanded **ex vivo**, and safely used in the cellular therapy protocols. At higher doses of infused cells, the procedure resulted in shortening of critical period of pancytopenia.

This work was supported by grant IGA NR/8003-3.

**0363**
CONTINUOUS INFUSION IDARUBICIN AND ORAL BUSULPHAN (IBU) AS CONDITIONING FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION
Cardarelli Hospital, NAPOLI, Italy

**Background.** One way for reducing the relapse rate after autologous stem cell transplantation (ASCT) in acute myeloid leukemia (AML) in first complete remission (CR) is the adoption of new conditioning regimens. We developed an original conditioning program, named IBu, consisting of the combination of high dose idarubicin (IDA), given at 20 mg/m^2/sqm as 3 days continuous infusion from day -13 to -11 and busulphan (Bu) at 4 mg/kg from day -5 to -2, whose feasibility was previously demonstrated in a phase II study on 14 patients (Ferrara et al., THJ 2001). **Aims.** To report results from a series of 80 AML patients autografted in first CR conditioned with IBu regimen. **Patients and Methods.** There were 50 males and 30 females with a median age of 53 years (16-77). All patients had non M3 AML autografted in first CR. Karyotype was evaluable in 75 cases, with favourable, intermediate and unfavourable cytogenetics being found in 4, 60 and 11 cases, respectively. All patients received peripheral blood stem cells (PBSC) collected after consolidation plus G-CSF. The median interval between CR achievement and ASCT was 3 months (5-10). The median number of CD34+ cells infused was 6.5×10^6/kg (2.1-29). In patients aged more than 60 years (n=24), IDA and Bu were reduced to two and three days, respectively. **Results.** One case of transplant related death (1.2%) occurred in a patient aged 55 years, due to septic shock. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 10 (7-21) and 12 (6-168), respectively. The median number of platelet and blood units transfused was 3 (0-8) and 2 (0-12), respectively. Extra-hematological toxicity mainly consisted of grade WHO III-IV stomatitis (62/82 or 77%) requiring in all cases total parenteral nutrition, while 2 patients had grade III hepatic toxicity and one experienced transient hallucinations. Furthermore, most patients had FUO, while 3 experienced documented infection. Median days of intravenous antibiotics, required in 75 cases, were 11 (4-28). IVER examination post-ASCT did not reveal any cardiac toxicity. Finally, median time of hospitalization was 28 days (22-49). At the time of writing, 48 patients (54%) are in continuous CR, with only 3 patients relapsing after more than one year from ASCT. One patient died in CR from gastric cancer. After a median follow-up for surviving patients of 29 months from ASCT, median overall and disease free survival are 52 months and 48 months, respectively, as shown in the figure. Patients aged more than 60 years did not experience more complications than younger patients. **Conclusions.** Our data demonstrate the efficacy of the IBu regimen in patients with AML, due to a substantial reduction of relapse rate. The most relevant toxicity of the regimen was severe mucositis requiring TPN.

X. Zhao,¹ J. Wan,¹ N.V. Tsvetaeva²
¹Peking University Institute of Hematology, BEIJING, China; ²Nina, MOSCOW, Russian Federation

**Backgrounds.** The beneficial effect of killer immunoglobulin-like receptors (KIRS) driven allogeneic NK cells has been proved in the T-cell-depleted hematopoietic stem cell transplantation (HSCT), but with the requirement (CR) the adoption of HLA-mismatched/haploidentical blood and marrow transplantation.

**Aims.** To report results from a series of 80 AML patients autografted in first CR conditioned with IBu regimen. **Patients and Methods.** There were 50 males and 30 females with a median age of 53 years (16-77). All patients had non M3-AML autografted in first CR. Karyotype was evaluable in 75 cases, with favourable, intermediate and unfavourable cytogenetics being found in 4, 60 and 11 cases, respectively. All patients received peripheral blood stem cells (PBSC) collected after consolidation plus G-CSF. The median interval between CR achievement and ASCT was 3 months (5-10). The median number of CD34+ cells infused was 6.5×10^6/kg (2.1-29). In patients aged more than 60 years (n=24), IDA and Bu were reduced to two and three days, respectively. **Results.** One case of transplant related death (1.2%) occurred in a patient aged 55 years, due to septic shock. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 10 (7-21) and 12 (6-168), respectively. The median number of platelet and blood units transfused was 3 (0-8) and 2 (0-12), respectively. Extra-hematological toxicity mainly consisted of grade WHO III-IV stomatitis (62/82 or 77%) requiring in all cases total parenteral nutrition, while 2 patients had grade III hepatic toxicity and one experienced transient hallucinations. Furthermore, most patients had FUO, while 3 experienced documented infection. Median days of intravenous antibiotics, required in 75 cases, were 11 (4-28). IVER examination post-ASCT did not reveal any cardiac toxicity. Finally, median time of hospitalization was 28 days (22-49). At the time of writing, 48 patients (54%) are in continuous CR, with only 3 patients relapsing after more than one year from ASCT. One patient died in CR from gastric cancer. After a median follow-up for surviving patients of 29 months from ASCT, median overall and disease free survival are 52 months and 48 months, respectively, as shown in the figure. Patients aged more than 60 years did not experience more complications than younger patients. **Conclusions.** Our data demonstrate the efficacy of the IBu regimen in patients with AML, due to a substantial reduction of relapse rate. The most relevant toxicity of the regimen was severe mucositis requiring TPN.
fore to address KIR (i.e. CD158a, CD158b, and CD158e) and CD94, NK2G2A recovery on the NK cells after HLA-mismatch/haploidentical HSCT (with T-cell repletion). Specifically, we wished to assess any differences in KIR recovery that may affect the cytotoxicity and alloreactivity of NK cells, and to compare results with those for HLA-match transplant or HLA-mismatch transplant (with T-cell depletion), as reported by Pathan et al. Results. We sequentially evaluated 24 patients before and after HSCT on day +30, +60, +90, +120 and +180, and their donors by flow cytometry. All the patients achieved engraftment and complete donor chimerism after transplantation. All patients were alive and CCR, except 5 who died of transplant-related complications after HSCT; three patients relapsed on days 370, 330, 270 respectively. The recovery of CD94, CD94/NK2G2A, CD158e (KIR2DL1) on NK cells in recipients increased first compared with their donor values on day 30 after HSCT (p=0.013, p<0.0001, and p=0.063, respectively), then sequentially decreased from day 60 to day 180, to the donor values. By day 180, NK2G2A expression on NK cells was still maintained at higher levels compared with their donors’ values. The kinetics of reconstitution of CD158a and CD158b (KIR2DL) was opposite to the kinetics of CD158e recovery, diminishing significantly by day 30 in patients after HSCT compared with their donor values (p=0.016 and p<0.001 respectively), then sequentially increasing by days +60 to +180 after HSCT. However, the kinetics of reconstitution of all KIRs and CD94, CD94/NK2G2A on the CD8+ lymphocyte after HSCT from day +90 to +180 resembled the kinetics of CD94/NK2G2A recovery on NK cells after HSCT from day +30 to +180. Meanwhile, the patients were classified into low or ‘high’ CD4+ cell dose groups based on whether they received less or more than a median CD4+ cell dose of 0.85 x 10^9 respectively. There were a significant difference in the incidence of II-IV acute graft-versus-host disease (GVHD) between the two groups, p=0.0226. NK cells expressed less KIRs in recipients with II-IV GVHD or receiving ‘high’ CD4+ cell dose compared with those with 0-1 aGVHD or receiving ‘low’ CD4+ cell dose by day 30/60 after HSCT. Furthermore, the dose of CD4+ cells inversely correlated with the KIRs (CD158a, CD158b, CD158a/CD158b+, CD158e) expression on NK cells by day 30 and 60. Summary/Conclusion. These results suggested that both of the T cells in grafts and the occurrence of aGVHD affected the KIRs early reconstitution on NK cells in vivo after HSCT.

0365
EARLY AND LONG-TERM ENGRAFTMENT AFTER AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA PATIENTS


Hematology, BARI, Italy

Background. Autologous peripheral stem cell transplantation (PBSCT) has been increasingly performed in acute leukemia patients without an HLA-matched related donor; the use of mobilized PBSCT has resulted in a wide range of malignant and non-malignant diseases. In spite of its benefits to the patients, there are numbers of obstacles limiting the wide use of SCT. Because of ethical and technical problems, animal models of SCT are used widely to study basic mechanisms underlying SCT and SCT-related complications such as veno-occlusive disease (VOD) and graft versus host disease (GVHD). The majority of mice models of SCT and GVHD are based on the use of radiotherapy as a conditioning regimen. However, these models can not cover the variety of SCT in clinical settings. Many patients are conditioned with chemotherapy which may affect the occurrence and rate of transplantation related complications. Aims. To establish a murine model of SCT and GVHD using the chemotherapy as a conditioning regimen. Methods. One hundred and twenty female BALB/c mice were divided in two main groups. Group I received Cy (100 mg/kg/day for two days) followed by liposomal Bu (15 mg/kg/day four days). Bu-Cy subgroups received the same dose of the drugs but in reversed order. Forty two of the recipients (group I) were transplanted by bone marrow stem cell (Sca-1) of male BALB/c (syngeneic) and forty two recipients from group II were transplanted by bone marrow stem cell (Sca-1) of male C57BL/6 (allogeneic). The chimerism and engraftment were surveyed by FISH analysis. Conclusions. Engraftment was established in both groups successfully and complete donor chimerism after transplantation. All patients showed aGVHD. The number of CD34+ cells infused correlated with the neutrophils (p=0.08, p=0.005) and platelets counts (p=0.67, p<0.05) at 12 months after PBSCT; this correlation was better than the total dose of CD34+ cells at 6 (p=0.51, p=0.5) and 12 months (p=0.48, p=0.06) for the platelets count. Conclusions. We suggest that the study of CD34+ cells subsets is useful for the evaluation of long-term hematopoietic reconstitution after autologous PBSCT transplantation and that the cell doses of CD34+/CD38- and CD34+/CD90+ are good predictors of neutrophils and platelets long-term engraftment. In the clinical setting, in patients with a borderline or suboptimal CD34+ cell dose, measurement of CD34+/CD90+ and CD34+/CD38- cell numbers may provide additional information about the graft quality.

0366
MURINE MODEL OF STEM CELL TRANSPLANTATION AND GVHD BASED ON CHEMOTHERAPY CONDITIONING

B. Sadeghi, Z. Hassan, M. Abedi-Valugerdi, M. Hassan

‘Karolinska Institute, STOCKHOLM, Sweden; 1Stockholm University, STOCKHOLM, Sweden

Background. Stem Cell Transplantation (SCT) is a curative treatment for a wide range of malignant and non-malignant diseases. In spite of its benefits to the patients, there are numbers of obstacles limiting the wide use of SCT. Because of ethical and technical problems, animal models of SCT are used widely to study basic mechanisms underlying SCT and SCT-related complications such as veno-occlusive disease (VOD) and graft versus host disease (GVHD). The majority of mice models of SCT and GVHD are based on the use of radiotherapy as a conditioning regimen. However, these models cannot cover the variety of SCT in clinical settings. Many patients are conditioned with chemotherapy which may affect the occurrence and rate of transplantation related complications. Aims. To establish a murine model of SCT and GVHD using the chemotherapy as a conditioning regimen. Methods. One hundred and twenty female BALB/c mice were divided in two main groups. Group I considered for syngeneic SCT and Group II considered for allogeneic SCT. Each group was divided into two subgroups. Cy-Bu subgroups received Cy (100 mg/kg/day for two days) followed by liposomal Bu (15 mg/kg/day four days). Bu-Cy subgroups received the same dose of the drugs but in reversed order. Forty two of the recipients (group I) were transplanted by bone marrow stem cell (Sca-1) of male BALB/c (syngeneic) and forty two recipients from group II were transplanted by bone marrow stem cell (Sca-1) of male C57BL/6 (allogeneic). The chimerism and engraftment were surveyed by FISH analysis. Results. Engraftment was established in both groups successfully and started from day +15. Also there were differences in time period of engraftment. In allogeneic group we could show the occurrence of GVHD as well. GVHD has shown symptoms of acute GVHD and occurred between day +30 and day +40 post transplantation. Interestingly this conditioning is not myeloablative and can consider as non-myeloablative conditioning model of SCT. Summary/Conclusion. We have established a new murine model of SCT using chemotherapy which is compatible and comparable with non-myeloablative model of conditioning in human. This model can also be used to study the basic mechanisms underlying GVHD that might be caused by the effect of the conditioning regimen on different cell sub-populations.
**0367**

**IN VIVO LUCIFERASE EXPRESSION OF TRANSDUCED HEMATOPOIETIC STEM CELL POPULATIONS USING THE NON-INVASIVE BIOLUMINESCENT IMAGING**


University Medical Center, UTRECHT, Netherlands

The homing and outgrowth of luciferase gene-transduced hematopoietic cells can be visualized in live animals on sequential time points by bioluminescent imaging (BLI), using a highly sensitive liquid nitrogen cooled charge-coupled camera (CCCD). A safe transduction of bone marrow (BM) hematopoietic cells was optimised for the retroviral vector encoding the Green Fluorescent Protein-luciferase (GFP-Luc) fusion gene. To validate the signal of the BLI in relation to the number of transduced hematopoietic cells, different cell doses were transplanted. There is a linear correlation of the number of cells and the bioluminescence signal in the BM compartment during the first 5 weeks after transplantation. However, after a longer period of time the variation increased between the individual mice. Studying the fate of different transduced murine HSC populations after transplantation into lethally irradiated mice, not-treated BM was compared to Sca-1 positive cells from 5Fluorouracil-BM, a technique to enrich for the primitive hematopoietic stem cell. After transplantation of the total cell population with 20% transduced cell, different foci in the BM showed luciferase activity, predominantly in the femurs and sternum. Luciferase activity in mice transplanted with transduced BM cells decreases below detection level after 6 weeks, suggesting that only committed progenitors were transduced in this cell sample. Mice transplanted with transduced Sca-1 positive cells reached a maximum level of luciferase expression at week 4-5 and thereafter a consistent signal during the 7 months, indicative for the activity of the transduced primitive stem cells. The transduction of primitive stem cells was confirmed by a secondary transplantation in which long-term expression of luciferase was observed. These results show that the BLI might be of value to study different populations of hematopoietic stem cells or for monitoring and quantitate proliferation of locally active hematopoietic cells.

**Infectious diseases (including supportive care)**

**0368**

**RELATIONSHIP BETWEEN HTLV-1-ASSOCIATED ANTIBODIES, TAX-SPECIFIC CYTOTOXIC T LYMPHOCYTES, AND PROVIRAL LOAD AMONG HTLV-1 CARRIERS**

M. Akimoto,1 T. Kozako,1 T. Sawada,1 K. Matsuhashi,1 K. Uozumi,2 N. Arima,1 C. Tei1

1Kagoshima University Hospital, KAGOSHIKA, Japan; 2Kagoshima University, KAGOSHIKA, Japan; 3Clinical Research Center, Eisai Co., Ltd., TOKYO, Japan

**Background.** Previous studies have demonstrated that higher anti-HTLV-1 antibody titer and lower anti-Tax antibody titer in human T-lymphotropic virus type 1 (HTLV-1) carriers imply a higher HTLV-1 proviral load and greater risk of ATL. However, it is still not fully understood how these factors are correlated with each other in HTLV-1 carriers. **Aims.** The present study was performed to examine the relationship among anti-HTLV-1 antibody, anti-Tax antibody, Tax-specific CTLs, and proviral load to clarify the significance of these factors. **Methods.** Forty-five HTLV-1 carriers were examined. Anti-HTLV-1 antibody was measured by ECLIA and anti-Tax antibody was measured by ELISA. Sixteen distinct HLA-A*0201 and HLA-A*2402 tetramers were prepared to detect HTLV-1 Tax or Env epitope-specific CTLs. Quantification of HTLV-1 DNA was performed by real-time PCR in a Light-Cycler System. **Results.** There were significant positive correlations between the frequency of Tax301-309-specific CTLs and both anti-HTLV-1 titer (r = 0.349, p < 0.001) and anti-Tax titer (r = 0.582, p = 0.003), whereas the frequency of Tax11-19-specific CTLs was correlated with neither antibody titer. However, both the frequency (median 0.14% vs. 0.00%, p = 0.002; Mann-Whitney U test) and the prevalence (100% vs. 30.8%, p = 0.029; Fisher’s exact test) of Tax11-19-specific CTLs in the anti-Tax-positive group were significantly higher than those in the negative group. The frequency (median 0.19% vs. 0.09%, p = 0.035) of Tax301-309-specific CTLs in the anti-Tax positive group was also significantly higher than that in the negative group, whereas the prevalence of the CTLs (84.6% vs. 60.0%, p = 0.245) was not different between the two groups. The proviral load ranged from 4.6 to 592.4, with a median value of 62.3/1000 copies. The proviral load in the Tax11-19-specific CTL positive group was significantly lower than that in the CTL negative group (median 24.1 vs. 69.5, p = 0.017), although no difference was observed between the Tax301-309-specific CTL-positive and negative groups (median 69.0 vs. 33.5, p = 0.291). However, multivariate regression analysis showed a positive correlation between anti-HTLV-1 titer and proviral load (r = 0.510, p = 0.001), and negative correlations between HLA-A*0201 positivity and proviral load (r = -0.413, p = 0.004) and between the frequency of Tax301-309-specific CTLs and proviral load (r = -0.413, p < 0.007), whereas HLA-A*2402 positivity and the frequency of Tax11-19-specific CTLs had no independent effect on HTLV-1 proviral load. **Summary/Conclusions.** Anti-Tax titer, the frequency of Tax301-309-specific CTLs, and HLA-A*0201 positivity (which confers efficiency to the Tax11-19-specific CTL response) may prevent growth of HTLV-1-infected cells in HTLV-1 carriers, whereas higher anti-HTLV-1 titer is a risk factor for higher HTLV-1 proviral load.

**0369**

**WHAT IS THE VALUE OF THE NECROPSIC STUDY ON CLINICAL HISTORY OF PATIENTS WHO DIED OF MALIGNANT HEMATOLOGIC ILLNESSES?**

M.J. Moreno,1 F. Arriba,1 V. Pérez,1 A. Jerez,1 C. Castilla,1 M.L. Lopez,1 F.P. Pastor,1 J.M. Moraleda,1 V. Vicente1

1Hospital Morales Meseguer, MURCIA, Spain; 2Hospital Reina Sofia, MURCIA, Spain

**Background.** During decades the autopsy has been important in order to complete clinical expressions understanding for clinical manifestations knowledge. In the last two decades many outstanding advances on diagnostic procedures of malignant hematologic illnesses had taken place (new image tests, new techniques like flow cytometry, molecular biology, microbiology and biological markers). **Aims.** It is necessary to wonder if the necropsy study continuous being worthy for the comprehension of the clinical profile presented by patients affected of malignants hematologic illnesses. **Patients and Methods.** We have compared the available clinical evidences in the clinical history and the results of the necropsy study in 24 patients with Acute Leukemia and 10 patients with other hematologic neoplasias that had undergo autologous progenitor cell transplantation. **Results.** The necropsy study provide contributed data that didn’t figure in clinical valuation: 1) opportunist infection for...
a not suspected germ in 3 patients (Tuberculosis, Citomegalovirus, Aspergillus); 2 erroneous interpretation of the final symptoms in different aspects: we suspect opportunist infection that was not confirmed in the autopsy, and that was turn into leukemic infiltration (2 cases); compatible clinic with gastrointestinal acute graft-versus-host disease that change to leukemic infiltration (1 case) or fungal infection (1 case). Finally, the autopsy disclosed unexpected involvement of different organs by opportunist infections in 10 patients. Conclusion. In spite of the advance on the diagnostic procedures, we confirm the profitability of the autopsy as source of valuable information for the clinical manifestations in patients with malignant hematologic illnesses.

0370
CMV INFECTION AND DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION
A single center experience
Y. Flossand,1 J. Rislien,2 B. Fossum Lland,3 E. Holter,4 H. Rollag,5 Y. Floisand1
1Rikshospitalet National Hospital, OSLO, Norway; 2Rikshospitalet University Hospital, OSLO, Norway

Background/Aims. CMV infection and disease remains a significant cause of morbidity and to some degree mortality after allogeneic stem cell transplantation (ASCT). The aim of this retrospective study was to evaluate the incidence of CMV infection and disease after ASCT to our center and to identify possible risk factors. Patient/Material/Methods. From 1994 to 2004 380 patients underwent ASCT. Conditioning regimens were oral Busulfan 16 mg/kg and iv Cyclophosphamide 60 mg/kg (BuCy2) (N=297) or Cyclophosphamide and ATG (N=10), 23 patients received other previous regimens. GVHD prophylaxis was cyclosporine A (CyA) and a conventional short course of Methotrexate. Established adult GVHD ≥ grade II was treated with increased immunosuppression with CyA and corticosteroids, occasionally ATG and other immunosuppressants in steroid refractory GVHD. Blood products were leukocyte depleted or CMV negative to CMV negative recipients. CMV surveillance was routinely performed by measurement of pp65 or PCR 1-2x/week until tapering of immunosuppression. CMV infection was diagnosed by detection of matrix protein pp65 or CMV DNA by PCR in two consecutive samples, which prompted treatment with ganciclovir or foscarnet. CMV disease was diagnosed according to the criteria of the 4. International Cytomegalovirus Workshop, Paris, 1993. Results. CMV infection was diagnosed in 22% (N=73) and CMV disease in 6% (N=19). Gastrointestinal (GIT) CMV disease was diagnosed in 11; lung in five, lung and GIT in two and spleen in one case. Two CMV infections were primary, whereas primary CMV disease was not observed. Of the patients who were CMV negative pretx (N=95); infection developed in 3 in patients receiving grafts from CMV positive donors. In the group of patients receiving grafts from family donors (n=220) the incidence of CMV infection and disease was 18.6% and 4% respectively. In the group receiving grafts from matched unrelated donors (MUD) (n=110) these incidences were 29% and 9%. CMV infection and disease were diagnosed at a median of 45 and 46 days post transplant. Late CMV infection, after day 100, was diagnosed in 9 patients. Using logistic regression analysis, the following factors were found to be statistically significant for development of CMV infection and disease: graft from MUD, use of corticosteroids, and CMV seropositive recipient pretx. Mortality in patients with CMV infection and disease was 47% and 68%, compared to 44% in the total material. Conclusions. In this retrospective single center study the incidence of CMV infection and disease was 22% and 6%. The low incidence of infection might be due to the fact that two positive tests and antiviral treatment were required for diagnosis. Grafts from MUD, use of corticosteroids and seropositive recipient pretx were identified as risk factors. Based on our results, CMV disease did not seem to be the direct cause of death, but the overall mortality is high, reflecting the severity of immunosuppression.

0371
AMPLIFICATION OF AN ASPERGILLUS SPP. SPECIFIC REGION OF THE 18S rRNA GENE BY REAL-TIME POLYMERASE CHAIN REACTION ON SERUM SAMPLES AS DIAGNOSTIC TOOL FOR INVASIVE ASPERGILLUS IN FEBrile HEMATOLOGICAL PATIENTS
C. Cattaneo,1 C. Bottelli,1 L. Bassani,1 D. Colombo,Bra; E. Borlenghi,2 M.A. Capucci,1 A. Caruso,3 G. Rossi3
1Spedali Civili, BRESCIA, Italy; 2Lab. Microb. Ped., PO dei Bambini, BRESCIA, Italy

Background. Invasive aspergillosis (IA) is responsible for about 5-10% cases of fever of unknown origin during neutropenia in hematological cancer patients. The diagnosis of invasive aspergillosis is conventionally based on indirect criteria devised by international cooperative study groups (EORTC-MSG) which include the determination of galactomannan (GM) antigenemia. However the precise cut-off values of GM antigenemia are still debated and the sensitivity and specificity are still suboptimal. DNA-based methods have shown potential utility in the diagnosis of invasive fungal infections and they are more specific than GM test. Aims. We retrospectively evaluated a new Aspergillus specific real-time polymerase chain reaction (AspRT-PCR) test in the serum of patients affected by hematological malignancies at the onset of fever, in order to evaluate the correlation between the result of AspRT-PCR with the subsequent clinical diagnosis. Methods. Twenty-three patients affected by acute leukemia (n=9), chronic lymphocytic leukemia (n=2), myelodysplastic syndrome (n=2), chronic lymphocytic leukemia (n=2) were evaluated. They underwent a complete microbiological screening, chest radiograph and CT scan. GM antigenemia >1 was considered positive. AspRT-PCR was performed with an Aspergillus gene-specific Taqman probe and Applied Biosystems 7700 instrument. The Aspergillus-specific probe was designed using Primer Express software, within a conserved region of the 18S rRNA gene of A. fumigatus, A. flavus, A. nidulans, A. niger, A. terreus, A. versicolor, but not homologous to other sequenced pathogenic fungi or mammalian DNA. Thirty sera obtained from healthy voluntaries were evaluated as control. Results. All the healthy voluntaries were found negative and no cross amplification was observed. According to the EORTC-MSG diagnostic criteria, we classified 5 patients as having probable aspergillosis, 8 as possible aspergillosis and 10 as no aspergillosis. GM antigenemia was positive in 5/5 patients with probable aspergillosis and in 1/15 patients with possible/no infection. AspRT-PCR was positive in all the patients (8/8) with probable infection and in 3 of the 8 patients with possible aspergillosis. Two of the patients classified as no aspergillosis showed a positivity of RT-PCR. Considering the groups of probable infections as true infection and possible/no infection as true negative, the frequency of AspRT-PCR was significantly higher among truly infected patients (p=0.0075). The sensitivity and negative predictive value were 100%. Specificity was 72% and the positive predictive value 50%. In three of 5 patients with probable aspergillosis, RT-PCR became positive earlier than galactomannan antigen (median 5 days, range 5-9). Conclusions. AspRT-PCR for invasive aspergillosis showed an excellent sensitivity and negative predictive value. Moreover, it could be an earlier mark for early diagnosis in comparison with GM test. Although these results have to be confirmed on larger number of patients. Further studies are needed to discard factors accounting for false positive results.

0372
INFECTIOUS COMPLICATIONS IN HAEMATOLOGICAL PATIENTS WITH CENTRAL VENOUS CATHETERS: A PROSPECTIVE ANALYSIS OF RISK FACTORS AND ETIOLOGICAL AGENTS
C. Nador,1 A. Nosari,2 A. De Gasperi,1 L. Marbello,3 M. Nichelatti,4 A. Corti,5 V. Mancini,6 A. Molteni,7 C. Baraté,8 F. Ricci,8 D. Ciappana,8 F. Garrone,9 E. Ravelli,1 A. Greco,1 M. Turri,1 E. Morra1
1Lab. Microb. Ped., PO dei Bambini, BRESCIA, Italy; 2Lab. Microb. Ped., PO dei Bambini, BRESCIA, Italy

Background. The use of central venous catheters (CVCs) in haematological patients is associated with various complications, among which infections are the most frequent and life-threatening. Aims. The aim of this single-centre, prospective study was to evaluate the epidemiology and the outcome of catheter-related infectious complications in haematological patients. Methods. Data concerning catheterizations of patients with haematological malignancies were collected between September 2002 and December 2004. The study cohort included 279 patients (137 male and 142 female, mean age 49.7, range 17-75) for a total of 388 catheterizations: 120 acute myeloid leukaemia (43%), 24 acute lymphoblastic leukaemia (8.6%), 9 chronic lymphocytic leukaemia (3.6%), 4 multiple myeloma (1.1%), 21 other haematological malignancies (7.5%). In acute leukaemia pts CVCs were used for chemotherapy administration and support therapy during aplasia, while the principal use in lymphoma and multiple myeloma pts was to harvest peripheral blood stem cells. A catheter-related bloodstream infection (CR-BSI) was defined by demonstration of the same microorganism both in the catheter and in the peripheral blood cultures, when no other source of infection other than the catheter itself was found. Results. Mean duration of catheterization was 18.8 days, while mean neutropenia with ANC < 0.5×109/L during catheter in situ maintained was 7.4 days and mean severe neutropenia with ANC <0.1×109/L was 4.7 days. In particular in acute myeloid leukaemia pts mean duration of catheterization was 23.8 days, mean neutropenia with ANC < 0.5×109/L was 11.3 days, mean neutropenia with ANC < 0.1×109/L was 7.3 days. Exit tunnel infections occurred in 19 cases (2.6 per 1000 catheter days), while catheter-related bloodstream infections haematologica/themehematologyjournal | 2006; 91(s1) | 137
occurring in 49 cases (6.7 per 1000 catheter days). Gram-positive CR-BIs were 76%, among which Staphylococcus epidermidis and Streptococcus were prevalent (58% and 12%, respectively). The remaining were Gram-negative CR-BI, most of which caused by E.coli, Pseudomonas aeruginosa and Enterobacter spp (31%, 23% and 15%, respectively). No fungal CR-BI was diagnosed. During hospitalization two patients (0.7%) died due to their haematological disease; catheter removal because of infectious complications was necessary in 14 cases (5.6%), of which 6 showed CR-BI. At univariate analysis, significant risk factors for CR-BI were number of days/catheter (p<0.0001), chemotherapy dose (high vs. standard dose; p<0.015), duration of neutropenia (p<0.001) and thrombocytopenia (p<0.001). At multivariate analysis, only days/catheter and duration of neutropenia appeared significant risk factors for CR-BI. Conclusion. The incidence of CR-BI is greatly increased by risk factors connected to haematological diseases and consequent chemotherapy administration, such as duration of catheterization, neutropenia and thrombocytopenia. In particular, patients affected by acute leukaemia are at a higher risk for CVC-related infections due to the use of aggressive and/or high-dose chemotherapy cycles during induction and salvage therapies, and a longer duration of severe neutropenia.

## RESPIRATORY VIRAL INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

M. Gilmore, A. Fortune, P.V. Browne, E. Vandenberge, S.R. McCann, B. Crowley, E. Conneally
St James Hospital., DUBLIN, Ireland

**Background.** Acute respiratory viral infections are generally self limiting, but can lead to morbidity and mortality in immunocompromised patients. The incidence of respiratory viral infection in our unit has varied from 30-100%. Aims. 1. To establish the incidence of respiratory viral infection in our unit. 2. To identify high risk patients requiring treatment and determine outcome. Methods. During the study period, July 2003-June 2005, all symptomatic patients had samples of respiratory secretions, nasopharyngeal aspirate (NPA) or bronchoalveolar lavage evaluated for respiratory viruses. Symptoms included cough, fever and coryza. Results were phoned to the referring doctor. Results. There were 40 positive results in thirty-eight patients. The following viruses were identified and patients were commenced on appropriate therapy: oseltamивir for influenza A or B; Nebulised ribavirin for 5 five days and alternate day intravenous immune globulin for RSV and parainfluenza.

<table>
<thead>
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<th>Virus</th>
<th>Count</th>
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</tr>
<tr>
<td>Parainfluenza 2</td>
<td>2</td>
<td>5%</td>
</tr>
<tr>
<td>Parainfluenza 3</td>
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<tr>
<td>Influenza</td>
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<td>21%</td>
</tr>
<tr>
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</tr>
<tr>
<td>Adenovirus</td>
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<td>0%</td>
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</tbody>
</table>

There were no deaths associated with respiratory virus infection during the study period (July 2003-June 2005) One patient with chronic GVHD required non invasive ventilation for acute respiratory distress due to RSV. Two patients required a second course of treatment due to persistent symptoms. Two transplants were deferred due to Influenza A. Conclusion. A high index of suspicion with early investigation and prompt isolation is required to reduce the morbidity and mortality associated with these infections in immunocompromised patients. The presence of lymphopenia, GVHD, and steroid administration are risk factors for poor outcome.

## REVERSE SEROCONVERSION OF HEPATITIS B VIRUS AFTER ALLOGENIC OR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

Keio University School of Medicine, TOKYO, Japan

**Background.** Reactivation of hepatitis B virus (HBV) in patients with antihepatitis B surface antigen antibody (HBsAb) has been known as reverse seroconversion, and recognized as a rare complication after hematopoietic stem cell transplantation (HSCT). However, its precise incidence has yet to be fully elucidated. Aims. We retrospectively analyzed the incidence of HBV reverse seroconversion in patients undergoing allogeneic or autologous HSCT for hematologic diseases. Patients and Methods. Eighty-three patients undergoing allogeneic HSCT (allo-HSCT; n=73) or autologous HSCT (auto-HSCT; n=10) between March 1992 and December 2005 were HBsAb-positive before transplant, and could be evaluated. Median age was 44 years-old (range 18-61) in allo-HSCT recipients, and 52 years-old (range 26-66) in auto-HSCT recipients. Diagnoses were acute leukemia in 25, non-Hodgkin’s lymphoma (NHL) in 22, multiple myeloma in 20, chonic myelogenous leukemia (CML) in 12, myelodysplastic syndrome in 7, and aplastic anemia in 1. Stem cell sources were bone marrow or peripheral blood stem cells (PBSCs) from related donor (n=23), bone marrow (n=30) or cord blood (n=2) from unrelated donor for allo-HSCT, and PBSCs (n=28) for auto-HSCT. Only 2 of 55 allogeneic donors were HBsAb positive. For conditioning, patients received myeloablative (n=4) or reduced-intensity regimen (n=11) for allo-HSCT, and high-dose melphalan regimen (n=17), MCVAC regimen (n=9), or total body irradiation-based regimen (n=2) for auto-HSCT. Results. Three of 55 patients (5.5%) and 3 of 28 patients (10.7%) experienced HBV reverse seroconversion after allo- and auto-HSCT, respectively. Time to reverse seroconversion from HSCT was 7.8, 10.5, and 53.6 months in allo-HSCT recipients, and 4.6, 6.1, and 6.6 months in 3 auto-HSCT recipients. Underlying diseases were acute leukemia, CML, multiple myeloma in allo-HSCT recipients, while multiple myeloma in 3 auto-HSCT recipients. In 4 (allo-HSCT 3, auto-HSCT 1) out of 6 patients, clinical hepatitis was diagnosed. Conclusions. HBV reverse seroconversion after HSCT is not infrequent, and close HBV monitoring is strongly recommended in HBsAb-positive patients. Furthermore, it is suggested that allo-HSCT recipients might be at higher risk of developing clinical hepatitis due to HBV reverse seroconversion than auto-HSCT recipients.

## HEPATITIS B AND REACTIVATION OF HBV DURING TREATMENT OF DLBCL PATIENTS: AN UNDETERMINED EVENT

O. DeHenau, L. Ysebrant, I. Ahmad, M.C. Ngrabacu, J. Bennani, A. Georgala, N. Meuleman, D. Bron
Institut J. Bordet, BRUSSELS, Belgium

**Background.** Hepatitis due to HBV reactivation after high dose chemotherapy for cancer patients is a well-recognized complication in chronic HBV carriers. The clinical consequences of hepatic injury range from asymptomatic liver dysfunction to massive hepatic necrosis and death by liver failure. Aim. The aim of this study is to compare the occurrence of hepatitis and HBV reactivation in Diffuse Large B Cell Lymphoma (DLBCL) patients with distinct HBV serologic pattern. Methods. We reviewed the medical files of sixty two patients with DLBCL followed since 1997 until now. All patients were treated according to the IFCT protocol of the lymphoma study: we defined 2 groups of patients based on the serologic HBV pattern at diagnosis. The first one included patients who had no antibodies anti-Hbc and no antigen HBs (AbHBc- / AgHBs- ), the second group included patients who had antibodies anti-Hbc but no antigen HBs (AbHBc+ /AgHBs- ). The third group included patients who carried antigen HBs (AgHBs+). The endpoint of this study was the time to diagnosis of hepatitis which was defined by serum ALT level above 100 UI for at least three consecutive days. We also reviewed the severity of hepatitis and occurrence of HBV reactivation, defined as a raise in serum HBV DNA level or a re-appearance of AgHBs in serum. Results. We identified forty one patients in the AbHBc- /AgHBs- group, thirteen in the AbHBc+ / AgHBs- group and four in the AgHBs +. None of the patients had hepatitis before treatment. Seventeen patients developed hepatitis during or after the treatment: Nine in AbHBc- /AgHBs- group (9/41 = 22%), four in AbHBc+/AgHBs- (4/13= 31%) and four in AgHBs+ group (4/4 = 100%). The rate of hepatitis was significantly higher in AgHBs+ group than in AbHBc-/AgHBs- group (100% vs 21%, p=0.001) and than in AbHBc+/AgHBs- group (100% vs 31%, p=0.015). A trend to higher rate of hepatitis development was observed in AbHBc+/AgHBs- group comparatively to AbHBc-/AgHBs- (31% vs 21%, p=0.076). All the patients in the AgHBs+ group developed HBV reactivation and severe hepatic complications. Three of them died from liver failure. Interestingly, one patients of the AbHBc+/AgHBs- developed a HBV reactivation with acute hepatitis (ALT >1000 UI). In the others patients hepatitis was transient, ALT levels did not exceed 300 UI and no seroconversion occurred. Conclusions. Despite the small number of patients, we observed a significant higher risk to develop severe hepatitis in DLBCL patients with AgHBs+ (chronic HBV carriers). In those cases hepatitis was due to HBV reactivation and associated with a high mortality. Hepatitis related to HBV reactivation occurred also in AbHBc+/AgHBs- patients, who have presumed resolved hepatitis B. These results emphasize the need for a careful follow-up for chronic HBV carriers and patients with pre-
sumed resolved hepatitis B. Prophylaxis for HBV reactivation should be administered to all chronic HBV carriers before chemotherapy regards to the high risk of severe hepatitis and HBV related death. The opportunity of viral prophylaxis in patients with antiHBc antibodies but no HBs antigen deserves to be further investigated.

**0376**

**ORAL VALGANCICLOVIR IS AN EFFECTIVE PRIMARY PREEMPTIVE THERAPY OF CYTOMEGALOVIRUS DISEASE IN PATIENTS WITH ALLOGENIC STEM CELL TRANSPLANT**

A.C. Candoni, R. Mestrioni, C. Fil, S. Buttignol, M. Cerno, R. Fanin

University Hospital, UDINE, Italy

Cytomegalovirus (CMV) infection is a common complication after allogeneic SCT, Valgancyclovir hydrochloride (VALCYTE-VGC) is a pro-drug of ganciclovir, orally available, that has been used in CMV infection in high-risk solid organ transplants (donor positive, recipient negative); there were only a few data about this drug in allogeneic SCT. The primary aim of our study was the assessment of efficacy and safety of VGC as preemptive therapy of CMV disease after allogeneic stem cell transplantation. This study is ongoing and here we are reporting the preliminary results. During a five-month-period VGC was administered to 10 consecutive patients (pts) with a CMV infection which was diagnosed on a median time of 56 days (range 59-450) from transplant. There were 6 males and 4 females (myelofibrosis 2, leukaemia 4, myeloma 2, lymphoma 2). The median age was 55 years (range 43-66); 7/10 pts underwent stem cell transplantation from unrelated and 3/10 from related donors; 7/10 pts received a reduced intensity conditioning regimen (RIC); CMV prophylaxis consisted in acyclovir in all cases. Pre-transplant CMV serology showed that in 100% of cases either recipient and/or donor were positive (D+/R+ = 5/10, D-/R+ = 5/10). At the onset of CMV infection 9/10 pts have an acute or chronic graft versus host disease for which were received therapy including prednisone plus other drugs. The pp65 antigenemia assay was positive in all cases with a median number of positive nucleic of 21±25. The starting treatment dosage of VGC was 900 mg twice a day and it was continued until the CMV antigenemia and PCR became negative in two consecutive samples. All 10 cases obtained a clearance of antigenemia after a median of 8 days of VGC therapy (range 5-16 days); viremia became negative in all cases. Median length of maintenance therapy with VGC (900 mg once-daily) was 21 days (range 8-32). Only one patient developed a mild deterioration of renal function that required dose adjustment (VGC 450 mg once-daily). None of the pts developed gastrointestinal disorders; mild anemia was reported in 8/10 (50%) pts, neutropenia in 8/10 (50%) pts and thrombocytopenia in 4/10 (40%). Conclusions: 1) Preemptive therapy with VGC after related and unrelated allogeneic SCT seems to be safe and effective (with a rapid clearance of antigenemia and viremia). 2) The simple once or bi-daily VGC regimen can improve the compliance of the pts. 3) Regular blood counts should be performed to early detect cytopenia. 4) The optimal dose and duration of VGC therapy in this setting need to be established with additional prospective studies.

**0377**

**BAL AS DIAGNOSTIC TOOL IN SEvere Pneumonia in PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: SINGLE CENTER REPORT ON 16 PATIENTS**

A. Manna,1 S. Cordani,1 M. Vignali,2 G. Benini,3 L. Cadenotti,1 P. Canessa1

1ASL La Spezia, LA SPEZIA, Italy; 2Microbiology Laboratory, OSPEDALE S.BARTOLOMEO SARZANA, Italy; 3Oncohematology Department, LA SPEZIA, Italy; 4Pneumology Department, OSPEDALE S.BARTOLOMEO SARZANA, Italy

Pneumonia is one of the most frequent life-threatening complications in patients affected by hematological malignancies despite recent improvements in support therapy; in these patients a timely identification of the microbial agent is crucial. The aim of this study was to evaluate the utility of broncho-alveolar lavage (BAL) in etiological diagnosis of pulmonary inflammatory infiltrates in this setting. Over 2 years period 16 patients affected by hematological malignancies (2 Myeloma, 1 Essential Thrombocytopenia, 5 Non Hodgkin Lymphoma, 4 Acute Myeloid Leukemia, 1 Myelofibrosis, 2 Chronic Lymphocytic Leukemia, 1 CML BC) age 58-76 years, showing clinical and radiological signs of Severe Pneumonia and failing to respond to antimicrobial therapy were studied. Together with initial routine serological and microbiological diagnostic tests on blood, urine and sputum (if available) broncho-alveolar lavage (BAL) was performed to identify the etiological agent. According to ATS (American Thoracic Society) criteria a bacterial cut-off > 10^4 CFU/mL or the isolation of a pathogen which doesn't ordinarily colonize the upper respiratory tract (M. tuberculosis, Pneumocystis J. Legionella sp, Aspergillus sp) defined infectious pneumonia. Results: the final diagnosis obtained by means of BAL among the 16 patients enrolled was: a) infectious pneumonia in 7 patients: the etiological agent was 2 polimicrobial infections (Mycobacterium T plus E.Coli, Mycobacterium T plus Pseudomonas sp), 2 Aspergillus sp (1 diagnosed by galactomannan detection on BAL and serum), 1 MRSA, 1 Corynebacterium sp. b) non infectious lung disease in 6 patients with alternative diagnosis: 2 alveolar drug damage, 2 BOOP, 1 T cell lymphoma, 1 bronchial infiltration of CCL c) 3 unknown diagnosis. Routine laboratory results were diagnostic only in one case (serum galactomannan detection). In conclusion: discrimination between infectious and non infectious diseases that mimic pneumonia is laborious namely in hematological patients; in our experience BAL procedure had a substantial impact on the etiological diagnosis and allowed a change of therapeutic strategy in 10 of 16 cases (62%).

**0378**

**SURVIVAL AND DISEASE COMPLICATIONS OF THALASSASMA MAJOR - 14 YEARS EXPERIENCE AT KING ABDULLAZIZ UNIVERSITY HOSPITAL, JEDDAH, KSA**

S.K. Al Jaouni

King Abdulaziz University, JEDDAH, Saudi Arabia

Background. Treatment of thalassemia major is complex, expensive and requires a multidisciplinary approach. Optimal clinical care is demanding and expensive but achievable. In spite of medical treatment improving dramatically, complications and deaths still occur. Aim. To assess the prevalence of mortality and disease complications among patients with thalassemia major at our center. Methods. A retrospective chart review was done of all patients diagnosed as Thalassemia Major (TM) between 1990 and 2004. The patients were followed and treated at King Abdulaziz University Hospital (KAUH), an academic tertiary care medical center. All 360 patients (203 males & 157 females) were transfeuson dependant since early childhood and treated with parenteral Deferoxamine. Approximately 98% were B-TM and 2% were HbE_o. The data had been collected by means of specially prepared forms (from Hematology Clinic, Day Care and Medical Records Department). The mean of serum ferritin has been available for all patients yearly. Comparison of ferritin levels between groups was performed by Student’s t test. Results. Out of 360 patients, 295 (81.4%) patients were alive, 27 (7.2%) patients had died, 15 (4.2%) patients underwent BMT and 25 (6.9%) patients’ follow-up were lost. Twelve (3.3%) patients died from heart disease. 7 (1.9%) patients died from infections, all patients were splenectomised. The serum ferritin levels for patients who died were significantly higher than for those patients who survived (7,500 vs. 3,200; p<0.01). Conclusions. Cardiac constitutes the first important cause of death followed by infection. Infection among thalassemics is still a risk factor which needs to be addressed carefully. Splenectomized thalassemic patients required special attention to avoid and prevent fatal infections. Complications and deaths among thalassemics are iron related organ dysfunction and age related. The majority of patients were on non-optimal chelation therapy and non-compliance. Poor compliance with parental chelation started at the adolescence age. Prevention program of inherited blood diseases should be implemented as a priority in the region.

<table>
<thead>
<tr>
<th>Causes of Death</th>
<th>Number</th>
<th>Percentage</th>
<th>Mean Age</th>
<th>Age Range</th>
<th>Mean S. Ferritin (ng/mL)</th>
</tr>
</thead>
<tbody>
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<td>3.3</td>
<td>20</td>
<td>16-24</td>
<td>7500</td>
</tr>
<tr>
<td>Infection</td>
<td>7*</td>
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*Splenectomized patients.
0379
EFFECTIVENESS OF NONINVASIVE VENTILATION IN TREATMENT OF ACUTE RESPIRATORY FAILURE IN ONCOHEMAOTOLICAL PATIENTS

B. Gil,1 A. López,1 M.J. Moreno,1 J.A. Barnes,2 V. Perez,3 M.M. Osma,1 B. Gil,1 A. Carrillo1
1Hospital Morales Meseguer, MURCIA, Spain; 2Intensive Care Unit, Spain; 3Oncolhematology Unit, Spain

Background. Use of noninvasive ventilation has been recommended in the immunosuppressed patients to avoid the endotracheal intubation complications. Aims. Analyses the effectiveness and safety of noninvasive ventilation (NIV) in the treatment of acute respiratory failure (ARF) in oncohematological patients. Methods. Observational prospective study included 42 patients among 135 patients with disseminated malignant neoplasias admitted in our intensive care unit (ICU) because ARF and treated with NIV. Success of NIV was defined as avoidance of endotracheal intubation and survival to ICU. The quantitative variables were expressed as mean±standard deviation and qualitative ones like percentage. After univariate analysis, achieves multivariate analysis by logistic regression. Results. In the period between January 1997 and September 2005 were admitted 135 patients with disseminated neoplasia and ARF. The mean age was 62±17 years and 65.2% was males. The malignant etiology was: 68 patients with metastatic solid organ neoplasia, 50 patients with lymphoma, 24 with leukemia and 18 with myeloma. Pneumonia was the most frequent cause of ARF (31 patients, 23%) and next acute respiratory distress syndrome (29 patients, 21.5%). Do not intubation order was indicated in 63 patients (46.7%). The majority of patients were ventilated with VISION® ventilator (92.6%) and the remains patients with BIPAP STD® ventilator. BIPAP ventilation mode was used in 96.3% and CPAP mode in the rest patients. Initial ventilator pressure levels were IPAP of 15±2 and EPAP of 7±1 cms of H2O. Respiratory parameters before and after one hour of NIV therapy were, respectively: pH: 7.32±0.11 and 7.34±0.07 (p<0.005); PaO2/FiO2: 145±57 and 162±45 (p<0.001); respiratory frequency: 36±5 and 32±5 bpm (p<0.001). Complications due to NIV were present in 52 patients (38.5%): naso-frontal cutaneus injury (36.3%); ocular irritation (5.9%); claustrophobia (3.7%); nosocomial pneumonia (3.7%); vomiting (3%); neumothorax (1.5%); aspiration pneumonia (0.7%). Duration of NIV therapy was 2.8±2.5 days and 40.5±38.3 hours. The success of NIV was 56.3% and hospital mortality was 56.3%. The length of ICU and hospital stays were 2.4±10.2 and 2.7±16.8 days, respectively. The variables associated to NIV failure were: PaO2/FiO2 before NIV beginning (OR: 0.902, CI-95%: 0.845, 0.964; p=0.002), PaO2/FiO2 one hour after NIV (OR: 0.886, CI-95%: 0.827, 0.949; p=0.001), respiratory frequency one hour after NIV (OR: 1.784, CI-95%: 1.274, 2.759; p=0.001) and highest SOFA (Sepsis Organ Failure Assessment) score (OR: 1.751, CI-95%: 1.050, 2.499; p=0.001). The variables associated to hospital mortality were NIV unsuccessful (OR: 8.566, CI-95%: 2.467, 29.770; p=0.001) and highest SOFA score (OR: 1.188, CI-95%: 1.049, 1.345; p=0.007). Conclusions. NIV treatment in oncohematological patients has high success rate. The patients who present NIV unsuccessful and severe multorgan failure have worse outcome.

0380
INCIDENCE AND RISK FACTORS OF INVASIVE FUNGAL INFECTIONS IN 246 PATIENTS UNDERGOING RELATED OR UNRELATED ALLOGENEIC BONE MARROW TRANSPLANTATION.

A.C. Candoni, S. Lovato, E. Simeone, E. Calistri, S. Buttignol, R. Fanin
University Hospital, UDINE, Italy

Introduction. Allogeneic bone marrow transplantation (BMT) is increasingly used to treat hematologic diseases. Invasive fungal infections (IFI) remain an important cause of morbidity and mortality in this setting. Patients and Results. To evaluate the epidemiology, outcome and risk factors of proven or probable IFI in allogeneic BMT recipients, we retrospectively examined the medical records of 246 consecutive adult patients (pts) who underwent allogeneic BMT (150 in related and 96 from unrelated donor) at our Department between 1992 and 2004; 193/246 (78%) pts received a myeloablative conditioning regimen and 53/246 (22%) a non myeloablative one. The median age of patients was 42 years (range 19-66). We identified 31 cases of IFI with an overall incidence of 13%; the incidence after related BMT (R-BMT) was 8% (12/150) and after unrelated BMT (UR-BMT) (p<0.05). The incidence was the same in the myeloablative (24/193, 12%) and non myeloablative (7/53, 13%) setting. IFI occurred after a median of 41 days from BMT (range 5-1440). There were 28 cases with proven or probable IFI (Aspergillus 22, Candida 4, Fusarium 1, Mucor 1) and 3 cases with possible IFI (all with lung localization). The sites of infection were: lung only 21/31 (69%), CNS 4/31 (12%), multiple sites 6/31 (19%); 13/31 (42%) cases occurred during pre-engraftment phase while 18/31 (58%) occurred after engraftment (with 12/18 cases after day 100). Advanced hematologic disease (relapsed or refractory) at time of transplant, history of pre-transplant IFI, presence of acute or chronic graft-versus-host-disease (GVHD), age >50 years, presence of other opportunistic infections were identified as risk factors (p<0.05). In the UR-BMT setting the incidence of IFI was significantly higher in patients who received a combination of immunosuppressive agents in the conditioning regimens (ATG ± Thalidomide ± Campath). In this setting the incidence of IFI in UR-BMT was significantly higher than in R-BMT probably as a result of increased immunosuppressive conditioning regimens in this setting. IFI can develop late after engraftment (after day 100 from transplant) and without neutropenia. 8/31 patients with IFI, status of hematologic disease (relapsed/refractory) at transplant and history of pre-transplant IFI are important predisposing factors. Retrospective studies, like this one, can be useful in order to identify high-risk BMT patients for which targeted and more effective strategies should be explored to prevent and treat IFI.

0381
INVASIVE FUNGAL INFECTIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

Y. Floisand,1 B. Fossum Løland,1 J. Reisslien,1 U. Randen,2 G. Gaustad,2 L. Brinch2
1Rikshospitalet National Hospital, OSLO, Norway; 2Rikshospitalet University Hospital, OSLO, Norway

Background. From 1989 to 2004, 360 patients underwent allogeneic stem cell transplantation (ASCT) for malignant hematologic disease. The temporal distribution differed between Candida and Aspergillus. Candida infections occurred at mean 66 days (range 9-285 post-Tx). Aspergillus infections at a mean of 146 days (range 32-519). Methods. In two patients a definitive diagnosis was established using a conditioning regimen consisting of oral Busulfan 16mg/kg and iv Cyclophosphamide 60mg/kg (Bu4Cy2). 242 and 118 patients received grafts from family donors or matched unrelated donors (MUD), respectively. Methods. In this retrospective study we analyzed the incidence and outcome of invasive fungal infections (IFI). From 1999 oral Busulfan was used in 59% of patients receiving grafts from family donors or unrelated donors (MUD). Routine primary antifungal prophylaxis was not given. Results. The incidence of IFI was 12.2% (n=44), 37 proven and 7 probable; Aspergillus species in 39%, Candida albicans in 39% and non-albicans species in 16% of the infections registered. In two patients a definitive diagnosis could not be made. In one patient both Aspergillus and Candida species were isolated. Eleven patients had evidence of IFI before ASCT and secondary prophylaxis with Amphotericin-B or Fluconazole was given. The temporal distribution differed between Candida and Aspergillus. Candida infections occurred at mean 66 days (range 9-285 post-Tx). Aspergillus infections at a mean of 146 days (range 32-519). In patients developing IFI before day 100, 63% had acute GVHD grade II. Patients developing IFI after day 100 all had chronic GVHD with a risk of 6% and 16% in limited and extensive disease. The incidence of IFI was 9.2% and 18.6% in patients receiving grafts from family donor and MUD respectively. In logistic regression analysis the following were identified as factors predisposing to IFI: 1) graft from MUD, 2) corticosteroids; 3) acute GVHD. Stem cell source (bone marrow / peripheral blood stem cells) and disease were not found to be significant contributors. The mortality in patients with IFI was 71%, 77% and 58% in the non-albicans, albicans and aspergillus groups, respectively. 63% (N=27) and 4% (N=18) of patients had concomitant GVHD or CMV infection/disease. Summary. In this retrospective single center study of ASCT patients not receiving primary antifungal prophylaxis, documented IFI were found in 44 of 360 patients (12.2%), which is of the same order as in previous studies. The mortality was high; 71-77% among patients with Candida infections and 88% in patients with aspergillus infection. GVHD, corticosteroid therapy and graft from MUD were identified as risk factors.
INTENSIFIED ENVIRONMENTAL SURVEILLANCE IN A STEM CELL TRANSPLANTATION DEPARTMENT - A PROSPECTIVE STUDY

A. Nikitin, V. Anttila, M. Richardson, L. Volin, T. Ruutu
Dept. Med., Helsinki Univ. Central Hosp., HELSINKI, Finland; Dept. Bact&Immunol, Haartman Institute, HELSINKI, Finland

Invasive aspergillosis is a serious complication with high mortality in allogeneic stem cell recipients and patients with acute leukemia. Construction work close to a haematology ward is a known risk factor for aspergillus infections. At the Helsinki University Central Hospital, heavy construction work was performed from mid October until the end of year 2005 immediately adjacent to the 13-bed HEPA-filtered stem cell transplantation ward, located on the ground floor of the building. A protective barrier was built around three close-by ventilation ducts and around the construction area. The function of the air filters was followed by daily checking of the air pressure of ventilation channels. No increase in the pressure was seen. Regular surveillance sampling was performed in the ward. Particle counts were measured for particles above 0.3 microns in all patient rooms five times a week using Particle Scan Pro, (IO Air). The median particle count was 63-420 particles/litre. One peak of 1084 particles/litre was noticed. This was associated with heavy drilling during reconstruction work inside the hospital, four floors above the ward. The particle counts of the outside air at the hospital main entrance were significantly higher, between 110806 and 185645 spores/litre. Sampling for fungal spores was performed with a Surface Air Sampler, SAS100 (pbi International, Italy). The samples were taken once a week from five different locations; three patient rooms, the construction work in the hospital main entrance. The samples from patient rooms were negative on 31 and positive on two occasions, one with Aspergillus niger (1 CFU/m³) and the other with nonpathogenic, environmental fungi. The samples from the construction area and the hospital main entrance were all positive, with 2-21 (median 9) CFU/m³ and 1-31 (median 7) CFU/m³, respectively. To rule out colonisation of the patient rooms and the patients, fungal cultures were performed. Surface samples from three different patient rooms were obtained once a week using contact plates. Of the 33 samples, 23 were negative and seven were positive but only for nonpathogenic fungi. Three samples were positive for aspergilli, two with Aspergillus fumigatus and one with Aspergillus niger. Swab samples were taken from both nares and the mouth of all patients and cultured for fungi on three occasions. All 70 nasal samples from 24 patients were negative. Of the 35 mouth samples, 18 were negative. Of the positive samples, 16 grew yeasts and one grew Aspergillus niger. This patient had been diagnosed with pulmonary aspergillosis prior to the beginning of the construction work. 35 patients were treated on the ward during this period. 15 allogeneic and 7 autologous stem cell transplantations were performed. Acute GVHD was treated in 11 patients. With a follow up time of 155 days from the beginning of the construction work, no aspergillus infections have been diagnosed in these patients. In conclusion, there were no indications of malfunctions of the HEPA-filtered stem cell transplantation ward and no invasion of fungal spores to the ward was seen and the incidence of invasive aspergillus infections did not seem to increase.

HICKMAN CATHETER-RELATED COMPLICATIONS IN ADULTS WITH HAEMATOLOGICAL/ONCOLOGICAL DISEASES: SINGLE CENTER EXPERIENCE OF 243 DEVICES

University Hospital JM Morales Meseguer, MURCIA, Spain; University Hospital Virgen de Arrixaca, MURCIA, Spain

Background. The use of tunneled central venous catheters facilitates the management of haematological and oncological patients, but is not exempt from complications. Aims. We describe our experience with Hickman catheters in a tertiary care hospital in Spain between 1992 and 2005, trying to quantify the complications and characterize them. Patients and Methods. A retrospective analysis was performed on 243 consecutive double lumen Hickman-Broviac catheters (109 of fine diameter ‘9F- and 134 of large diameter ‘15F’) inserted in 190 patients with haemopathies (151) or solid tumours (9). Catheters were inserted by the Haematologist under fluoroscopic guidance. Results. Six early complications occurred, being the most relevant a subcutaneous tunnel necrosis. In 39.3% of the catheters a late complication was observed: 28.1% infectious, 9% mechanical, and 2% both of them. The infection location: 76.2% bloodstream, 13.8% exit site and 10% tunnel infection. The most common microorganisms isolated were coagulase-negative Staphylococcus (38.8%), Pseudomonas sp. (15.8%), Klebsiella sp. (10%), Escherichia coli (8.8%) and other Gram negatives (20%). The main mechanical complications were: accidental removal (4%), device breaking and symptomatic thrombosis (1.6% in each case). The overall catheter days at risk (CVC-days) were 20.902 (median: 54 days, range: 2-486 days). The overall complication rate was 4.9/1000 CVC-days (infectious rate 3.6/1000 CVC days: mechanical rate: 1.3/1000 CVC-days). The complication rate of gross catheters was 5.9/1000 CVC-days and 5.3/1000 CVC-days in the fine diameter devices. Complications were less likely to develop in catheters inserted in patients with haemopathies compared with those with solid tumours (0.8/1000 CVC-days vs 7.4/1000 CVC-days, p=0.002). Conclusions. In our experience, the Hickman catheter-related complication rate was 4.9/1000 CVC-days. Complications are more frequent in patients with solid tumours, but we did not found an statistical significancy in the risk of complication related to the catheter size.

ORAL VALGANCICLOVIR TREATMENT FOR CYTOMEGALOVIRUS DISEASE IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

Università Cattolica del Sacro Cuore, ROMA, ITALY

Background. Valganciclovir is a valyl ester of ganciclovir which is hydrolyzed to ganciclovir before reaching the systemic circulation, having higher bioavailability than oral ganciclovir. Aims. Despite the efficacy of valganciclovir has been demonstrated in immunosuppressed patients (CMV retinitis in AIDS patients, solid organ transplant recipients, patients treated with alemtuzumab), no data exist for the establishment of an effective and pre-emptive therapy in stem cell transplantation (SCT). Methods. Starting from July 2004, valganciclovir at a dose of 900 mg os once a day, was administered in 8 out-patients submitted to allogenic SCT as pre-emptive therapy on the basis of detection of primary or reactivated CMV infection by positive antigenemia (Ag), or positive PCR. The median age was 45 years (range 21-54 years). They were affected by acute myeloid leukemia (4), aplastic anemia (1), acute lymphoblastic leukemia (2) and low grade non-Hodgkin lymphoma (1). Six patients obtained complete remission after transfusion. Acute GVHD occurred in 7 patients and chronic GVHD was noticed in 5 patients. Immunosuppressive regimen consisted of cyclosporine and methotrexate for all patients with addiction of steroids and mycophenolate or tacrolimus based on the development of acute or chronic GVHD. Antigenemia and PCR DNA for CMV were monitored twice a week. The median time of positivization of Ag and/or PCR for CMV was 45 days after transplant (range 35-65). Among evaluable patients, the mean baseline antigenemia level was 2/200.000 cells (range 1/200.000-5/200.000), whereas the mean level of DNA viral copies was 6 x 10³ /ml (range 0.320 x 10³ -24 x 10³). Results. The positivization of PCR and/or Ag for CMV occurred in 6/8 patients (75%) at a median of 2 weeks from starting valganciclovir (range 2-3 weeks). One patient required further CMV treatment for a 2nd re-activation, at 252nd day, but obtained a rapid negativization after 1 week of therapy. Two patients, not achieving negativization, were shifted to foscarnet but developed CMV pulmonary disease and died at 189 days and 431 days. The cause of the death was not attributable to CMV because they also developed recurrent of their malignancy. No significant increased myelo or nephro-toxicity was observed. Conclusions. Oral Valganciclovir was well tolerated and showed efficacy and safety without significant hematological and/or extra-hematological toxicity; moreover it allowed good compliance and outpatient management.
Hodgkin's lymphoma (NHL, 12 patients) were stratified by disease and randomly allocated to receive (prophylaxis group, 21 patients) or not receive (control group, 19 patients) prophylactic antimicrobials just prior to administration of high-dose chemotherapy. Prophylactic antimicrobials consisted of ciprofloxacin (500 mg twice daily p.o.), fluconazole (100 mg twice daily p.o.) and acyclovir (400 mg every 8 h p.o.), starting 1 day before high-dose chemotherapy (high-dose melphalan for MM and BEAM for AML) and continuing until absolute neutrophil count reached 500/mm³ after nadir or infection occurred. Lenograstim 5 µg/kg/day was given from day 1 of ASCT. Results. At least one episode of fever occurred in 15/19 (79%) patients in the control group, compared with 12/21 (57%) patients in the prophylaxis group (p=NS). Microbiologically or clinically documented infections occurred in 4 patients (21%) in the control group, but none in the prophylaxis group (p=NS). Documented infections in the control group included 3 staphylococcal bacteremias and 1 herpes skin infection. No deaths, invasive fungal infections, or serious adverse events occurred in either group. The median duration of fever (9 days in the control group and 11 days in the prophylaxis group) was shorter in the prophylaxis group (9 days in the control group and 11 days in the prophylaxis group), and hospital stay after ASCT (19 days in both groups) did not differ between the groups. Median time to neutrophil engraftment was 10 days in both groups and median time to platelet engraftment was 11 days in the control group and 12 days in the prophylaxis group. Summary/Conclusions. This small-sized prospective randomized phase II comparison showed no beneficial effect of antimicrobial prophylaxis in ASCT.

0386
PREDICTIVE FACTORS OF SEPTIC SHOCK AND MORTALITY IN NEUTROPENIC PATIENTS
R. Jeddì, M. Zarrouk, Y. Benabdennbi, K. Kacem, R. Belakhal, L. Aissaoui, H. Benabid, Z. Belhadjali, B. Meddeb
Aziza Othmana Hospital, TUNIS, Tunisia

Neutropenia is the major risk factor for developing a serious infection. Bacteriaemia still causes significant mortality (15-25%) among neutropenic patients with cancer. The purpose of this study was to identify risk factors for septic shock and for mortality in neutropenic patients with leukaemia and bacteriaemia. Consecutive sample from 20 patients with acute myeloid leukaemia and bacteriaemia was studied during 1 year (January 2003-December 2005). All patients received empiric antibiotic therapies for febrile episodes with ceftazidime plus aminocillin. 110 neutropenic febrile episodes were noted: clinically documented 14.54% (16/110), microbiologically documented 16.36% (18/110), and fever of unknown origin 69.09% (76/110). Gram-negative organism caused 8 febrile episodes (8/18); Pseudomonas (5), klebsiella pneumoniae (3), Gram-positive organism caused 10 episodes: Staphylococcus (6), streptococci (2), enterococci (2). Pulmonary infection accounted for 25% of clinically documented infections. 14 of the 110 febrile episodes were associated with septic shock causing mortality in 7 patients. The following variables influencing septic shock and mortality were analysed using binary logistic regression technique: site of infection, bacteria isolated, serum lactate, serum bicarbonate. In univariate analysis variables associated with septic shock were: pulmonary infection (OR=17, p<0.001), serum bicarbonate <17 mmol/L (OR=68, p<0.001) and serum lactate >3 mmol/L (OR=62, p<0.001). Variables associated with mortality: pulmonary infection (OR=33, p<0.001) and serum bicarbonate <17 mmol/L (OR=61, p<0.001). In multivariate analysis two variables were associated with septic shock and mortality: pulmonary infection (OR=5, p=0.043) and serum lactate >3 mmol/L (OR=10, p=0.003). Elevated serum lactate (>3 mmol/L and low serum bicarbonate (<17 mmol/L) at the onset of bacteriaemia showed strong value in predicting septic shock and mortality in neutropenic patients.

0387
ANALYSIS OF COMPLICATIONS OF CENTRAL VENOUS CATHETERS IN ADULT HAEMATOLOGY PATIENTS.
S. Tamiazzo, D. Gioia, A. Bellora, L. Gambarini, M.G. Candeo, B. Allione, F. Salvi, A. Levis
SS Antonio e Biagio Hospital, ALESSANDRIA, Italy

Background. Central venous catheters (CVC) are routinely employed in haematological patients, but their use may be complicated by many adverse events, such as catheter related infections (CR-I) and thrombosis (CR-T), that can induce an advanced removal. Aim of the Work. To analyse the fate of CVCs implanted from 2002 to 2004 in our haematological patients, with particular attention to: a) unfavourable events; b) relationship between surveillance culture and subsequent events. Methods. The records of patients whose CVC was implanted between January 2002 and December 2004 were reviewed retrospectively. All patients underwent high dose chemotherapy and/or stem cell transplantation. All patients received prophylaxis with ciprofloxacin or levofloxacin during aplasia periods. A protocol of periodic CVC surveillance cultures was started. Results. A total of 210 CVC was considered. Distribution of patients by class of age was: 44 (21%) < 40 y; 70 (37%) from 40-60 y; 88 (42%) > 60 y. Diagnosis distribution was: acute leukemia 98 (47%); lymphoma 75 (36%); multiple myeloma 30 (14%); others 7 (3%). 163 catheters (78%) were Groshong type. CVCs were inserted via subclavian vein in 196 cases (94%) and jugular vein in the remaining 12 cases (6%). Median CVC duration was 6 months, range 7 days to 18 months. Removal was independent from any CVC-related complication in 167 cases (80%), while it was performed in advance as a consequence of infections, malfunction and thrombosis in 25 (12%), 17 (8%) and 1 (<1%) respectively. At least one episode of subcutaneous fibrositic event was evident in 118 cases (56%). Ninety nine of these subcutaneous infections (84%) were managed without CVC removal. Complete microbiological information on surveillance cultures were available in 157 cases, with one or more positive culture in 53 of them (84%). A subclavian thrombosis was demonstrated in 5 (2%) patients. Advanced CVC removal was statistically independent from age, diagnosis, subcutaneous infections and positive surveillance culture. No endocarditis was demonstrated. Among patients positive for surveillance cultures, Gram+ micro-organisms were more frequent then Gram- ones: 45 (85%) vs. 8 (15%). Cultures during a fever episode were performed in 145 cases with positive results in 77 (53%). Among patients with central position, during fever episode, Gram+ micro-organisms were isolated in 55 (75%), Gram- in 14 (18%), mycobacterium in 2 (3%) and both Gram+ and Gram- in two subsequent cultures in 3 (4%). A high concordance (89%) was evident among cultures of surveillance and those performed during fever episodes. Septic events were more frequent in patients with a positive surveillance culture than in those without (62% vs 34%, p<0.01). A similar predictive value was demonstrated by subcutaneous infections (48% vs 22%, p<0.01). Conclusions. Haematology patients frequently required an advanced CVC removal, mainly for infection complications. Both positive surveillance cultures and subcutaneous infections are highly predictive of subsequent septic events and can be useful to choose an empiric antibiotic therapy in case of fever.

0388
VARICELLA-ZOSTER VIRUS INFECTIONS AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION: RELATIONSHIP WITH CD4+ CELL COUNTS.
M. Albo, I. Figueroa, C. Ares, C. Poders, E. Benitez, J. Plaza
Complejo Hospitalario de Vigo, VIGO, Spain

Introduction. Patients’ undergoing haematopoietic stem-cell transplants (H SCT) are at high risk of varicella zoster virus (VZV) reactivation and antiviral prophylaxis only appears to delay events until cessation of prophylaxis. The relationship between CD4+ lymphocyte depletion with CD4+ lymphocyte count at the time of VZV reactivation has been extensively studied in HIV infection. This relationship is not well described in the oncology population, although several studies suggest that there is indeed increase risk for viral infections with low CD4 counts. Patients and Methods. Patients: 136 patients undergoing an auto and 26 an allo-HSCT in our center from 1995 to 2005. The median age was 47 years (6-69). Methods. We report patients presenting VZV reactivation. Its clinical characteristics and CD4+ lymphocyte count were reviewed. Results. 25 patients (11.79%), 6 patients (23%) who had undergone autologous and 19 (10%) who had undergone allogeneic-HSCT, were reviewed. VZV reactivation a median of 147 days (range 25-630) post transplantation. 28% occurred in the first 3 months and 88% in the first year. In 3 patients reactivation occurred after the first year, all were allo-transplant. Infection occurred in a localised dermatal distribution in 92% of cases. 2 patients had disseminated cutaneous involvement. No patients had visceral dissemination or died. For patients (56) had a CD4+ lymphocyte count of <200 cells/microl. Conclusion. 1. VZV reactivation may be a significant infectious complication during H SCT recovery. Such infection is usually mild (85% of cases). 2. Almost all patients had a CD4+ lymphocyte count <200 cells/microl. Our data indicated that it is not the only risk factor associated with reactivation. Immune system alterations in post transplant period are complex, and the role of monitoring lymphocyte subsets is uncertain. It neither seems to correlate with severity of disease. 3. We should investigate if these patients are candidates for vaccination because antiviral
prophylaxis only appears delay events as suggest the largest period of latency observed in allotransplant patients.

0389
AUDIT OF THE USE OF CT SCANNING AND RISK STRATIFICATION IN THE DIAGNOSIS OF INVASIVE Fungal INFECTIONS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES
M.W. Besser,¹ E.J. Gudgin,¹ M.T.G. Gaskarth,¹ N.J. Screaton,¹ A.K. Dixon,¹ J.L.O. Craig,¹ R.E. Marcus¹
¹West Suffolk Hospital, BURY ST. EDMUNDS, United Kingdom; ²Dept.Haematology, Addenbrooke’s Hospital, CAMBRIDGE, United Kingdom; ³Dept.Radiology, Addenbrooke’s Hospital, CAMBRIDGE, United Kingdom

Background. Invasive fungal infections (IFIs) are a major cause of mortality and morbidity in neutropenic patients, but accurate early diagnosis remains difficult. Bronchoscopy is often problematic in such patients. As an alternative, computerized tomography (CT) examinations of the chest are non-invasive, readily available and show characteristic appearances if performed early in the course of the disease. The British Society for Medical Mycology (BSMM) has proposed standards for the diagnosis of patients with IFIs. Aims. We aimed to audit the radiological diagnosis of IFIs following these guidelines. In addition, we sought to assess the impact of risk stratification on the diagnosis of IFIs. Risk stratification models divide patients into low and high-risk groups. High-risk categories for invasive pulmonary aspergillosis are: - Neutropenia (neutrophils <= 0.2 x 10⁹/L) due to intensive chemotherapy, if prolonged for > 21 days or concomitant steroid administration. - Post-allogeneic transplant if engraftment delayed or on steroids for graft versus host disease (GvHD). - Fungal spore exposure in neutropenia or recent invasive mould infection. Methods. 32 febrile, neutropenic patients initially treated with triple antibiotic therapy were enrolled in the study. CT chest studies were requested for the following indications: - Fever unresponsive to 72 hours of antibiotics (18/39), or to 7 days of antibiotics in presence of another probable bacterial focus of infection (3/39). - Respiratory symptoms or signs (17/39, 8 with chest radiograph changes). - Positive fungal sputum cultures (1/39) CT was performed a median of 16 hours (range 1 hour - 45 hours) following request. A total of 39 examinations were performed in 32 patients. Patient details are outlined in Table 1. Statistical significance for a difference in the incidence of fungal infections between the low and high-risk groups was tested using the chi-square test. Results. CT diagnosis of pulmonary IFI was made in 11 patients (2 of whom had IFI with bacterial super-infection). Other diagnoses made were bacterial bronchopneumonia (9/39), atypical chest infection (1/39), GvHD (3/39), viral pneumonitis (2/39) and Pneumocystis carinii pneumonia (1/39). Bronchoscopy was performed in 4 cases, one of these was positive for Candida albicans. One patient died of IFI during the audit period. Patient risk was classified as high (HR: 27/39) or low (LR: 12/39) for invasive mould infections according to the Martino and Viscoli criteria. IFI was not confirmed in a single LR case, but was diagnosed in 11/27 HR patients (p=0.009). If CT imaging had been limited to high risk patients who met above criteria, a total of 12/39 examinations (51%) could have been avoided without missing a single case of IFI. Conclusion. Identification of patients at low risk of IFI by risk stratification may save resources, and reduce the use of empirical antifungal agents. CT is a useful diagnostic tool in IFIs.

Table 1. Patient characteristics (n=32)

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</table>

0390
CLINIC-ANALYTIC PROFILE AND USEFULNESS OF BONE MARROW SMEAR EXAMINATION AND SEROLOGICAL METHODS IN PATIENTS WITH VISCERAL LEISHMANIASIS: EXPERIENCE OF OUR CENTER
General Hospital of Cartellon, CASTELLON, Spain

Background. The Mediterranean area is an endemic region of visceral leishmaniasis (VL). With the advent of human immunodeficiency virus (HIV) infection, the number of cases of VL has dramatically increased in this area over the last years, mainly in adults. Aims. To analyze the clinic-analytic profile and usefulness of methods used in diagnosis of visceral leishmaniasis in our center. Patients and Methods. A total of 58 cases of VL were reviewed retrospectively from January 1989 to September 2005. Sex, age, clinic and analytic profile, diagnostic methods and HIV infection were studied. The median age was 29 years (range 0-81 years) with 72% males (n=42) and 28% females (n=16). At diagnosis 97% presented fever (n=56), 91% splenomegaly (n=58), 71% hepatomegaly (n=53) and 19% pancytopenia. In 76% of patients (n=44) the hemoglobin was <100 gr/L, 34% (n=20) neutrophil count <1000 cells/mm³ and 41% had thrombocytopenia (c<100x10⁹/L). HIV infection affected 21 patients (56%) and the median hemoglobin of our series was 89 gr/L (range 53-135). The methods used in our center for the diagnosis of VL are bone marrow smear examination and serology methods (Indirect immunofluorescent antibody test (IFAT) and ELISA). The statistical analysis was performed using the program SPSS v10.0. Results. The serodiagnosis of VL was positive in 26 cases and direct examination of the bone marrow smear yielded the diagnosis in 52 cases. The sensitivity of serologic studies was significantly lower in HIV(+) than in HIV patients (p=0.031). The 6 cases with negative examination of the bone marrow smear were HIV (+p=0.057). Conclusions. The diagnosis of VL should be based in a direct examination of the bone marrow smear in combination with another diagnostic procedure. A negative serology is possible in HIV+ patients. When PCR is not available the diagnosis method of choice in HIV/leishmanias co-infected patients is direct examination of the bone marrow. In HIV(-) patients the sensitivity of serological methods is better than that of direct examination.
DEEP VEIN THROMBOSIS AND PULMONARY EMBOLISM CAN BE TREATED AT HOME IN PATIENTS WITH CANCER

University of Palermo, PALERMO, Italy

Background. Outpatient treatment of deep vein thrombosis (DVT) has become a common practice in uncomplicated patients. Scanty data are present in patients with comorbidity (such as cancer) or concomitant symptomatic pulmonary embolism (PE). Cancer patients with Venous Thromboembolism (VTE) are often excluded from home treatment because of high risk of bleeding and recurrent thrombosis. We tested the feasibility and safety of the home-treatment program in cancer patients with acute VTE. Material and Methods. Consecutive cancer patients having a confirmed episode of DVT or PE were treated as outpatients unless they required admission for other medical problems, were actively bleeding or had pain treated with i.v. narcotics. As anticoagulants, patients received standard therapy with Low Molecular Weight Heparin (LMWH) followed by warfarin or LMWH alone, at therapeutic dosages; all of them were treated for 6 months. At the index visit, an educational program for self-injection and clinical surveillance was implemented. Results. Over a period of 3 years, 207 patients with cancer and acute VTE (139 with DVT and 68 with PE) were evaluated; 56 (17.4%) of them had metastatic disease. Treatment with standard anticoagulation (LMWH followed by warfarin) was given to 106 (51.2%) while LMWH alone to 102 (48.8%) patients. One hundred and twenty-seven patients (61.3%) (91 with DVT and 36 with PE) were entirely treated at home. In the remaining patients, reasons for hospital admission (n. 80) were poor compliance (22, 27.5%), concomitant serious illness (26, 65%) and refusal of home-treatment (6, 7.5%). There were no differences between patients treated at home and those hospitalized with regard to gender, mean age, site of cancer, presence of metastases and choice of anticoagulants (Table).

Table 1. Clinical events at 6 months of follow-up.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>48 DVT</th>
<th>32 PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>45.8%</td>
<td>62.5%</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>54.2%</td>
<td>37.5%</td>
</tr>
<tr>
<td>Age (y) Median</td>
<td>60.5</td>
<td>60.5</td>
</tr>
<tr>
<td>Age (y) 25-75%</td>
<td>57.3</td>
<td>60</td>
</tr>
<tr>
<td>DVT (%)</td>
<td>39.9%</td>
<td>28.1%</td>
</tr>
<tr>
<td>PE (%)</td>
<td>60.1%</td>
<td>71.9%</td>
</tr>
<tr>
<td>Proximal DVT (%)</td>
<td>42.9%</td>
<td>40.6%</td>
</tr>
<tr>
<td>Distal DVT (%)</td>
<td>57.1%</td>
<td>59.4%</td>
</tr>
<tr>
<td>Symptoms of PE (%)</td>
<td>25.0%</td>
<td>24.4%</td>
</tr>
<tr>
<td>ETP (%)</td>
<td>25.0%</td>
<td>24.4%</td>
</tr>
</tbody>
</table>

Table 1. The IC50% for each parameter of TG.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lag-time (50%)</th>
<th>Max Cmax (50%)</th>
<th>ETP (50%)</th>
<th>Cmax (50%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bemiparin</td>
<td>&gt;1 IU/mL</td>
<td>&gt;1 IU/mL</td>
<td>0.86 IU/mL</td>
<td>0.85 IU/mL</td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>0.62 IU/mL</td>
<td>0.58 IU/mL</td>
<td>0.95 IU/mL</td>
<td>0.85 IU/mL</td>
</tr>
<tr>
<td>Nadroparin</td>
<td>0.50 IU/mL</td>
<td>0.55 IU/mL</td>
<td>0.65 IU/mL</td>
<td>0.45 IU/mL</td>
</tr>
<tr>
<td>Dalteparin</td>
<td>0.50 IU/mL</td>
<td>0.50 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.38 IU/mL</td>
</tr>
<tr>
<td>Tinzaparin</td>
<td>0.50 IU/mL</td>
<td>0.35 IU/mL</td>
<td>0.25 IU/mL</td>
<td>0.18 IU/mL</td>
</tr>
<tr>
<td>UFH</td>
<td>0.05 IU/mL</td>
<td>0.10 IU/mL</td>
<td>0.25 IU/mL</td>
<td>0.18 IU/mL</td>
</tr>
</tbody>
</table>

Summary/Conclusions. Our study reinforces the concept of LMWH heterogeneity and the important effect exerted by the additional anti-IIa activity of LMWHs, combined with their anti-Xa activity. Thus, their characterization can be made through their ability to inhibit TG and not only their anti-Xa/anti-IIa ratio. Furthermore, the anti-IIa inhibitory activity of heparins is primarily expressed by prolonging the lag-time and the Tmax and by reducing the TG velocity. The clinical relevance of our findings has to be studied, while the use of TG assay should be considered as a potent method to monitor anticoagulant treatment with LMWHs in the routine hematological laboratory.

RISK OF RECURRENT VENOUS THROMBOEMBOLISM ASSOCIATED WITH PREGNANCY IN WOMEN WITH A HISTORY OF VENOUS THROMBOSIS

A. Gerhardt, R.E. Scharf, R.B. Zott
Universitätsklinikum Düsseldorf, DUSSELDORF, Germany

Background. Previous estimates of the rate of recurrent venous throm-

A GLOBAL ASSAY FOR THE ASSESSMENT OF LOW MOLECULAR WEIGHT HEPARINS

A. Petropoulou, G. Gerotziafas, M.M. Samama, I. Elalamy
Hotel-Dieu Hospital, PARIS, France

Background. Low molecular weight heparins (LMWHs) are derived from unfractioned heparin (UFH) by depolymerization. Thus, they present biochemical and pharmacological differences and the ratio of the anti-Xa/anti-IIa activities varies from one product to another. LMWHs have no effect on prothrombin time and there is no global clotting assay for the in vitro assessment of their antithrombotic activity. Furthermore, the anti-Xa activity measurement, which is routinely used in clinical practice for monitoring the anticoagulant treatment with LMWHs, has a limited predictive value concerning the clinical outcome (thrombosis or bleeding). Aims. The aim of the present study was to assess the LMWHs global antithrombotic activity by using a rather physiologically relevant system. For this purpose we used the Thrombograms-Thrombinscope assay, a dynamic assay which describes all the phases of thrombin generation (TG) process (initiation, amplification and inhibition of TG as well as the integral amount of generated thrombin). Methods. TG was assessed after tissue factor (TF) pathway activation in platelet rich plasma (PRP) (1.5x105 platelets/µL) using diluted thromboplastin (Dade Innovin®, 1:1000 final dilution). We studied five different LMWHs (bemiparin, enoxaparin, nadroparin, dalteparin and tinzaparin), as well as UFH at five different prophylactic and therapeutic anti-Xa final concentrations. These agents were added to control plasma from 14 healthy volunteers with equivalent anti-Xa concentrations. TG was initiated by adding the triggering solution containing CaCl2 and the hemo
geric substrate. The analyzed TG parameters are the lag-time, the maximal concentration of thrombin (Cmax), the time to reach Cmax (Tmax), the TG velocity and the endogenous thrombin potential (ETP).

Results. Bemiparin had almost no effect on TG, with concentrations below 0.60 anti-Xa IU/mL. Enoxaparin, nadroparin and dalteparin showed a similar potential in inhibiting TG at equal anti-Xa concentrations. Tinzaparin proved to be the most active LMWH in inhibiting TG and had a similar potency to UFH. Tinzaparin and UFH, with the lowest anti-Xa/anti-IIa ratio, exerted their inhibitory effect mostly by prolonging the lag-time and Tmax and by reducing TG velocity, especially at concentrations below 0.40 anti-Xa IU/mL. Besides, UFH totally inhibited TG, as expressed by ETP, at a concentration over 0.40 anti-Xa IU/mL. For a given anti-Xa/anti-IIa ratio characterizing each LMWH the IC50 for each parameter was different. The IC50 for the reduction of the velocity of TG was lower as compared to the IC50 for the other parameters. (Table 1).

Table 1. The IC50% for each parameter of TG.

<table>
<thead>
<tr>
<th>Antithrombotic Activity</th>
<th>Lag-time (50%)</th>
<th>Max Cmax (50%)</th>
<th>ETP (50%)</th>
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boembolism (VTE) during pregnancy in women with a history of VTE have vary between 0 and 15%. Therefore, the decision to administer or withhold heparin especially in the antepartum period has been discussed controversial. In a recent study by Brill-Edwards et al. (N Engl J Med 2000;343:1439-44), no recurrences of VTE occurred in women (n=44) who had a previous episode of thrombosis that was associated with a temporary risk factor and who also had no evidence of thrombophilia. Based on these results, antepartum heparin prophylaxis is not routinely recommended in women without thrombophilia whose previous episode of thrombosis was associated with a temporary risk factor (ACCP guidelines 2004). The aim of our study was to evaluate the risk of recurrent pregnancy-associated thrombosis in women with a history of VTE. Materials and Methods. We retrospectively studied 198 women with at least one pregnancy (275 pregnancies in total) after a previous episode of VTE. Thirty-six women (81 pregnancies) were excluded from the analysis because of antepartum heparin prophylaxis. Results. In the subgroup of women without heparin prophylaxis (n=135), 15 (7.7%) thromboembolic events occurred antepartum in 194 pregnancies. Further subgroup analysis, stratified for the nature of first VTE, gave the following number of antepartum VTE per number of pregnancies: 2 VTE/19 pregnancies (10.5%) in 14 women (first VTE: immobility), 4 VTE/35 pregnancies (12.1%) in 24 women (first VTE: surgery), 5 VTE/69 pregnancies (7.2%) in 46 women (first VTE: oral contraception), 2 VTE/25 pregnancies (8%) in 24 women (first VTE: pregnancy); 2 VTE/15 pregnancies (13%) in 11 women (first VTE: idiopathic). Nine of the 15 women with VTE (7/18 women with first VTE triggered by temporary risk factor; 2/2 women with first idiopathic VTE) had a heterozygous factor V Leiden G1691A or prothrombin G20210A gene mutation. In Conclusion, the risk of recurrent antepartum VTE was similar in women with and without factor V Leiden G1691A or the prothrombin G20210A gene mutation and did not differ between women with first VTE triggered by a transient risk factor or an idiopathic first VTE. In addition to recommended postpartum heparin prophylaxis, our data support the need for a routine antepartum prophylaxis in women without thrombophilia whose previous episode of thrombosis was associated with a temporary risk factor.

0394 MAJOR HAEMORRHAGE BEFORE VENOUS THROMBOEMBOLISM: DIFFERENT OUTCOMES DEPENDING ON THE BLEEDING SITE J.A. Nieto Rodríguez,1 M.J. Bruscas Alijarde,2 M.D. Ruiz Ribó,2 E. Grau Segura,2 R. Lecumberri,1 A. Grau Martín,1 E. Rague Sanz,1 R. Guijarro Merino3 1Hospital Virgen de la Luz, CUENCA, Spain; 2Hospital Luis Alcañiz, VALENCIA, Spain; 3Clínica Universitaria de Navarra, PAMPLONLA, Spain; 4Hospital de Figueres, GERONA, Spain; 5Hospital de Terrassa, BARCELONA, Spain; 6Hospital Carlos Haya, MALAGA, Spain

Background. Patients with a recent episode of major bleeding are usually excluded from clinical trials. The management of these patients is not evidence based and their outcomes are unknown. Aims. To study outcomes of patients with VTE and a recent episode (<30 days) of major bleeding before VTE diagnosis, according to the bleeding site and the time interval between bleeding and VTE. Methods. Analysis of the data from a prospective, multicentre registry of VTE (RIETE) entering consecutive patients with VTE diagnosed by objective tests. Patient characteristics, antithrombotic treatments and 3-month outcomes were recorded. Results. Of the 12,502 patients enrolled up to July 2005, 306 (2.5%) patients had had a recent episode of major bleeding, 106 (35%) gastrointestinal (GI), 94 (30%) intracranial, and 96 (32%) from other sites. When compared with the group of patients without recent haemorrhage, the mortality rate (14.1% vs. 8.0%), major haemorrhage rate (6.2% vs. 2.3%) and fatal haemorrhage rate (2.6% vs. 0.5%) were significantly higher (p<0.01) in the recent bleeding group. With the exception of the intracranial site, previous bleeding patients had an increased risk of new bleeding: GI HR 1.9, 95% CI: 1.9-4.9, (p<0.01); Other HR 1.4, 95% CI: 1.2-2.3; Other other HR 2.0, 95% CI: 1.2-3.3, Other Other HR 2.0, 95% CI: 1.2-3.3. Episodes of major bleeding were associated with previous GI haemorrhage (HR: 2.8, 95% CI: 1.4-5.3). A time interval of less than 2 weeks between major bleeding and VTE diagnosis was associated with an increased risk of the postmenopausal age group, VTE in 194 pregnancies occurred after live birth in the 153 women without heparin prophylaxis. Nine of these 16 women had a heterozygous FVL or prothrombin G20210A gene mutation. In Conclusion, the risk of recurrent antepartum VTE was similar in women with and without factor V Leiden G1691A or the prothrombin G20210A gene mutation and did not differ between women with first VTE triggered by a transient risk factor or an idiopathic first VTE. In addition to recommended postpartum heparin prophylaxis, our data support the need for a routine antepartum prophylaxis in women without thrombophilia whose previous episode of thrombosis was associated with a temporary risk factor.

0395 SUBOPTIMAL DOSES OF LOW MOLECULAR WEIGHT HEPARIN IN THE TREATMENT OF VENOUS THROMBOEMBOLIC DISEASE M.J. Bruscas Alijarde,1 J.A. Nieto Rodríguez,2 M. Pérez Pinar,2 M.T. Orue Lecue,2 I. López Lagunas,2 P. Román Sánchez,2 E López Chuliá,2 J. Sanchis Cervera2 1Hospital Virgen de la Luz, CUENCA, Spain; 2Hospital de Navarra, PAMPLONLA, Spain; 3Hospital San Agustín, ASTURIAS, Spain; 4Hospital General de Requena, VALENCIA, Spain; 5Hospital Arnau de Vilanova, VALENCIA, Spain; 6Hospital de la Plana, CASTELLON, Spain

Background. A number of patients with venous thromboembolism (VTE) are treated with suboptimal doses of low molecular weight heparin (LMWH). However, there are no clinical trials that have established the efficacy of these doses. Aims. The objective of this study was to evaluate the evolution of patients treated with suboptimal LMWH (60-149 UI/Kg/d) as compared with patients treated with standard doses (150 UI/Kg/d). Methods. Analysis of data from a prospective, multicentre registry of VTE (RIETE) entering consecutive patients with VTE diagnosed by objective tests. Patient characteristics, antithrombotic treatments and 3-month outcomes were recorded. Results. Up to July 2005, 10,524 were diagnosed with deep vein thrombosis (DVT) or pulmonary embolism (PE). During the first two weeks, major bleeding rate (4.8% vs. 1.6%) and renal failure (15.6% vs. 13%). Standard doses of LMWH were more frequently used in patients with proximal DVT (80.6%, PE (42.5% vs. 28.8%). At the end of the follow-up, there were no significant differences in the rates of mortality (7.7% vs. 7.8%), VTE recurrence (2.7% vs. 2.3%), or fatal haemorrhage (3.2% vs. 2.6%) between the suboptimal and the standard group. Neither were there any differences in the subgroup of patients with PE (mortality, 10.1% vs. 9.7%; recurrence, 2.7% vs. 2.5%; fatal haemorrhage, 0.9% vs. 0.6%) nor in the whole group after a 2-week follow-up (mortality, 2.7% vs. 2.9%; recurrence, 0.8% vs. 0.8%; fatal haemorrhage, 0.3% vs. 0.3%). During the first two weeks, major bleeding rate was significantly higher in the suboptimal LMWH group (2.0 vs. 1.1%; p=0.05). In multivariate models, entering relevant baseline risk factors, etiology, and mortality nor new bleeding in patients treated with the use of suboptimal doses of LMWH. Conclusion. Suboptimal doses of LMWH were more frequently given to patients with prior recent bleeding, renal failure and less critical clinical manifestations, and were not associated with an increased mortality or recurrence rate.

0396 ELEVATED PROTHROMBIN FRAGMENT F1+2 LEVELS DURING PREGNANCY IN WOMEN WITH PREVIOUS VENOUS THROMBOEMBOLISM A. Gerhardt,1 S. Marzotko,2 H.G. Bender,2 R.E. Scharf,2 R.B. Zottz1 1Universitätsklinikum Düsseldorf, DUESSELDORF, Germany; 2Universitätsklinikum Düsseldorf, DUESSELDORF, Germany

Background. Changes in blood coagulation and fibrinolysis during pregnancy create a state of hypercoagulability. This phenomenon predisposes to venous thromboembolism. Women with prior venous thromboembolism are believed to have a higher risk of venous thromboembolism in a subsequent pregnancy. The risk is higher if the past episode was unprovoked, and the risk is higher if the past episode was associated with biochemical abnormalities such as factor V Leiden G1691A (factor V Leiden). Since the positive predictive value of factor V Leiden and other thrombophilic conditions for a pregnancy associated thrombosis is low, additional indicators of hypercoagulability are needed. Indicators of hypercoagulability in normal pregnancy are increased levels of prothrombin fragment 1+2. Aims. We hypothesized that women with factor V Leiden or a previous venous thromboembolism are at a higher hypercoagulable state during subsequent pregnancies than women without prior thromboembolic complications or without factor V Leiden. Methods. In a prospective study, we determined prothrombin fragment F1+2

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over pregnancy among 109 women (175 measurements) with previous venous thromboembolism, and among 75 pregnant women (75 measurements) without previous venous thromboembolism. The prothrombin fragment F1+2 levels were statistically analyzed over time using a mixed model. This model allows a longitudinal analysis of the influence of a between-subjects factor (e.g., history of thrombosis) on prothrombin fragment F1+2 levels, the importance of prothrombin fragment F1+2 levels independent from the interaction of the history of thrombosis and weeks of gestation representing a change of risk factor-dependent differences over time (weeks of gestation). Results. Among women with a previous history of venous thrombosis, prothrombin fragment F1+2 values were significantly higher during the course of pregnancy than among pregnant women without a previous history of venous thrombosis (p<0.001). The results were adjusted for the physiological increase of prothrombin fragment F1+2 over pregnancy and independent from heparin prophylaxis. In addition, factor V Leiden was independently associated with increased levels of prothrombin fragment F1+2 (p<0.05). Conclusion. Thus, determination of indicators of hypercoagulation like prothrombin fragment F1+2 represent an additional approach independent from known and unknown risk determinants of thrombosis to identify women at risk for venous thromboembolism during pregnancy.

RECURRENT FETAL LOSS: PROSPECTIVE EVALUATION OF THE EFFICACY OF THREE DIFFERENT THROMBOPROPHYLAXIS REGIMENS: ASPRIN VERSUS LOW MOLECULAR WEIGHT HEPARIN VERSUS LOW MOLECULAR WEIGHT HEPARIN PLUS ASPRIN

A. Chistiolini, F. Torelli, A. Giancotti, P. Pignoloni, B. Mutto, C. Cosimo, C. Santoro, M.G. Mazzucoconi

University La Sapienza Rome, ROME, Italy; University La Sapienza, ROMA, Italy

Background. Growing evidence suggests that thrombophilia is associated with an adverse pregnancy outcome, but there is a lack of controlled trials of antithrombotic prophylaxis to prevent pregnancy complications. Aims. The aim of our study was the prospective evaluation of the efficacy of three different thromboprophylaxis regimens in women with two or more unexpected pregnancy losses. Methods. A total of 361 women with pregnancy loss were studied for thrombophilia: 226 (72.6%) did not present thrombophilia; 99 (27.4%) were positive for one or more thrombophilic parameters. 94/361 women got pregnant (none of these patients presented APA syndrome); 56 (59.6%) with negative congenital thrombophilic screening, 38 (40.4%) with positive congenital thrombophilic screening. These 94 patients were randomly assigned to one of the three thromboprophylaxis regimens from the 8th week of pregnancy: low dose aspirin 100 mg daily (arm A), enoxaparin 40 mg daily (arm B), aspirin 100 mg plus enoxaparin 40 mg daily (arm C). All patients with thrombophilia were treated with implemented enoxaparin at the 36th week of pregnancy and the 6th week after delivery. Results. Thromboprophylaxis was associated with 73 (77.7%) live births and 21 (25.3%) pregnancy losses: in a total of 305 previous pregnancies, we observed 37/305 (12.1%) live births and 268/305 (87.9%) pregnancy loss (p<0.0001). In the 56 patients with negative thrombophilia screening, thromboprophylaxis was associated with 49 (87.5%) live births and 7 (12.5%) pregnancy losses: in a total of 150 previous pregnancies, these negative patients had 18/150 (12%) live births and 132/150 (88%) pregnancy losses (p<0.0001). Considering the three different therapeutic regimens, we noted in arm A: 14/19 (73.7%) live births and 5/19 (26.3%) pregnancy losses; arm B: 16/18 (88.9%) live births and 2/18 (11.1%) pregnancy losses; arm C: 19/19 (100%) live births and 0/19 (0%) pregnancy losses. In the 38 patients with positive thrombophilia screening, the thromboprophylaxis was associated with 24 (63.2%) live births and 14 (36.8%) pregnancy losses: in a total of 155 pregnancies, these positive patients had 19/155 (12.2%) live births and 136/155 (87.8%) pregnancy losses (p<0.0001). In these patients, considering the three different therapeutic regimens, we noted in arm A: 3/12 (25%) live births, and 9/12 (75%) pregnancy losses; in arm B: 10/15 (66.7%) live births and 5/15 (33.3%) pregnancy losses; in arm C: 11/13 (84.6%) live births and 2/13 (15.4%) pregnancy losses. Conclusions. Our study shows that thromboprophylaxis therapy is effective in women with recurrent pregnancy losses. In the negative thrombophilic patients, thromboprophylaxis is effective (87.5%) live births. In these patients, no difference was found in the three therapeutic regimens. In the positive thrombophilic patients, therapy with enoxaparin or aspirin plus enoxaparin was more effective than aspirin treatment (p=0.0169 and p=0.0048, respectively). No difference was found between enoxaparin versus aspirin plus enoxaparin.

A THROMBOELASTOGRAPHIC STUDY IN WHOLE BLOOD EMBOLIZING A FIBRIN POLYMERIZATION INHIBITOR (PFA-200) AND AN INHIBITOR OF ACTIN POLYMERIZATION (CYTOCHALASIN D)

A. Petropoulou, G. Gerotziafas, M.M. Samama, T. Chakroun, M. Hatmi, I. Elalamy

Hotel-Dieu Hospital, PARIS, France; Institut Pasteur, PARIS, France

Background. Minimal tissue factor (TF) triggered whole blood thromboelastography (TEG) provides a valuable tool for studying the kinetics of thrombus formation (expressed by the parameters θ, k and λ-angle) and the physical characteristics of the thrombus, such as its firmness and size. In the present study, the elastic modulus shear expressed by the parameters of maximal amplitude (MA) and the G respectively. Aims. We studied the influence of fibrin polymerization and platelet functional status on the thromboelastographic trace after minimal TF pathway activation in whole blood using increasing concentrations of a fibrin polymerization inhibitor (Gly-Pro-Ar-Gro-OH, AcOH; Fetalbloc-FG) and an inhibitor of actin polymerization (Cytochalasin D). Methods. Coagulation was triggered in a plastic disposable cup containing 20 µl CaCl2 (0.2M) and 10 µl of diluted thromboplastin by the addition of 380 µl whole blood, supplemented with Fetalbloc-FG or Cytochalasin D. Data acquisition was done during
60 minutes, and five coagulation parameters were analysed: (1) R-time (min): time from the start of the sample run to the point of first significant clot appearance corresponding to an amplitude of 2 mm, (2) k-time (min): time from R-time until the level of clot firmness reaches an arbitrary value of 20 mm, (3) α angle (degree): reflects the kinetics of clot development, (4) MA (mm): maximum amplitude reflects the maximum strength of the clot, and (5) G: reflects clot firmness. Results. Pefabloc-FG at concentrations higher than 5 mg/mL prolonged the R and k-times and decreased the α angle in a concentration-dependent manner but it did not modify MA and G. Pefabloc-FG at 5 mg/mL, completely inhibited thrombus formation. Cytochalasin D did not modify R-time but decreased the α-angle, MA and G. The effect of cytchalasin D was pronounced on MA and G. A combination of Pefabloc-FG (0.5 mg/mL) and cytochalasin D (50 µM) significantly decreased α-angle compared to as well as their single effect. However, G was dramatically reduced in the presence of cytochalasin D, without any additional effect of Pefabloc-FG. Conclusions. This study confirms the importance of fibrin polymerization on the kinetics of thrombus formation and demonstrates the close association between the quality of the thrombus and the functional status of platelets. Normal platelet contractile forces are of major importance for the maximum amplitude of TEG which is related to the strength and elastic modulus of the thrombus.

0400

PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES HAVE A HIGH INCIDENCE OF ANTI-ADAMTS13

S. Austin,1 A. Lawrie,1 R. Starke,1 M. Scully,1 S.J. Machin,1 H. Cohen,1 I. Mackie1
1University College London, LONDON, United Kingdom; 2University College London Hospitals, LONDON, United Kingdom

Background. Thrombotic thrombocytopenic purpura (TTP) and antiphospholipid syndrome (APS) are autoimmune diseases associated with thrombosis. TTP is associated with thrombocytopenia, microangiopathy, variable micro-organ ischaemia and reduced ADAMTS13 activity. The mechanism of thrombosis in APS is unclear, but catastrophicic cases of APS can result in similar microangiopathic features to those of TTP. Aim. Autoantibodies that neutralise ADAMTS13 are commonly found in patients with acquired idiopathic TTP, but their incidence in other thrombotic microangiopathies is less well investigated. Method. In our ongoing study we have currently assessed 76 patients with antiphospholipid antibodies (66/76 primary APS, 8/76 SLE, 2/76 other secondary APS). Results. We found IgG ADAMTS13 antibodies (Imubind ELISA Kit, American Diagnostica Inc) in 59/76 (71%). The ELISA normal reference range was <9.6, and these patients had a mean of 15.3 micrograms per millilitre (range 9.7–53.5). Of 43 patients assessed for ADAMTS13 activity, 12 (27.9%) had reduced ADAMTS13 activity by collagen binding technique (median 18%, range 0.4–85%, NR 66–126%). 7 patients were positive for IgG anti-ADAMTS13 and had low activity, however, some patients were only abnormal in one or the other assay. Low ADAMTS13 activity was not associated with excessively high VWF: antigen suggesting that ADAMTS13 was not depleted due to high VWF turnover. Antibodies to ADAMTS13 in TTP are primarily inhibitory. In the APS population, it is not known whether ADAMTS13 IgG antibodies neutralise activity or cause immune complex formation with subsequent removal. In addition, some patients may have other classes of antibody to ADAMTS13 (eg. IgM or IgA). We intend to further characterise these antibodies. Conclusion. We hypothesise that the presence of ADAMTS13 antibodies in APS may contribute to the pathophysiology of thrombosis in APS.

0401

B-VITAMIN SUPPLEMENTATION INCREASES MARKERS OF ENDOTHELIAL FUNCTION IN PATIENTS WITH VENOUS THROMBOEMBOLISM

C.A. Rodrigues,1 R.C. da Silveira,1 M.A.E. Noguti,1 V.M. Morelli,1 V. D’Almeida,1 A.A. Garcia,1 E.H.A. Maffei,1 D.M. Lourenço1
1Universidade Federal de Sao Paulo, SAO PAULO, Brazil; 2Universidade de Sao Paulo, RIBEIRAO PRETO-SP, Brazil; 3Universidade Estadual Paulista, BOTUCATU-SP, Brazil

Background. Mild hyperhomocysteinaemia is associated with an increased risk of venous thromboembolism (VTE) and other cardiovascular events. The pathophysiological mechanism that explains this association is unclear, but in vitro studies suggest impaired endothelial function in hyperhomocysteineemic patients. Whether decreasing homocysteine with B-vitamin supplementation interferes with its effects on the endothelium is still to be determined. Aims. This study was designed in order to evaluate the correlation between homocysteine and markers of endothelial function and to evaluate the effect of vitamin supplementation on these markers in patients with VTE. Methods. This study was a multicentre, randomized, double-blind, placebo-controlled trial. We randomized 105 patients with a first event of objectively confirmed VTE, 14–75 years of age, with homocysteine level between 15 and 18 µmol/L, received antithrombotic therapy (folinic acid 5 mg, vitamin B6 50 mg and vitamin B12 0.4 mg) or placebo. Blood was collected at randomization and 8 weeks after the intervention period. Results. Ninety-nine (94.3%) completed the 8-week period of treatment. We compared patients with normal homocysteine above the highest tertile (12.6 micromol/L) with those below the lowest tertile (9.9 micromol/L). There was a significant difference in the plasma levels of plasminogen activator inhibitor type1 (PAI-1), Factor VIII·C and von Willebrand factor antigen (VWF) between the two groups. Vitamin supplementation decreased the homocysteine median levels from 10.7 to 8.1 micromol/L (29% reduction). There was a significant increase in the levels of tissue plasminogen activator (t-PA) from 6.1 to 9.0 mmol/L in the group treated with vitamins, (p=0.0008, Wilcoxon rank-sum test) and also in the group treated with placebo, although less evident (from 8.0 to 9.5 mmol/L, p=0.08). PAI-1 levels did not change after 8 weeks both in the vitamin and in the placebo groups. Both t-PA and PAI-1 levels significantly increased only in the group of patients above the highest tertile of homocysteine who had received vitamin supplementation (p=0.0004 and p=0.014, respectively). There was no change in the levels of these two markers in patients with homocysteine levels below the lowest tertile or in patients who received placebo with higher and lower homocysteine levels. VWF and factor VIII·C were unaffected by both vitamins and placebo, even in patients above the highest tertile of homocysteine. Conclusions. In patients with VTE, homocysteine reduction by B-vitamin supplementation caused a significant increase on t-PA. In patients with higher levels of homocysteine both t-PA and PAI-1 were increased by vitamins, although the basal levels of both markers were similar to the patients with lower levels.

0402

D-DIMER LEVEL IS ASSOCIATED WITH THE SEVERITY OF PULMONARY EMBOLISM

W. Ghannina,1 M. Abdelnoor,1 L.O. Holmen,1 B.E. Nielsen,1 S. Ross,1 P.M. Sandset7
7Oslofjord Hospital in Fredrikstad, FRODRIKSTAD, Norway; 2Ullevi University Hospital, OSLO, Norway; 3Oslo University Hospital, OSLO, Norway

Background. PE is a potentially fatal condition with a 1-month mortality rate reaching 15%. D-dimer is widely used as an initial test in the work-up of suspected patients. While the presence of PE is often determined by clinical, biochemical and radiological parameters. Patients and Methods. From Feb 2002 to Dec 2003, 99 consecutive patients were diagnosed with PE using 4-detector row CT at the Oslofjord Hospital Trust Fredrikstad, Norway. All patients had elevated ST-A latest D-dimer (cut-off ≤ 0.4 mg/L). Pulmonary artery obstruction index (PAOI) and Right Ventricular/Left Ventricular ratio (RV/LV) were assessed retrospectively. Troponin T (TNT) was assayed in 67 patients within 48 hour following the establishment of diagnosis; levels >0.11 ng/ml were regarded as indicating myocardial injury. Results. The median value for D-dimer was 5.0 mg/L (inter-quartile range: 1.8–12.2). There was a significant linear association (p=0.0001) between log-D-dimer and between log-RV/LV (r=0.48, log-PAOI) (r=0.45), PAO2 (r=0.40), F(A-a)O2 gradient (r=0.45) and log-duration of hospital stay (r=0.25). The multivariate analysis showed an increased association between Log-D-dimer and between Log-RV/LV ratio (p=0.05, p=0.0005) and log-PAOI (r=0.52, p=0.0005) after adjusting for age, gender and for the duration of symptoms. A significant association was found between D-dimer and the most proximal level of PE. Moreover, a significant dose-response relationship was found between the level of D-dimer (low level = lower quartile of D-dimer values, n=26; intermediate level = interquartile range, n=48; high level = upper quartile, n=25) and between TNT (table) and the frequency of thrombolysis. Of the 96 patients who received thrombolysis (PE was overlooked in 3 patients), 84 patients (87%) were treated with heparin, while 12 patients (13%) received systemic thrombolysis. In the subgroup of patients with D-dimer in the upper quartile, 8 patients (33%) received thrombolysis, compared to 4 in the intermediate and none with low D-dimer. There were no in-hospital
we have shown that the level of D-dimer is related to the severity of PE assessed by various radiological, biochemical and clinical markers. Hence D-dimer could be of value as prognostic marker for the severity of PE. However, the low mortality rate precludes us from making conclusions regarding the predictive value of D-dimer on mortality. The prognostic value of D-dimer and its clinical significance need to be evaluated in properly designed prospective studies.

| Table 1 |
| D-dimer mg/L | Low (0.5-1.8) | Intemed. (1.9-12.1) | Hight (12.2-20) | p-value |
| N=26 | 20 (17) | 18 (16) | <0.0005 |
| N=48 | 21 (19) | 16 (21) | <0.0005 |
| N=25 | 12 (10) | 12 (34) | <0.0005 |

*p TNT was measured in 67 patients; 18 in Low, 31 in intermediate and 18 in the high D-dimer category.

0404
THE VALUE OF THE DETERMINATION OF ACTIVATED PROTEIN C RESISTANCE (APC-R) IN HEMODIALYSIS PATIENTS

E. Androulakis,1 G. Tzenakis,1 H. Kallinou,1 K. Nenakias,1 X. Manidakis,1 G. Stylianou,1 D. Dalianys,1 E. Malliaraki,1 K. Dafnis1
1 Iraklion University Hospital, IRAKLION, Greece; 2Nephrology Dept. University Hospital, IRAKLION, Greece; 3Int. Medicine Dept S. Dimitrios Hospital, THESSALONIKI, Greece; 4Microbiology Lab. Univ. Hospital, IRAKLION, Greece; 5Haematology Lab. Univ. Hospital, IRAKLION, Greece; 6Clinical Biochemistry Univ. Hospital, IRAKLION, Greece; 7Nephrology Dept. of Iraklion University, IRAKLION, Greece

Background. The genetic mutation of factor V Leiden which is characterized by an increased resistance to activated protein C (APC-R) is one of the most common inherited thrombophilia factors. Recent studies suggest that about 4-8% of the general population is heterozygous for factor V Leiden. These rates are higher in some populations such as the Northern Europeans. In Greece and Sweden some studies showed increased rates above 10%. Non molecular laboratory tests can demonstrate high sensitivity (99.6%) and specificity (99.7%) the presence of this mutation. These tests can be performed in plasma samples analyzers and with low cost. On the other hand, fibrinolytic pathways may contribute in the formation of thrombi in in these patients. Aim. The aim of this study was to establish the value of APC-R determination in hemodialysis patients by accessing the association between increased APC-R and vascular access thrombosis. Methods. In this retrospective study, 75 patients (36 men, mean age 63±10.9y and 39 women, mean age 62±12.2y) were selected from the hemodialysis Unit of the University Hospital, Iraklion Greece, between July 2003 and March 2005. The mean time on hemodialysis was 74.6±48.1 months. All patients were tested for antithrombin III, protein S, protein C, activated protein C resistance (APC-R), Lupus anticoagulant, antiphospholipid antibodies (panel), factors VIII and XI, homocysteine and lipoprotein(a). All patients were divided into two groups, those with access thrombosis (42 patients) and those with no access thrombosis (33 patients) and we assessed the prevalence of each thrombophilia factor to both groups. Results. Statistical analysis showed that among all tested thrombophilia factors only the presence of APC-R had a statistically significant association with access thrombosis. Overall, nine patients (12%) had an increased resistance to activated protein C. All these patients had at least one episode of access thrombosis (100%). Univariate analysis to estimate crude (unadjusted) odds ratio showed a 2 times higher risk for access thrombosis. However, this knowledge is inconclusive. Conclusion. This prospective randomized study was conducted in order to detect potentially existing differences in activation of coagulation and fibrinolytic pathways between open and laparoscopic surgery. Methods. From January to September 2005 40 patients ASA1 and ASA2 were randomly assigned to undergo laparoscopic (group A n=20) or open cholecystectomy (group B n=20) by the same surgical and anesthesiology team. Demographic data were comparable. Blood samples were taken a) preoperatively, b) at the end of the procedure, c) 24 hrs postoperative.

0405
ALTERATIONS OF HEMOSTASIS AFTER LAPAROSCOPIC AND OPEN SURGERY

C. Tsiminikakis,1 A. Skordylaki,1 F. Samiotaki,1 C. Oikonomoy,1 H. Antoniou,1 C. Bongiomi,1 F. Marikakis,1 N. Tsagarakis,1 T. Diamantis1
1University of Milan, MILAN, ITALY; 2CIRF/Center of Pharmacoeconomics, Facult, NAPOLI, ITALY; 3Maggione Hospital, MILAN, ITALY

Aims. This prospective randomized study was conducted in order to detect potentially existing differences in activation of coagulation and fibrinolytic pathways between open and laparoscopic surgery. Methods. From January to September 2005 40 patients ASA1 and ASA2 were randomly assigned to undergo laparoscopic (group A n=20) or open cholecystectomy (group B n=20) by the same surgical and anesthesiology team. Demographic data were comparable. Blood samples were taken a) preoperatively, b) at the end of the procedure, c) 24 hrs postoperative.
ly and d) 72 hrs postoperatively. The following parameters were measured: platelets, soluble fibrin monomer complexes (ET-test), fibrin degradation products (FDP), D-Dimers (D-Di), fibrinogen (FB), activated partial thromboplastin time (APTT), prothrombin time (PT). Thrombin-antithrombin III complexes (TAT) were measured at 24 hrs and 72 hrs postoperatively. Prothrombin fragment 1+2(F1+2) was measured at 24 hrs and 72 hrs postoperatively in 11 patients of group A and 13 patients of group B respectively. Results. Preoperatively, values of all haemostatic parameters were within normal limits in both groups. Immediately postoperatively, values of the coagulation markers TAT and F1+2 were significantly increased in the open surgery group as compared to the laparoscopic surgery group (p<0.05). Values of marker D-Dimers were also significantly increased in the open surgery group (p<0.01) immediately postoperatively and remained like that throughout the whole period of observation. Values of the coagulation marker FIB decreased slightly in both groups at 24 hrs postoperatively but there was a significant increase in the open surgery group as compared to the laparoscopic group (p<0.01) which remained like that thereafter. The APTT and PT values began to rise slightly in both groups but there was not observed a significant difference at any time between the two groups. The coagulation marker F5, test became positive twice in both the open surgery group starting immediately postoperatively and only once at 72hrs postoperatively in the laparoscopic group. Concentration of the fibrinolysis marker FDP was also higher in the open surgery group. In the laparoscopic surgery group starting immediately postoperatively and this difference became significant 72 hrs postoperatively (p<0.05). No patient from either group suffered thromboembolism or abnormal bleeding as a post-operative complication. Conclusions. Open surgery as compared to laparoscopic procedures leads in activation of the clotting system of a higher degree than in laparoscopic surgery group implying thus a greater thromboembolic risk for patients undergoing open surgery. Subclinical fibrinolysis is also more profound at the open surgery group. Although of a lower degree, hypercoagulability is still observed in patients undergoing laparoscopic surgery and therefore routine thromboembolic prophylaxis should be considered.

ROLE OF THE V617F MUTATION OF THE JAK2 IN PATIENTS WITH THROMBOSIS

Hospital de Sant Pau, BARCELONA, Spain

Background. Polycythemia Vera (PV) and Essential Thrombocytemia (ET) are Chronic Myeloproliferative Diseases (MPD) characterized by overactive hemopoiesis. Thrombosis is their main clinical complication. A single point mutation of JAK2 (Val617Fhe) has been detected in most PV and in half the patients with ET. On the other hand, many patients suffer from thrombosis without an underlying cause. However, an underlying MPD has been demonstrated especially in patients with thrombosis in uncommon locations, such as Budd-Chiari syndrome. The diagnosis of this underlying MPD is often difficult and requires sophisticated methodologies. Before the advent of the JAK2 mutation, X-chromosome inactivation patterns and in vitro erythroid colony formation have been used. These methodologies are cumbersome and its use is restricted to some laboratories, but the investigation of the single point mutation (Val617Fhe) of JAK2 is now readily available. Aims. The presence of the JAK2 mutation was assessed in a cohort of patients with thrombosis. Methods. A cohort of 309 patients with thrombosis were recruited from November 1997. Their DNA samples were analyzed by the allele-specific PCR methodology (1). DNA samples from 25 patients with PV and 18 patients with ET were used as positive controls. Results. In PV patients, 24 out of 25 cases showed the JAK2 mutation (96%). As for the patients with thrombosis, 1 out of the 309 patients with thrombosis was positive. This case was a 69-years-old man with 3 episodes of deep venous thrombosis and two of superficial venous thrombosis. His thrombophilic study was negative. This patient has been controlled in our department since 1997 and his Hb ranged from 160-168 g/l. Platelets and leukocytes were always normal. Conclusions. An obscure MPD is a very improbable cause of thrombosis. The investigation of the mutation V617F of the JAK2 gene should be reserved for special cases, such as patients with thrombosis in uncommon localization or patients with increased cell counts.

Reference


EVALUATION OF NEW COMMERCIAL ELISA KITS IN THE LABORATORY DIAGNOSIS OF ANTIPHOSPHOLIPID SYNDROME IN VIEW OF THE REVISED CLASSIFICATION CRITERIA OF THE ANTIPHOSPHOLIPID SYNDROME

K. Devreese
Ghent University Hospital, GHENT, Belgium

Since the publication of the 1999 Sapporo criteria for the classification of the antiphospholipid syndrome (APS) new clinical and laboratory insights have led to a recent update. These revised criteria now include testing for the presence of IgG and IgM β-2-glycoprotein I (β2GPI) with a positive titer being defined as higher than the 99th percentile of the normal population. aCL continues to be measured by a standardised ELISA. aCL positivity continues to be detected according to the ISTH guidelines. We have evaluated a newly developed Asserachrom® Anti-phospholipid antibodies immunoassay line (Diagnostica Stago, Asnières, France) for the detection of antiphospholipid antibodies (APA) in a lupus anticoagulant (LAC) positive (n=157) and a LAC negative (n=134) population. The Asserachrom® APA Screen has been proposed to be used as a first screening assay for the qualitative detection of APA. Positive samples can be further investigated by the Asserachrom® APA IgM test to determine the isotype and the quantitative antibody level. As 0 to 10% of APS patients are only positive for anti-β2GPI (Myakis S., J. Thromb Haemost, 2006) the Asserachrom® anti-β2GPI IgG and Asserachrom® anti-β2GPI IgM have also been proposed to be used in parallel to the Asserachrom® APA Screen. Despite that anti-prothrombin antibodies (aPT) are not included in the updated laboratory criteria they have been taken into account in this evaluation (Asserachrom® anti-prothrombin IgG, M). This new line of ELISA’s uses monoclonal antibody based standardisation in accordance with the recommendations of the Standardisation Group of the European Forum on Antiphospholipid Antibodies for the APA, β2GPI assays. Imprecision characteristics performed with the included control material for all ELISAs were good, with coefficient of variation (CV) ranging from 4.9% to 13.9%. Cut-off values calculated with 99th percentile, as advised by the updated laboratory criteria, are higher than those currently proposed by the manufacturer (calculated with 97.5th percentile). The Asserachrom® APA Screen showed 2.6% false positive and 0.7% false negative results when compared with the Asserachrom® APA IgM which is acceptable. 49 patients out of 271 (18.1%) were positive for b2GPI antibodies. For 23 patients out of those 49, the Asserachrom® APA Screen was negative. This is in agreement with the above observation that the anti-β2GPI may be the only test positive (Myakis S., J. Thromb Haemost, 2006). 20 patients out of 271 (7.4%) had a positive titer for aPT antibodies, 40.0% of them (8/20) were negative with the Asserachrom® APA Screen. In conclusion, the Asserachrom® anti-phospholipid antibodies line shows good performance characteristics and is a practical tool in the laboratory diagnosis of the APS. Own cut-off values should be calculated for each laboratory with the 99th percentile. As the anti-β2GPI may be the only test positive, the Asserachrom® APA Screen, the Asserachrom® anti-β2GPI IgG and the Asserachrom® anti-β2GPI IgM should be performed in parallel.
of Fluvastatin on both PARs and TF expression in this experimental setting. Methods. Ten patients with APS and previous history of thrombosis received Fluvastatin (40 mg/day) for one month. Blood samples were obtained before treatment and after one and three month of treatment. Monocytes were isolated from peripheral blood mononuclear cells by magnetic depletion of non-monocytes. TF and PARs expression at both mRNA and protein levels were measured by real time RT-PCR, western blot, and flow cytometry. Results. Analysis of mRNA of the four PAR described to date in humans (PAR-1 to PAR-4) revealed that PAR1 was de most abundant member of the PAR family in the monocytes of APS patients. Significantly increased expression of PAR2 was also observed in relation to the control group. PAR3 expression was also demonstrated, but not significantly altered versus healthy controls. PAR4 expression was absent. Monocytes from all the APS patients studied showed significant inhibition of TF expression at both mRNA and protein levels after one month of Fluvastatin treatment ($p<0.002$). These levels then suffered a slowly recovery, although remained significantly lower than control values after three months of the end of the treatment. Interestingly, mRNA expression levels of PAR2 strictly paralleled this behavior in response to fluvastatin treatment. Conclusion. These results provide the first demonstration of increased PAR expression in monocytes from APS patients. Statins drugs indirectly downregulate thrombin generation at the cellular levels. Our study for explaining the anti-thrombotic properties of statins couples the downregulation of TF with inhibition of PAR expression. Thus, PAR blockade might also draws increasing attentions to its therapeutical applications for anti-thrombosis. Supported by FIS 03/1033.

0409 PROCOAGULANT FACTORS IN PATIENTS WITH CANCER
M.S. Molnar, H. Guglielmone, M. Lavarda, M.L. Rizzi, G. Jarchum Sanatorio Allende, CORDOBA, Argentina

Background. Clotting activation and thromboembolic manifestations are common features in patients with cancer. Tumor cells can directly activate the clotting through two procoagulants: tissue factor (TF) and cancer procoagulant (CP). Aims. the aim was to evaluate the levels of the TF and CP in patients with different tumors in order to: 1) to establish an association between these markers and tumor localization, 2) to establish a correlation between the levels of procoagulants and status of disease, 3) to evaluate if the treatment with chemotherapy induced some modifications on the levels of procoagulants, 4) to evaluate the possibility of using procoagulants as predictor factors in the development of thrombosis. Methods. Sixty-one patients with different types of cancer (lung, breast, digestive and genitourinary) and 20 normal controls were included. The activity of TF and CP was studied in blood serum. Statistical analysis of the data was performed by the two-tailed Fisher exact test. Results. TF was increased in 72.5% and 0% ($p<0.01$) of cancer patients and normal controls, respectively. The PC was demonstrated increased in 88% of the cancer patients but in healthy controls it was increased in only 15% ($p<0.01$). The patients with genitourinary cancer presented the highest values of both procoagulants coinciding with a major prevalence of thrombotic events. The activity CP was found in 95% of patients with stages I and II but in patients with stages III and IV disease it was found in 85% (ns). They were not different in levels of both procoagulants between the patients treated with chemotheraphy and those with other treatments. Conclusions. TF and CP are elevated in patients with cancer. The highest values of both procoagulants are in the genitourinary cancer group in agreement with the greater presence of thrombosis observed in this group. A clinical follow up would be an important aspect to have a more clear idea on the potential value of these procoagulants and the tendency to develop thrombosis in patients with cancer.

0410 THROMBOPHILIC RISK FACTOR OF C46T POLYMORPHISM IN THE FACTOR XII GENE FOR VENOUS THROMBOSIS
University Hospital Valme, SEVILLA, Spain

Background. Currently the dates in relation to thrombophilic risk factor of C46T polymorphism in the factor XII gene are contradictory. Tira-do et al. are suggesting that the polymorphism itself is an independent risk factor for venous thromboembolism, another hand, Bertina et al., in their study, are showing similar results for the frequencies of the C46T genotypes in patients and controls. Aims. The objective of this study is to define the prevalence of C46T polymorphism in the factor XII gene in healthy people and patients with venous thromboembolism and to establish his thrombophilic risk factor. Methods. A prospective study case/control we were included 516 subjects (219 patients and 297 controls). The patients with venous thromboembolism are diagnosed: 161 Deep Venous Thrombosis, 34 Pulmonary Embolism and 24 with both disease. The controls are healthy persons, blood donors, they were included in study voluntarily. The sex and age of the patients and the controls have a similar distribution. The detection of polymorphism factor XII 46C/T by PCR in real time, in liquid phase, in a LightCycler (Roche diagnostics) thermal cycler was made. The sequences of the allele primers are, forward: TTCTTCTgCTTCCAgTCCC and reverse: ATggCCTATggCAgTgTA. Statistical methodology, the descriptive was made by groups in patients and controls; to estimate the risk by square-chi proof. Results. The results of prevalence of C46T polymorphism Factor XII gene in patients and controls are: patients CC 152 (60.3%), CT 75 (29.4%), TT 12 (4.6%); in the next table are showed. The estimate risk to have got a venous thromboembolism event in relation to genotype TT of C46T polymorphism factor XII gene, with CI 95% is 5.6 (1.2;10.56) $p=0.012$. Conclusion. The allele homozygous T of C46T polymorphism in the factor XII gene is a thrombophilic risk factor for venous thromboembolic disease

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Table 1.

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<th>Total</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
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<td>Patients</td>
<td>219</td>
<td>132 (60.3%)</td>
<td>75 (34.2%)</td>
<td>12 (5.5%)</td>
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<tr>
<td>Controls</td>
<td>297</td>
<td>206 (67.3%)</td>
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Bleeding disorders

L. Scalone,1 A. Gringeri,1 S. Von Mackensen,1 L.G. Mantovan1
1University of Milan, MILAN, Italy; 1RCCS Policlinico Hosp & Univ. of Milan, MILAN, Italy; 1University Hospital Hamburg-Eppendorf, HAMBURG, Germany; 1Center of Pharmacoeconomics, MILAN, Italy

Background. The adoption of modern treatment strategies in hemophilia care have significantly prolonged patients’ life expectancy and efforts have been made to improve their health and wellbeing. Aims. Our objective was to evaluate Cost of Care and Health-Related Quality-of-Life (HRQoL) of adult hemophilic patients without inhibitors. Methods. We conducted the naturalistic, multicenter, longitudinal Cost Of Care of HEMophilia (COCHE) study involving >18-year-old patients sequentially enrolled at 23 Italian Hemophilia Care Centers. Information collected was on socio-demographic and clinical data, resource absorption, HRQoL, treatment satisfaction. Results. 232 patients were enrolled (median age 54.5, 18-74), 86.6% with hemophilia A, 72.4% severely affected. At enrolment 31.8% of patients had chronic hepatitis C, 25.0% hepatitis B, 15.9% HIV infection. Most of the patients (87.8%) had some or severe orthopedic problems. The total World Federation of Hemophilia Orthopedic Joint Score (OJS, the higher the score, the worse the functioning) was 0.5 in 74 patients (52.2%), 6-16 in 81 patients (53.2%), 17-66 in 75 patients (53.6%). During the follow-up 81% of patients bled at least once, with a median of 1.44 episodes per month. One to six target joints were present in 56.5% of patients. Compared with general population HRQoL appeared compromised in physical sphere of health but not significantly reduced in the mental one: the mean (SD) SF-36 Physical Component Summary (PCS) of the study, the better the HRQoL score was 42.5 (10.2) and Mental Component Summary (MCS) score was 45.5 (8.5). The mean (SD) EQ-5D Visual-Analogue Scale (EQ-VAS), evaluating general HRQoL, was 66.2 (18.4). The major direct cost driver from the Italian National Health Service’s perspective was attributable to treatment with coagulation factor concentrates (96.6% of total costs). The use of strategies aimed at preventing long-term consequences such as arthropathy can have important repercussions on patients’ health status, on their wellbeing and on a more efficient resources consumption.

0413

PROPOSED GUIDELINES FOR ANTICOAGULATION DURING CARDIAC SURGERY USING ARGATROBAN
A. Laber, E. Martin, H. Kloecker
University of Louisville, LOUISVILLE, KY, USA

Background. Anticoagulation during cardiac surgery is essential and almost always done with heparin. Some patients with contra-indications to heparin, like allergy or heparin-induced thrombocytopenia, require surgery. Knowledge about the use of alternative anticoagulants to heparin, like direct thrombin inhibitors (DTI) is limited. Some major issues with DTI during surgery such as, bleeding, optimal laboratory monitoring, and safety have not been systematically addressed. In these situations, physicians have to make clinical decisions based on personal or anecdotal experience. Previously, we have described our experience with the direct thrombin inhibitor argatroban for anticoagulation during cardiac surgery. Here, we systematically...
The aim of this study is to develop guidelines for the appropriate use of argatroban during adult cardiac surgery. Methods. The information on all reported adult cases of argatroban use during cardiac surgery was reviewed. This analysis focused on patient characteristics, type of surgery, argatroban dosing schedule, monitoring of anticoagulation, morbidity and mortality. Results. Twenty-one cases have been reported on patients undergoing off-pump coronary surgery with the argatroban initial dose 5 mcg/kg/min infusion adjusted to maintain an activated clotting time (ACT) range between 200-300 s. Three intra-operative thrombi occurred in two patients when the ACT was less than 280 s. None had coagulopathy. Six cases reported the use of argatroban during CPB dosed with a bolus of 0.1-0.3 mg/kg followed by an infusion of 5-10 mcg/kg/min to keep ACT greater than 400 s. Intra-operative thrombotic complications were not reported in this group; however, one clot in the pump was noted after the procedure when the ACT was between 300-350 s. All six cases required larger volumes of peri-operative blood products and had severe coagulopathy. Of the 21 cases, 7 had an indication for continued anticoagulation following surgery. Four cases did not report further use of argatroban after surgery. Three patients received argatroban after surgery without complications. Conclusions. Argatroban, with ACT monitoring, can be safely used for anticoagulation during cardiac surgery using the following proposed guidelines. We recommend an ACT level of greater than 300 s for off-pump cardiac surgery and greater than 400 s for CPB. It would be advisable to use an arbitrary upper limit of ACT for both on and off-pump procedures to prevent severe coagulopathy. ACT monitoring seems to be a clinically reliable test to predict coagulation status in patients undergoing cardiac surgery with argatroban and should be checked often (i.e. every 15 minutes) to allow for proper adjustments of the dose. Prospective studies to evaluate the optimal dose and monitoring effect of this agent during cardiac surgery should be supported.

References

0414
PILOT STUDY TO ESTABLISH PREFERENCES TOWARDS COAGULATION FACTOR CONCENTRATES USED TO TREAT HAEMOPHILIC PATIENTS WITH INHIBITORS

L. Scaloni,1 A. Gringeri,1 F. Borghetti,2 S. Ravera,1 A. Casati,1 L.G. Mantovani2
1University of Milan, MILAN, Italy; 2IRCCS Polichinico Hosp & Univ. of Milan, MILAN, Italy

Background. Haemophilia is a very expensive disease. This situation becomes extreme when patients develop inhibitors that compromise the effectiveness of treatment, with potential increase of morbidity and mortality. Treatment of haemophilia is the result of interactions between patients, physicians, pharmacists and budget holders, each carrying their own set of preferences. Aims. A pilot study was conducted to identify which characteristic of coagulation products are considered more important to treat patients with inhibitors: these characteristics will be included with a price proxy characteristic in a Discrete Choice Experiment, with the objective to elicit preferences and willingness to pay towards treatments of patients with inhibitors. Patients and methods. The sample was identified during focus groups with patients and clinicians and rated from 0 (not important) to 10 (very important) by 35 people (adult patients, caregivers, physicians, pharmacists). Results. The following median (mean) scores were found: viral safety: 10 (8.9); time to stop bleeding: 9.5 (9.0); risk of anamnestic response: 9.0 (8.5); possibility of undergoing major surgery: 9.0 (8.5); regular use in prophylaxis: 9.0 (8.4); time to pain recovery: 9.0 (8.5); number of injections to stop bleeding: 8.0 (7.9); time to prepare and give/have the injection: 7.0 (6.6). All groups of respondents considered as more important viral safety, possibility of undergoing major surgery, risk of anamnestic response, time to stop bleeding, while time to prepare and give/have the injection was considered the least important. Different preferences were attributed to time to pain recovery, considered more important by patients; regular use in prophylaxis, considered more important by caregivers. Conclusions. Viral safety and effectiveness are considered as the most important characteristics in the treatment of haemophilic patients with inhibitors. Different levels of preferences are present between patients, or their caregivers, and physicians. Understanding these differences is important to guide optimal therapeutic strategies in patients with inhibitors.

Table 1. Proposed guidelines for argatroban use during cardiac surgery in adults.

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<tr>
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<th>Off pump cardiac surgery</th>
<th>On pump cardiac surgery</th>
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<tr>
<td>Argatroban dose</td>
<td>5 mcg/kg/min infusion</td>
<td>0.1 mg/kg bolus followed</td>
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<tr>
<td></td>
<td>Adjust according to ACT</td>
<td>Adjust according to ACT</td>
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<tr>
<td>Target ACT</td>
<td>&gt;300 s</td>
<td>&lt;400 s</td>
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<tr>
<td>Monitoring</td>
<td>ACT every 15 min</td>
<td>ACT every 15 min</td>
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<tr>
<td>Low ACT level</td>
<td>300 s</td>
<td>400 s</td>
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<tr>
<td>High ACT level</td>
<td>500 s (arbitrary level)</td>
<td>600 s (arbitrary level)</td>
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<tr>
<td>Risks</td>
<td>Intra-operative thrombi</td>
<td>Coagulopathy with high</td>
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<td></td>
<td>formation with ACT &lt;</td>
<td>ACT levels</td>
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0415
PRELIMINARY RESULTS FROM THE EUROPEAN ESCHOOL FIELD STUDY

S. von Mackensen,1 A. Gringeri,2 K. Berger,2 P. Giaranfre,3 R. Rolf,4 L. Mantovani,1 L. Laszlo,1 M. Serban,2 W. Schramm2
1Institute for Medical Psychology, HAMBURG, Germany; 2Dept. of Internal Medicine - Haemophilia, MILAN, Italy; 3Medical Economics Research Group, MUNICH, Germany; 4Oxford Radcliffe Hospitals NHS Trust, OXFORD, United Kingdom; 5Region Sane, University Hospital, MALMOE, Sweden; 6Centre of Pharmacoeconomics, MILAN, Italy; 7National Medical Centre, Haemophilia C, BUDAPEST, Hungary; 8University of Medicina & Pharma-

Background. The ESCHOOl Study is a retrospective/prospective prevalence-based cohort study of clinical, quality of life and health economic outcomes in haemophilia treatment in Europe and is sponsored by the EU. 1732 patients with haemophilia from 4 years on have been recruited from 19 European countries, out of them 78% have been enrolled in the study. The ESCHOOl Study consisted of a pilot testing phase where the study procedure was feasibility tested in 70 patients and a field testing phase in which more then 1.300 haemophilia patients participated. Aims. Objectives of the study were a) to make validated instruments available for the assessment of patients’ health status, quality of life and health care and its cost on an European basis, b) to identify models of health care of haemophiliacs in terms of clinical characteristics, their possible costs and impact on quality of life, c) to provide policy recommendations for optimal care of haemophilia patients based on clinical, quality of life and health economic information. Methods. In the ESCHOOl Study patients with haemophilia were asked to complete a comprehensive questionnaire concerning socio-demographic (such as age, gender), psycho-social (such as quality of life, coping) and health economic (such as days lost of work, costs of care) information and to fill in a diary during a 6-months period. In parallel, clinical information concerning bleeding history, inhibitor history, concomitant disease, arthropathy assessment, surgery, treatment modality, type of product and medical visits was obtained from physicians. Results. In the pilot testing 70 patients (41 adults, 29 children) were included from the steering committee countries (Italy, Germany, Romania, Hungary, U.K.) and France. According to the feedback evaluation, patients and parents found the questionnaire good and interesting, but many said it was too long and repetitive. All respondents found the questions relevant for haemophilia. The extended completion time (55 min) led to the decision to shorten the questionnaire for parents and adults. The pilot testing questionnaire was divided into two parts in order not to lose information, where one part was administered at baseline the other part was given at follow-up. Suggestions from physicians were implemented in the medical documentation. In the field testing 1.345 patients (931 kids, 1,412 adults) were enrolled from 19 countries. 87% had haemophilia A and were severely affected (72%). In 11% inhibitors occurred and one third of the patients received prophylactic treatment. 45% of the patients suffered from chronic pain and 40% reported target joints. Viral infections were found in 42% of the patients (hepatitis C) and 8% for HIV. 5% of the patients underwent an orthopaedic surgery. Conclusions. The feasibility testing of...
the study documents revealed that the original questionnaires had to be modified for the field testing. Preliminary results of the field testing revealed differences between countries concerning clinical status and treatment modalities and their impact on costs and quality of life. These results underline the importance of the aim of the ESCHoQol Study: to compare CoQl outcome of haemophilia care in Europe in order to recommend future improvements.

0416
INTRACRANIAL HEMORRHAGE IN HEMOPHILICS RECEIVING NO PROPHYLACTIC REPLACEMENT THERAPY

A.M. Petrescu,1 M. Serban,2 W. Schramm3
1University of Medicine and Pharmacy, TIMISOARA, Romania; 2Ludwig Maximilians University, MUNCHEN, Germany

Background. Intracranial hemorrhage (ICH) in hemophiliacs is the primary cause of death through bleeding. The mortality risk associated with ICH was 70% before the introduction of cryoprecipitate; it decreased to 25-30% thereafter but remained still very high. Aims. Analysis of the frequency, type, severity, and consequences of the ICH in hemophiliacs from Romania, receiving no prophylactic replacement therapy. Methods. The study was conducted on a cohort of 212 hemophiliacs, 121 hemophilia A, and 32 with hemophilia B; 58.35% with severe disease, 50.48% with sporadic disease. The period of the study was 1990-2005. Results. Intracranial hemorrhage was registered in 18/212 (8.49%) of the studied patients, being a very rare bleeding (0.46% of the registered bleedings). ICH was the manifestation at diagnosis 35.52% of the patients. It appeared mostly in case of severe hemophilia (77.78%) at different ages. Head trauma registered in 46.49% of cases in patients aged between 1-6 years, was complicated by ICH in 9.18% of the cases. Except for the neonatal period, the risk for ICH after head trauma was higher in school aged boys (10.71%). ICH appeared even after minor head trauma (14.56%), sometimes being apparently spontaneous. Birth trauma represented the most important risk factor for ICH, determining 7/18 (38.59%) of the ICH cases, respectively 3.3% of the patients, all with severe hemophilia. ICH was rapidly recognized and treated in only one case, although in five cases hemophilia has been diagnosed in the family before. Four patients with ICH were treated in our clinic, one being also operated, and neither has complications. The long term complications of the ICH (neurological, sensorial and psychological sequelae) were very frequent (72.22% of the ICH cases), generally extremely severe (epilepsy-76.92%, hydrocephalus-15.38%, palsy-15.38%, blindness-23.08%, mental retardation-80.78%, a.o.), and usually associated. The risk for permanent damages was even higher in case of ICH associated to birth trauma: 91.67%. No deaths through ICH were registered in the period of study. Conclusions. ICH is a very dangerous bleeding in hemophiliacs receiving no prophylactic replacement therapy, being the cause of the most severe long term complications. The prevention, early recognition, and correct replacement treatment in case of ICH are essential in order to reduce their consequences. Many hemophilia centers have developed special protocols for the prevention of ICH associated to birth trauma or head trauma. Although the prophylactic replacement therapy proved not to be sufficient in order to prevent ICH, the efficiency of early home therapy in case of head trauma is well documented.

0417
PREDISCUSSION OF ANTI-HLA AND ANTI-GPIIb/IIIa ALLO-IMMUNIZATION IN PATIENTS WITH GLANZMANN THROMBOASTHENIA: EXPERIENCE OF A SINGLE CENTER


1Hematology, ROMA, Italy; 2Istituto Regina Elena, ROMA, Italy; 3Dipartimento di Medicina Sperimentale, ROMA, Italy

Background. Platelet transfusions, the main therapy of Glanzmann thrombosthenia (GT), can induce an allo-immunization against HLA antigens and GPIIb/IIIa complexes, with a possible reduction of efficacy of subsequent treatments. Aims. To investigate the development of allo-antibodies against HLA antigens and anti-GPIIb/IIIa complexes in GT patients transfused, and evaluation of efficacy of replacement therapy. Patients and methods. From 1975 onwards, we have followed 17 GT patients, 12 type I, 3 type III, 2 not classified: 8 men, 9 women; median age at diagnosis 9.8 years (range 1-44.5); median age at the time of this study, 35.5 years (range 23.6-62.5). Our patients showed at least once in their life the following symptoms: 10/17 epistaxis, 15/17 gastrointestinal hemorrhage; 5/17 oropharyngeal hemorrhage; 4/17 muscle hematoma; 2/17 bleeding for traumatic injury, 2/17 hematoma, 2/17 hematoma; 1/17 intracranial hemorrhage; 1/17 hematothorax; 1/17 otorrhagia. Five/9 women experienced meno-metrorrhagia. Ten major and 22 minor surgical procedures have been performed. Two spontaneous deliveries and 3 cesarian sections with 5 live births have been observed; moreover, 2 spontaneous abortions occurred, 1 spontaneous and 1 voluntary. Globally, 9/17 patients have been transfused with platelets and red blood cells (RBC); 5/17 only with platelets; 2/17 only with RBC. One patient has never been transfused. Platelet transfusions have always been hemostatically effective. Fifteen/16 transfused patients have been investigated for allo-antibodies, anti-HLA and anti-GPIIb/IIIa. Results. The positivity for allo-antibodies has been demonstrated in 4/15 patients (26.67%), isolated for anti-HLA in 2; isolated for anti-GPIIb/IIIa in 1; combined in 1. Conclusions. The prevalence of allo-immunization (25.7%) is inferior to recent literature data (80%). While positivity for anti-HLA (3/15, 20%) agrees with the recent literature data (22%), positivity for anti-GPIIb/IIIa (15%) is inferior (38%). Presence of allo-immunization did not compromise the efficacy of platelet transfusions.

0418
GENETIC ANALYSIS OF THE COAGULATION FACTOR VIII AND IX GENES IN HUNGARIAN PATIENTS WITH HAEOMOPHILIA

1National Medical Center, BUDAPEST, Hungary; 2Hem P.I Children’s Hospital, BUDAPEST, Hungary

Haemophilia A (HA) and haemophilia B (HB) are common X-linked bleeding disorders resulting from the inherited deficiency of coagulation factors VIII (FVIII) and IX (FIX), respectively. Female relatives of patients with haemophilia may be carriers and many of them request carrier status determination. Previously, large gene inversion detection in HA patients and linkage analysis using intragenic polymorphisms in HB and an inversion-negative HA patients were used for carrier and prenatal diagnosis in our laboratory, but in some cases linkage analysis had limitations. A high number of different mutations can be identified by direct sequencing of the FVIII and FIX genes, which is now the preferred method for genetic analysis. The aim of our study was to provide precise carrier status determination in affected families. Upon selection of 16 severe HA-patients without intron 1 and 22 inversions and 20 HB-patients we decided to identify the disease causing mutations by sequencing the promoter and the exons with flanking regions of the FVIII and FIX genes using dyeode chain-termination method. Previously unpublished mutations were identified in 10/16 (62.5%) of the HA patients examined. Distribution of the novel mutations in the FVIII gene are the following: 3 novel missense [370A>C (Lys48Gln), 1883T>C (Leu552Pro), 2084G>A (Gly619Asp)], 2 nonsense [480G>A (Trp68STOP), 3839T>A (Leu1204STOP)] mutations, 3 small deletions [2769delG, 1473delC, 4216delG], one splice site variant [A-1 >IVS2], and one ins del mutation [2293delTCAC]. Among the 22 HB-patients, one novel point mutations (4 missense, 1 nonsense and 1 small insertion) of the FVIII gene were also found. Among 20 HB-patients, one novel [g.10443A>T (Asp64Val)] and 17 published FIX gene mutations were found. The new mutation was detected in two unrelated Hungarian families sharing identical haplotypes. Our results further confirm, that HA and HB can be caused by a wide variety of point mutations and indicate that regarding HA, significant proportion of novel mutations can be identified upon sequencing previously untested populations.

0419
RELATIONSHIP OF PAI-1 4G/5G POLYMORPHISM AND BLEEDING RISK ASSOCIATED TO CARDIAC SURGERY


Hospital Universitario de Canarias, LA LAGUNA - TENERIFE, Spain

Introduction. Plasminogen activator inhibitor-1 (PAI-1) is an important inhibitor of the fibrinolytic system. Several studies have pointed out that patients with the 4G allele of PAI-1 polymorphism (homozygous 4G/4G and heterozygous 4G/5G) have a higher level of PAI-1 in plasma, when compared with homozygous 5G/5G. Aims. The primary target of this work was to analyse the influence of PAI-1 genotype on the bleeding risk of patients undergoing cardiac surgery with cardiopulmonary bypass. Methods. We have studied 26 cardiac surgery patients in our center (15
USE OF LOW DOSE RECOMBINANT ACTIVATED FACTOR VII INFUSION FOR TREATMENT AND PROPHYLAXIS OF BLEEDING EPISODES IN SEVERE FACTOR VII DEFICIENCY

O. Ottmann, 1 A. Will, 1 P. Bolton-Maggs, 1 P. Mohn, 1 A. Hague, 1 L. Birtwhistle, 1 R. McDermott, 1 S. Pearson, 1 T. Bloodworth, 1 B. Boardman, 1 C.R.M. Hay 2

1 Universitätsklinik Frankfurt, FRANKFURT, Germany; 2 Pendlebury Childrens Hospital, MANCHESTER, United Kingdom; 3 Manchester Royal Infirmary, MANCHESTER, United Kingdom

Introduction. Severe factor VII deficiency is a rare bleeding disorder which can be treated with fresh frozen plasma, prothrombin complex concentrates and plasma derived factor VII. Recombinant activated factor VII (rFVIIa, Novoseven) is now licensed for the treatment of factor VII deficiency as a recombinant dose of 15-50 micrograms per kilogram (mcg/kg) by bolus injection every 4-6 hours. Correlation is poor between factor VII activity and haemostasis, but levels of 10-15% of normal are generally sufficient to achieve haemostasis. Activated factor VII makes up 1% of the total and in combination with tissue factor is the initiator of coagulation. The licensed dose of rFVIIa raises plasma levels of factor VII above the physiological norm. The half-life in plasma is short (2.30-2.97 hours) requiring frequent bolus injections. Infusions of low dose rFVIIa seem attractive as it abolishes peak and trough levels and avoids exposure to blood borne viruses and prions. Patients and methods and outcomes. Six patients (5 children and 3 adults) with severe factor VII deficiency (levels <1%) were treated with rFVIIa (Novoseven; 0.5-1.2 mg/kg) during the intervention were excluded. Among others, main differences in bleeding at the first 4 hours (332±223 vs. 846±519 ml; p=0.002) and 24 hours after arriving at the intensive care unit (771±446 vs. 1379±582 ml; p=0.016). This smaller hemorrhagic risk correlates with significantly elevated PAI-1 levels at the moment of arrival at the unit in the patients carrying 4G allele (120,±105 vs. 56,9±7 ng/ml; p=0.019), and also higher levels of antithrombin (p=0.016), PT (p=0.019) and fibrinogen (p=0.027), and a higher corpuscular temperature (p=0.011). We did not find significant differences between both groups of patients for the rest of analysed parameters; all the results are exposed. Conclusions. Some authors have studied the relationship between PAI-1 polymorphism and the risk of ischemic complications (mainly coronary disease and stroke), but few reports exist that clinically correlate this polymorphism with hemorrhage. Our work demonstrates that patients undergoing cardiac surgery who are carriers of the PAI-1 4G allele, may have a significantly lower bleeding risk in the first 24 hours after surgery, when they are compared with homozygous 5G/5G. Although being preliminary, of these findings it is possible to decide that patients carrying the 4G allele could not need antifibrinolytics, and thus, to contribute to avoid possible thrombotic complications.

0420

FACTOR XI DEFICIENCY AND POST-PARTUM HAEMORRHAGE: BLEEDERS AND NON-BLEEDERS!

B. Myen, 1 S.R. Pavord, 1 L. Keat 2

1 Queen’s Medical Centre, University Hosp, NOTTINGHAM, United Kingdom; 2 Leicester Royal Infirmary, LEICESTER, United Kingdom; 3 Nottingham City Hospital, NOTTINGHAM, United Kingdom

Management of pregnant women with Factor XI deficiency poses a challenge to the clinician because of the variable bleeding tendency and risks associated with factor replacement. Factor XI deficiency is an uncommon bleeding disorder with an autosomal inheritance. The gene frequency is low, comprising only 7% of bleeding disorders in our local data base in the East Midlands (UK). In heterozygotes, there is mild or moderate reduction in Factor XI level, between 20-70 u/dL. Homozygotes or compound heterozygotes have severe reduction in levels, often <1 u/dL. There is no clear correlation between FXI level and bleeding tendency; it is generally trauma or surgery-related. There are few data on pregnancy complications and FXI deficiency. Our study objectives were to assess pregnancy outcome with respect to miscarriage rate and post-abortion bleeding, and post-partum haemorrhage. Since symptoms of bleeding disorders can be difficult to assess objectively, we applied the criteria employed by Bolton-Maggs. Two haematologists independently assessed each set of case-notes and classified each patient into either bleeder or non-bleeder groups. 34 women were identified on the local data-base. Thirty-one had moderate or mild deficiency, while 3 had severe reduction in FXI levels. The patients were evenly divided between bleeders (B) and non-bleeders (nB), with 18 and 16 respectively.

In 2 cases, all women had undergone surgical or dental challenges, and were classified as non-bleeders in the absence of Menorrhagia +/or mucous membrane bleeding. They had a total of 109 pregnancies, with 79 live births. Pregnancy and delivery was uneventful in the majority of cases, 71% overall (76% nonB; 65% B). Of those pregnancies resulting in a live birth 80% were uneventful (92% nonB; 72% B). The local incidence of PPH is 5%. The total number of instances of PPH in our study was ten (15%), 9 primary and 1 secondary. This increased incidence of PPH was statistically significant, with a p value of 0.002. All but two episodes occurred in the group of women with increased bleeding tendency. Of the women in this group, PPH occurred twice in 2 patients and once in 4 women. When the incidence in bleeders was compared to that of non-bleeders there was a highly significant difference, (p 0.000001).

In this study, 10 women suffered a total of 13 spontaneous miscarriages. One further woman had a total of 15 miscarriages and was excluded from this analysis. The total rate of miscarriage between 8 and 13 weeks, locally, is 10%. Eleven of the miscarriages in our study were within this time period. The twelfth miscarriage occurred at 22 weeks, in a young woman who had ruptured her membranes at 20 weeks. The cases appeared evenly divided between the two groups However, significant post-abortal bleeding was noted in 2 cases, both bleeders. In summary, although uneventful for the majority of women, factor XI deficiency caused pregnancy complications. Further studies are required to define the underlying factors of this group.

0421

Patient  Age/sex  Dose Mcg/kg/24 h  Length Days  Procedure

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<td>2 5 M</td>
<td>300</td>
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<td>2</td>
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She was treated with a bolus of 60 mcg/kg followed by an infusion of 250 mcg/kg/24 hours for 24 days. During this time she underwent insertion of an extra-ventricular shunt and of a central venous catheter. There was no excessive bleeding. She has required six further infusions (2-7 days duration) for hip bleeds, gastro-intestinal bleeding and central venous catheter changes with no evidence of excessive bleeding, at doses of 150-250mcg/kg per 24 hours. In emergencies the patients were given a bolus of rFVIIa (60 mcg/kg) immediately followed by an infusion of rFVIIa (1.2 mg diluted in 24 ml 0.9% saline) until the bleeding was controlled. The dose 1.2 mg in the infusions is dictated by this being the smallest viable currently available. In elective procedures an infusion of rFVIIa (17.1-300 mcg/kg/24h) was commenced 2-4 hours prior to procedure. Results and Discussion. Our results show there were no episodes of increased blood loss over the expected for the procedure. There were no episodes where extra doses of rFVIIa or other treatment were required. Therefore we conclude it is feasible, safe and effective to use low dose rFVIIa infusions in both emergency and elective situations. Using doses of 20mcg/kg per 24 hours via an infusion pump appears to be as effective as the licensed dose of 60-180 mcg/kg per 24 hours in adults and may reduce the theoretical risk of thrombo-embolic phenomena. The reduction in dose represents a large saving and requires less medical and nursing time refilling the infusion every 24 hours. It may be possible to further reduce the dose given in paediatric patients with smaller vial sizes or if the rFVIIa is shown to be stable for a longer period once reconstituted.
EVALUATION OF BONE MINERAL DENSITY IN CHILDREN WITH HEMOPHILIA: MANSOURA UNIVERSITY CHILDREN HOSPITAL (MUCH) EXPERIENCE: MANSOURA, EGYPT

M. Abdelrazik, M.R. Bassiony, M.A. El-Ziny, H.M. Rabea
Mansoura University Children Hospital, MANSOURA, Egypt

Background. Patients with hemophilia may be at risk for developing reduced bone mineral density for a number of reasons such as recurrent hemorrhosis and immobilization. Aim of the Work: To assess the bone mineral density (BMD) in children with hemophilia and, to correlate bone mineral density with findings regarding the joint disease (hemophilic arthropathy). Patients and Methods. Thirty hemophilic patients aged 4.97±3.64 years and 30 control healthy individuals (had no joint disease) aged 5.09±3.64 years were selected from the hematology unit and outpatient clinic of MUNCH respectively. Anthropometric measurements were done to all cases. Z score was used for weight, height, and Body Mass Index (BMI). Joint evaluation for hemophilic patients and controls was done using Colorado PE-0.5: Half Point Instrument before using Dual Energy X-ray Absorptiometry (DEXA). DEXA scanning was performed to all hemophilic patients and controls focusing on L2-L4. Results. There was no significant difference between hemophilic patients and controls as regard anthropometric measurements and their z-score. There was a significant difference between severe hemophilic patients (factor level assay less than 1%) and controls as regard BMD and BMD z-score (p=0.03, 0.005) respectively. There was a significant difference between severe hemophilic patients and controls as regard BMD and BMD z-score (p=0.01, 0.001) respectively. Also, in hemophilic patients, there was an inverse significant correlation between joint evaluation scores and BMD z-score (r= -0.365, p=0.04). Conclusions. Children with hemophilia could have reduced bone mineral density compared with age and gender matched controls. This reduction in bone mineral density was independent on difference in age and body size. Children with more established hemarthropic arthropathy exhibited the lowest BMD and BMD z-score. Early detection of osteopenic hemophilic children using DEXA scanning. 2. Bisphosphonates plus calcium for hemophilic children with reduced bone mineral density. 3. Evaluation of the effect of on demand versus prophylaxis replacement therapy in hemophilic patients on BMD and hemophilic arthropathy.

CLINICAL AND LABORATORY PECULIARITIES IN CHILDREN WITH VON WILLEBRAND DISEASE

G.T. Tashenova, 1 B.Z.H. Aldaniarova, 1 K.O. Omarova 2
1 Scientific Center of Pediatric and Child, ALMATY, Kazakhstan; 2 Scientific Center of Pediatrics and Child, ALMATY, Kazakhstan

Von Willebrand disease (vWD) is the most widespread (frequency 1%) hereditary form of hemorrhagic diathesis after hemophilia. The main causes of bleeding in patients with vWD are quantitative and qualitative abnormalities of von Willebrand factor (vWF). The main cause of quantitative defects in von Willebrand factor is a decrease in vWF. vWF of platelets has an influence on severity of disease symptoms. The prominence of the bleeding varies due to complicated pathogenesis and variability of the forms. The aim of this study was to establish the clinical and laboratory peculiarities of vWD in children. Materials and Methods. We have assessed hemostasograms in 150 children with vWD aged from 6 mo to 14 years, including 35 children with parents (13 fathers and 22 mothers). Results and discussion. vWD was more frequent in girls (60%). In majority of patients diagnosis was established during initial examination, in 45% during the second examination. Among patients were 2 infants, 8 children of 1-3 y.o., 11 of 4-6 y.o. and 14 patients aged from 7 to 14 years. An evaluation of patients genealogy has allowed to confirm familial character of the disease in the majority of patients 84%, an autosom al-dominant type of inheritance was observed in 60%. The first symptoms of the disease were apparent in earlier childhood (44%) and in childhood (52%). The main symptoms of the hemorrhagic syndrome were: nasal bleeding (92%), skin hemorrhages (40%), post-operative and post-traumatic bleeding (36%), subcutaneous hemorrhages (18%), tooth (18%) and gingival (6%) bleedings. Metrorrhagias were leadings symptoms in 11 girls aged 12-14 years. Intensity of the bleedings was moderate in the majority (68%) of children. Disease course was characterized with periods of bleeding and remission of various duration. The frequency of bleeding episodes was: monthly in 12%, several times per year in 24%, annually in 36%, more rare in 28%. Laboratory investigations were started from routine test: bleeding time (BT) and number of platelets (P). BT was increased in 68% of patients, P was 150-200 thousands/L in all patients. Diagnosis of vWD was established on results of culogographical investigations: vWF coagulation factors levels and platelet functions. Platelet adhesion to glass (normal value 30-40%) was decreased: in 12% of patients was 0%, in 20% was 1-10%, in 25% - 10-20% and in 40% of patients was 20-30%. Ristomycin-induced (1.2 mg/mL) platelet aggregation was decreased in all patients: in 72% was up to 17 sec, in 28% of patients up to 20 sec (normal value 5-10 sec). Platelet aggregation time changed: in 45% of patients to 55-60 sec, in 28% to 61-60 sec, in 16% to 71-80 sec and more than 80 sec in 8% of patients. 50% decreasing of factor VIII was noted in 50% of patients: in 27% to 20-50%, in 23% of patients to 11-20%. The most accurate indicator in diagnosis of vWD is estimation of blood vWF level. In the majority of children decreasing of this indicator was observed: 0-20% in 12% of patients, 20-40% in 12%, 40-60% in 36% and 40-60% in 40%. So, in the majority of cases vWD type I (plasma vWF protein decreasing) and type III (complete absence of vWF protein) were noted. Almost normal vWF level (60-80%) does not exclude vWD type II. In this case diagnosis can be established by vWF multimeric structure investigations, which are not available in our laboratory. Some clinical and laboratory changes were observed in both children and parents in 28 cases, in 7 cases more prominent changes were noted in parents in comparison with children. Thus, von Willebrand disease is one of the most widespread hemorrhagic diatheses with autosomal-dominant or, more rare, autosomal-recessive inheritance. Variability of the clinical and laboratory signs is typical for vWD. vWD can be suspected in cases of familial bleedings in both genders. Relapsing spontaneous nasal bleedings more frequent symptom of the disease. In patients with vWD vascular-platelet hemostasis alterations are seen: bleeding time increasing, ristomycin-induced platelet aggregation decreasing. Alterations of coagulative chain of hemostasis are characterized by factor VIII activity decreasing and moderate increasing of activated partial thromboplastin time. Most accurate investigation in diagnosis of von Willebrand disease in von Willebrand factor level estimation.

PLATELET FUNCTION TESTING IN URAEMIC PATIENTS

S.J. Ho, 1 T.A. Brighton, 2 R. Gemmell 3
1 St George Hospital, SYDNEY, Australia; 2 Prince of Wales Hospital, SYDNEY, Australia

Background. Chronic renal failure (CRF) is associated with excessive bleeding. Platelet dysfunction is probably the most consistent and important feature, particularly platelet-platelet and platelet-vessel wall interactions. The skin bleeding time (SBT) is the best-established predictor of haemostasis integrity and has been withdrawn from sale in Australia. Several newer rapid assays of platelet function are able to provide a means of assessing primary haemostasis, but have not been specifically assessed in uraemic patients. Aim of the study. A pilot study to examine various in vitro assays of platelet function in a heterogeneous group of uraemic patients. Methods. A pilot study to examine various in vitro assays of platelet function are able to provide a means of assessing primary haemostasis, but have not been specifically assessed in uraemic patients. Background. Several newer rapid assays of platelet function are able to provide a means of assessing primary haemostasis integrity and have been withdrawn from sale in Australia. These have been compared to the traditional in vivo assay of skin bleeding time. Methods. Single centre, prospective cohort study of patients referred to a tertiary nephrology unit. Patients with both acute and chronic renal impairment were recruited. Laboratory parameters analysed included full blood count, serum creatinine and urea, calculated GFR (Cockcroft formulae), APPT, PT, fibrinogen, SBT (Simple II), WBPA, FPA-100, TEG and CPA. If patients were on haemodialysis, blood samples were collected via central venous lines, heparin removed, or via arterio-venous fistulae pre-dialysis. Results. This study included 42 patients: 9 with CRF (GFR<30 mL/min) not receiving dialysis; 25 CRF on dialysis; 7 patients presented in acute renal failure; 3 patients assessed had normal renal function but with nephrotic syndrome and presented prior to renal biopsy: 22 patients were on low-dose aspirin and 4 patients were on clopidogrel without significant effect on SBT. There was a poor correlation between calculated glomerular filtration rate (GFR) and SBT (r2=0.1564) and no correlation with serum creatinine or urea. Of the 42 patients 30 patients had SBT >7 minutes, 26 patients had SBT >8 minutes, 22 patients had SBT >9 minutes, and 10 had SBT 15 minutes or greater. Overall no other measure of platelet function predicted for abnormal SBT (see Table 1). In 12 patients with normal SBT, FPA-100 WBPA and CPA assays were abnormal in 7 of 12, 5 of 11, and 5 of 7 respectively. Of 10 patients with SBT 15 mins or greater, 2 had normal WBPA, 4 had normal CPA-100 results whilst none of 5 patients tested had normal CPA readings. Overall 19 patients had abnormal TEG tracings, but these were...
We used data accumulated in our haemophilia centre in the course of 6 years between 2000 and 2005. Five patients with obvious bleeding or without history of haemophilia manifested a factor VIII inhibitor. The mean age of these patients was 36 years with a range of 12 to 65 years. The occurrence of factor VIII inhibitor was diagnosed by the von Willebrand factor assay and by a bleeding history. The incidence of factor VIII inhibitor in adult haemophilia patients in our study was 1.5% (50/3204). We used a specific assay (Helena Laboratories, San Jose, CA) for the determination of factor VIII activity and inhibitor titre. A positive inhibitor was determined as a bleeding time of more than 10 minutes with a normal plasma factor VIII activity.

**Table 1. Comparison of assays.**

<table>
<thead>
<tr>
<th>SBT</th>
<th>PFA-100</th>
<th>WBPA</th>
<th>CPA</th>
<th>TEG</th>
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<tbody>
<tr>
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<td>12</td>
<td>3/11</td>
<td>0/1</td>
<td>6/12</td>
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<td>1/12</td>
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**0425**

**ANALYSIS OF CLINICAL AND BIOLOGICAL FACTORS ASSOCIATED TO EXCESSIVE BLEEDING IN CARDIAC SURGERY**


Hospital Universitario de Canarias, LA LAGUNA - TENEFÍRE, Spain

**Background.** Bleeding is the most frequent and important complication associated to cardiopulmonary bypass (CPB) in cardiac surgery. The knowledge of physiopathological aspects related to the own CPB and the use of certain measures (less deep hypothermia, drugs like aprotinin, and better control of the intraoperative anticoagulation, among others) have significantly reduced this hemorrhagic risk. Excessive postoperative bleeding (EPBS) takes place when the hemorhage volume is superior to 1 liter in the first 24 hours after surgery. **Aims.** We have analysed what clinical and biological factors are associated to EPBS after cardiac surgery with CPB. **Methods.** We studied 26 patients undergoing cardiac surgery with CPB (15 men and 11 women; median age 67 years, range 40-85), in whom tranexamic acid was not administered during the intervention. Twelve coronary artery bypass operations, 10 valve replacements, and 4 mixed surgeries, were included. Those patients with EPBS were grouped as opposed to those who did not have EPBS, and differences between both groups in relation to physical factors (corporal temperature, haemodynamic indexes), biochemical (BUN, creatinine, CK-MB, CK-NAC, CK-MB isoenzymes, soluble TNF receptor, interleukin-6, complement system and leptin included) and hemogram findings, hemostatic parameters, transfusional requirements and used drugs, were analysed. Data were recorded at four moments: preoperative, arrival at the intensive care unit, after 4 hours of arrival and after 24 hours. The different used statistical tests are explained. **Results.** EPBS was observed in 15 patients (50%). In the preoperative moment, there were no differences between both groups, except for a lower plasma concentration of PAI-1 in the patients who showed EPBS. In the moment of arrival at the intensive care unit, those patients who made EPBS presented lower levels of C1q, C1 inhibitor, C7, Factor B of the complement, PAI-1, PT, and leptin, than the other patients. After 4 hours of arrival, the patients with EPBS presented lower levels of C1q, C1 inhibitor, C3, C7, Factor B, leptin, PT and fibrinogen. Finally, after 24 hours of arrival at the intensive care unit, the values of C1q, C4 and leptin, were significantly lower in the EPBS-group. We did not find differences in the following factors and parameters: lactic acid, interleukin-6, soluble TNF receptor, APTT, antithrombin, d-dimer, tPA, BUN, creatinine, leukocytes, platelets, CK-NAC and CK-MB, administered dose of dobutamine and noradrenaline, and haemodynamic indexes (cardiac index and systemic vascular resistance index). **Conclusions.** In our experience, several biochemical and hemostatic parameters could serve as predicting factors of EPBS in patients undergoing cardiac surgery. Specifically, some factors of the complement system and leptin (obesity-related protein) seem to play an important role. Our work supports that the activation of the complement system caused by the CPB, could play an important role in the postsurgery hemorrhage.

**0426**

**SUBJECTIVE TRAINING EFFECTS ON ADULT PATIENTS WITH HAEMOPHILIA ATTENDING A SPORTS THERAPY PROGRAMME**

S. von Mackensen,1 D. Czepa,1 M. Herbleb,1 R. Ziecio,1 T. Hilberg2

1University of Hamburg, HAMBURG, Germany; 2F-S University, JENA, Germany

**Background.** Only since some years sport activities have been recommended for haemophilia patients. Still now the importance of sports therapy as an integral element in haemophilia treatment has not yet been widely recognized. In the frame of the Haemophilia & Exercise Project (HEP) the success of a two years sport specific therapy was evaluated subjectively in terms of isometric muscular strength and proprioception and subjectively in terms of the WOMAC questionnaire and the orthopaedic joint score. Subjectively perceived training effects were tested with the a newly developed sport-specific questionnaire (HEP-Test). In addition quality of life was tested with the SF-36 and the haemophilia-specific quality of life questionnaire (Haem-A-Qol). **Aims.** Assessment of subjective training effects of a sports therapy programme for adult patients with haemophilia in terms of bodily condition and quality of life. **Methods.** Based on the contents of the training programme a sport-specific questionnaire (HEP-Test) was developed consisting of 33 items pertaining to 6 dimensions (physical status, mobility, strength & control, endurance, balance, feelings) and rated from 1 (very bad) to 5 (very good). The HEP-Test was pilot tested in 23 German adult haemophilia patients and tested for it’s feasibility in terms of acceptance, comprehensibility and relevance. Data were psychometrically analysed in terms of reliability and validity (criterion, convergent, discriminant). Correlation of the HEP-Test with subjective and objective measures were performed. **Results.** From the 23 enrolled patients 87% were severely affected by haemophilia. In 8.7% inhibitors occurred and half of the patients received prophylactic treatment (52.2%). 47.8% of the patients reported target joints. Viral infections were found in 65.2% of the patients (hepatitis C) and in 21.7% for HIV. Concerning the newly developed HEP-Test the mean completion time was 15 minutes; the questionnaire was well accepted and patients found it related to physical activities. Feasibility testing led to the omission of 9 items and suggestions for rewording of some items were given by patients. Psychometric testing revealed excellent characteristics for reliability (Cronbach’s α ranging from .82-.90). Validity testing showed high correlation between scales of HEP-Test, SF-36 and WOMAC. Acceptable to high correlation were found with the orthopaedic joint score and the isometric muscular strength test. Discriminant validity testing revealed significant differences for clinical subgroups. **Conclusions.** HEP-Test is a short questionnaire assessing subjective training effects. HEP-Test was well accepted by patients and showed quite satisfactory psychometric characteristics. Subjective training effects can be measured with the HEP-Test and should be combined with objective assessments in order to reveal aspects, which can not be measured objectively such as body perception.

**0427**

**THE USE OF FEIBA AND NOVOSEVEN FOR TREATMENT OF BLEEDING EPISODES IN PATIENTS WITH HEMOPHILIA A AND FACTOR VIII INHIBITOR: A SINGLE CENTRE EXPERIENCE**

P. Smejkal,1 J. Muzik,1 M. Penka,1 A. Bulikova,1 M. Matyskova,1 M. Slechtova,1 J. Kasaova,1 G. Chlupova,1 L. Dusek2

1University Hospital, BRNO, Czech Republic; 2Centre of Biostatistics and Analyses, MU, BRNO, Czech Republic

**Background.** Factor VIII replacement is impossible in hemophiliacs with high titre inhibitor, so FEIBA® and NovoSeven® are the main possibility in treatment of bleeding. **Aims.** Evaluate efficacy and consumption of the products in treatment of bleeding episode in hemophiliacs with factor VIII inhibitor. **Methods.** We used data accumulated in our hemophilia centre in the course of 6 years between 2000 and 2005. Five hemophiliacs with factor VIII inhibitor were treated on demand with FEIBA® (49-72 U/kg 8-12 h) or with NovoSeven® (dose 0.05-0.2 U/kg to 2 h). For efficacy we used evaluation criteria: excellent: bleeding stops within 8 hours from start of treatment, efficient: bleeding stops more than 8 hours following start of treatment, partially efficient: bleeding stops but recurring within 48 hours following stop of bleeding, inefficient: no stop of bleeding after 48 hours of treatment or need for another treatment. **Results.** Patients had 124 bleeding episodes, included 99 spontaneous bleeding episodes (88 hemarthroses, 6 muscle bleedings and 5 other sites bleedings) and 17 traumatic bleedings (9 hemarthroses, one muscle bleeding, 2 other sites bleedings and 5 multiple sites
bleedings) and 8 re-bleeding episodes. Bleeding episodes were treated mostly with NovoSeven® (7) and with FEIBA® (45). We evaluated all episodes (except the re-bleeding episodes) treated with NovoSeven® (71) or with FEIBA® (44). Median total dose per episode was 352 µg/kg and 190 U/kg, dose per infusion was 112 µg/kg and 60 U/kg. In episodes with re-bleeding treated with NovoSeven® (6) median total dose per episode was 382 µg/kg, dose per infusion was 110 µg/kg. Using FEIBA® one episode with re-bleeding had occurred total dose 95 U/kg, 47 U/kg per infusion (dosage was lower than average). The efficacy of NovoSeven® and FEIBA® was excellent in 70% and 47.7% of the episodes, efficient in 21.4% and 47.7%, partially efficient in 8.6% and 2.3%, inefficient in 0 and 2.3%. Separately we evaluated spontaneous hematomas. NovoSeven® was used in 54 episodes and FEIBA® in 34 episodes. Median total dose per episode was 352 µg/kg and 187 U/kg, dose per infusion was 112 µg/kg and 60 U/kg. In episodes with re-bleeding treated with NovoSeven® (5) median total dose per episode was 385 µg/kg, dose per infusion was 112 µg/kg. Using FEIBA® only one episode was with re-bleeding it had been mentioned above. The efficacy of NovoSeven® and FEIBA® was excellent in 70.4% and 47.1% of the episodes, efficient in 20.4% and 50%, partially efficient in 9.2% and 2.9%. Median (mean) interval between start of bleeding and start of treatment in spontaneous hematomas treated with NovoSeven® without re-bleeding was 2.4 h (3.9 h), with re-bleeding was 2.5 (2.7 h). Conclusion. In our experience treatment with NovoSeven® stopped bleeding earlier than with FEIBA®, however about 9% of the episodes were with re-bleeding although the dosage used in these episodes and intervals to start of treatment were on the same level as in episodes without re-bleeding. The question is why any bleedings treated in the same way were recurring and the other not.

0428
ORTHOPAEDIC STATUS OF PERSONS WITH HAEMOPHILIA IN A DEVELOPING COUNTRY
M. Serban,1 C. Jinca,2 L. Pop,3 D. Poenaru,1 H. Ionita,1 A. Lacatusu,1 M. Bataneant,3 M. Serban,1 M. Gafencu,2 S. Arghirescu,1 H. Ionita,1
1University of Medicine V. Babes, TIMISOARA, Romania; 2University of Medicine V. Babes, TIMISOARA, Romania; 3Clinical Centre Cristian Serban, BUZIAS, Romania; 4Ludwig Maximillian University, MUNICH, Germany

Background. The medical approach of haemophilia, prototype of rare diseases, cannot be considered without the quality of sanitary system in a country. As joints are target sites of bleeding, the orthopaedic status of persons with haemophilia (PWH) is accepted as reliable clinical reflection of diagnostic and therapeutic performances in this disease. Aims. We sought to perform a cross-sectional analysis of joint status in haemophilia B and haemophilia A in children and adolescents and in adults. Methods. A prospective study was conducted on 93 patients (77 with haemophilia A and 16 with haemophilia B) consecutively enrolled: 31.18% children and 68.81% adolescents and young adults; 92.47% with a rest-activity <10%. Number of total bleeds and joint bleeding events/patient/year, number of patients with target joints and number of target joints/patient, clinical score/joint, global clinical joint score and motor performance as well were analysed. For arthropathy assessment Petrini scale in children and Gilbert scale in adults were used. Results. 15.65 bleeds/patient/year and 11.44 joint bleeds/patient/year in children, vs. 25.34 and 20.28 respectively in adults were found. Only 27.5% of children, and 9.57% of adults were spared of joint bleeding. More than 50% of adolescents had target joints, 51.17% of them with more than one affected joint. The global joint score was 22.96±21.11 in children and 38.38±20.79 in adults; 23% of patients presented chronic pain, and 75.86% vs. 100% (children vs. adults) live with the burden of functional deficit. Conclusions. The deleterious impact of inadequate substitution in haemophilia is evident, not only on joint status, but on quality of life as well. This situation imposes an urgent improvement of the therapy, a costly action, but certainly a cost-efficient one from the point of view of medical economics.

0429
SEUM VASCULAR ENDOTHELIAL GROWTH FACTOR AND THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR ANTIGEN IN CHILDREN WITH DISSEMINATED INTRAVASCULAR COAGULOPATHY
S. Aytaç, B. Bayrakci, H. Okur, G. Tekinalp, A. Gürgey
Hacettepe University, ANKARA, Turkey

Background. Disseminated intravascular coagulopathy (DIC) is a syndrome characterized by systemic intravascular activation of coagulation resulting in depletion of platelets and coagulation factors. Hypercoagulability and hyperfibrinolysis were both thought to be important mechanisms of DIC. Vascular endothelial growth factor (VEGF) is one of the potent angiogenic polypeptides produced by multiple tissues. High amount of VEGF is stored within circulating platelets and is subsequently released during platelet aggregation. When chronic stimulation of VEGF continues vascular hyperpermeability and thrombosis may be induced. We speculated that extremely high value of VEGF in serum of the patients with DIC might be caused via VEGF release in activated platelets. Thrombin-activatable fibrinolysis inhibitor (TAFI) is considered as a modulator of fibrinolysis and therefore it might play an important role in the pathogenesis of DIC. We planned to evaluate the predictive value of serum VEGF and TAFI for the determination of DIC. Aim. To evaluate clinical and laboratory findings of 40 consecutive children in a single center, diagnosed as DIC according to ISTH criteria and compare serum VEGF, TAFI levels of these patients with 40 healthy children to clarify their roles in the pathogenesis of DIC. Methods. Forty patients who experienced DIC in our department (Pediatric Hematology Unit of Hacettepe University, Ankara) between December 2000- May 2005 were examined. At the time of diagnosis hemostatic datas of patients with DIC were noted, serum sample of patients with DIC was collected and stored at -80 °C. Results. The underlying diseases of the patients were congenital heart disease (7 patients), chronic renal failure (5 patients), malignancy (5 patients), metabolic disease (5 patients) and collagen tissue disease (2 patients) Twenty four patients had infection, which 17 of them were documented. Mean acute quantitative CRP level was 6, 0±6, 2. At the time of diagnosis median WBC count was 7050/mm³ (600-154000), platelet count was 70.000/mm³ (8000-624000) and hematoglobin level was 9,5 gr/dl (5,8-16,5). Low level of protein C and S levels were detected in 18 (32.5%) and 9 (22.5%) patients respectively. Fibromen levels were decreased only in six of patients. Majority of patients (87 (92.5%)) had prolonged prothrombin time over 6 seconds. D-dimer levels over 2 g/dl. were detected in 36 (90%) patients. No significant difference were observed in the VEGF levels between study group (320,25±327,33 pq/ml) and healthy controls (514,51±679,19) (p=0.603). There were significant difference in serum TAFI Ag levels between control group (88,9±16,9%) and in patients with DIC (82,3±14,3%) (p=0.007). Conclusions. We could not show a correlation between the platelet count and VEGF levels. High value of VEGF in serum of the patients may be over after the disease progression and serial analysis might be helpful. On the other hand TAFI Ag levels were significantly low in patients with DIC. As it has been suggested that TAFI Ag is mainly under genetic control, further combined approach measuring TAFI levels and TAFI gene polymorphism will be needed.

*This study was supported by Turkish Society of Hematology.
Dendritic cells and cellular immunotherapy

0430 EXPRESSION AND REGULATION OF ENDOTHELIAL PROTEIN C RECEPTOR IN MONOCYTE-DERIVED DENDRITIC CELLS


Unit di Ematologia e Trombosi, MILANO, Italy; Unit di Anatomia Patologica, MILANO, Italy

Background. Endothelial protein C receptor (EPCR) is a transmembrane protein, homologous to MHC class-I molecules, that enhances the rate of protein C activation on endothelial cells. It is reported that EPCR mediates the anti-apoptotic activity of activated protein C on endothelial cells. EPCR was identified also in polymorphonuclear leukocytes and in monocytes. We previously showed by immunohistochemistry that dendritic-like cells in the normal gut mucosa express EPCR. Aims of the study. 1. To characterize phenotypically the gut mucosa EPCR+/dendritic-like cell. 2. To study, in a model of dendritic cell generated in vitro, the expression of EPCR and its modulation. Methods. EPCR was identified by immunohistochemistry, immunofluorescence or flow cytometry. Dendritic cells in vitro were obtained from CD14+ peripheral blood leukocytes, cultured in the presence of interleukin-4 and GM-CSF (MoDCs). Specific messenger RNA (mRNA) was measured by RT-PCR. Results. We confirm that the gut mucosa dendritic-like cells have a phenotype characteristic of dendritic cells, namely they express CD80, CD83 and HLA-DR. We could not identify by immunohistochemistry EPCR+/dendritic cells in other tissues, such as lymph node, spleen, tonsil, liver, lung, and skin. EPCR surface expression on MoDCs was monitored by flow cytometry together with expression of the DC markers HLA-DR, CD1a, CD80 and CD83. After 7 days of culture, approximately 25% of immature DCs expressed EPCR on their surface. De novo expression of EPCR was not correlated with modulation of apoptosis or cell cycle. Lipopolysaccharide-induced terminal maturation of MoDCs down regulated the surface expression of EPCR by 40% while up regulating the expression of CD83. Incubation of cultured DCs with prostaglandin E2 up regulated EPCR mRNA and protein expression by about 3 fold at 50 hours. Flow cytometry studies were compounded by confocal microscopy, which showed that dendritic cell EPCR has the same membrane distribution pattern as in endothelial cells. Conclusions. Contact with bacterial antigens modulates EPCR expression on MoDCs, suggesting EPCR might be involved in antigen recognition or processing.

0431 HIGH AFFINITY CYTOTOXIC T CELLS CANNOT OVERCOME THE INTRINSIC RESISTANCE TO THERAPY OF (LEUKEMIC) PRECURSOR CELLS IN DORMANCY

I. Jedema, C.A.M. van Bergen, M.G.D. Kester, R. Willemze, J.H.F. Falkenburg

Leiden University Medical Center, LEIDEN, Netherlands

Most patients with leukemia treated with chemotherapy show a good initial response to therapy. However, despite multiple courses of treatment even in patients initially developing a complete clinical response late relapses of the same leukemia may occur, suggesting that a fraction of the leukemic precursor cells evades the treatment. The selectivity of chemotherapeutic interventions for cells in active cell cycle can explain why treatment with high dose chemotherapy is causing limited harm to normal tissues of the patient. In accordance, we and others have previously demonstrated universal protection of cells in dormancy to conventional chemotherapy. It has been hypothesized that treatment with cytotoxic T lymphocytes expressing a T cell receptor (TCR) with high affinity for the defined antigen (high affinity CTL) would not be hampered by the dormant state of leukemic precursor cells. To analyze this we determined the sensitivity of leukemic cells and normal hematopoietic cells in dormancy and in active cell cycle to cell death induced by high affinity CTL clones recognizing allo-HLA or minor histocompatibility antigens (mHag). We analyzed the sensitivity to antigen-specific killing by T cells of dormant and proliferating normal CD34+ precursor cells and CD34+ CML precursor cells, of normal B cells, T cells and monocytes, and of activated B cells (EBV-LCL) and activated T cells (PHA blasts). In the study we found that all activated, proliferating target cells were very efficiently lysed, resulting in 60-90% lysis already after 4 hours of exposure to the CTL clones (E/T ratios 1/1-5/1). In contrast, target cells in relative dormancy including the non-proliferating CML stem cell fraction, unmanipulated CD34 progenitor cells, and resting T and B cell subsets appeared to be protected from CTL-induced cell death (0-20% lysis). To investigate whether these target cells in dormancy were intrinsically resistant to the effector mechanism used by the T cells or that decreased avidity of the interaction between dormant targets and high affinity effectors was underlying the poor susceptibility, we artificially enhanced the avidity by exogenous loading of the target cells with saturation concentrations of the relevant peptide. This was sufficient to completely restore the sensitivity to levels comparable to activated proliferating target cells, suggesting that reduced avidity of the interaction is playing a significant role in the differential killing of activated and dormant target cells. The differential susceptibility of dormant and activated target cells for T cell recognition may also explain the differential capacity of T cell subsets to cause graft versus host disease (GvHD) in the absence or presence of the cytokine storm after alloSCT. We mimicked the cytokine production during GvHD by the addition of interferons. This resulted in a limited, but significant upregulation of the sensitivity of the initially resistant target cell types to recognition by the T cells. In conclusion, we here demonstrate that normal hematopoietic and leukemic cells in dormancy are relatively resistant to cell death induced by high affinity CTL clones. This selective resistance of cells in dormancy is caused by the diminished avidity of the interaction with the CTL.

0432 DIFFERENTIATION TOWARDS LEUKEMIC DENDRITIC CELLS IS HINDERED BY THE PRESENCE OF A FLT-3 INTERNAL TANDEM DUALPLICATION IN AML BLASTS

I. Houtenbos, M. Westers, J. Ossenkoppele, A. van de Loosdrecht

VU University Medical Center, AMSTERDAM, Netherlands

In the search for new treatment modalities to eradicate minimal residual disease (MRD) in acute myeloid leukemia (AML), immunotherapy provides an attractive option. AML blasts show differentiation towards leukemic dendritic cells (DC), providing the unique opportunity to generate DC harbouring the full range of tumour antigens. In a large cohort of AML samples (n=154) AML-DC were generated by two culture methods, i.e. in presence of cytokines GM-CSF, TNF-α, SCF, Flt-3L, IL-3 and IL-4 (n=147) or calcium ionophore (Cl) and IL-4 (n=108). Medi- an AML-DC yield, defined by phenotypical DC characteristics, in the cytokine-based cultures was 12% (range:0-70%). Considering cultures yielding ≥10% AML-DC successful, 58% (85/147) of cytokine-based cultures were successful and 61% (66/108) of Cl cultures. Overall, functional AML-DC generated with either method was possible in 66% (101/154) of patients. Identification of AML blast populations with DC differentiation capacity is important to select patients eligible for immunotherapy programmes. Interestingly, presence of Flt-3 internal tandem duplication (ITD) was strongly correlated with decreased DC differentiation capacity in both culture methods (cytokine-based culture: p<0.001; Cl-culture: p=0.03) suggesting that constitutive activation of tyrosine kinase receptors inhibits differentiation towards DC. In multiparameter regression analysis, powerful predictors for cytokine-based AML-DC culture outcomes were positive Flt-3 ITD (B=0.4; p<0.001), CD14 (B=0.28; p<0.001) and TNFαRII (B=0.22, p<0.001). This regression model predicts 88% of culture outcomes. ROC curves show high sensitivity (95%) and specificity (76%) with an AUC of 0.93 (p<0.001). In 25% of unsuccessful cytokine-based cultures, the Cl-based culture method provides an alternative. This percentage increases to 56% if Flt-3 ITD+ AML samples are left out, emphasizing Flt-3 ITD+ blasts' inability to differentiate towards leukemic DC. In conclusion, AML-DC cultures are successful in most patients. Selection of patients is well possible based upon the presence of Flt-3 ITD and the expression of CD14 and TNFα-RI. Based on these results, we are currently entering patients in a phase I/II clinical vaccination trial.

0433 THE NOTCH LIGAND DELTA-LIKE1 PROMOTES THE GENERATION OF INTERSTITIAL AND PLASMACYTOID DENDRITIC CELLS FROM HUMAN MYELOID COMMITTED PROGENITOR CELLS

S. Richter, B. Platter, I. Heinz, P. Reiser, H. Strobl

Medical University of Vienna, VIENNA, Austria; iSREC, EPALINGES, Switzerland

The Notch ligand Delta-like-1 (DL-1) and the ETS-transcription factor PU.1 are key regulators of dendritic cell development. We recently observed that ectopic expression of PU.1 in CD34+ cord blood progenitor cells promotes the development of human conventional CD1a+ DCs (cDC), compromising Langerhans cells and interstitial type DCs.
DL-1 induces CD11a+ DCs from human progenitors at the expense of CD14+/CD11b+ monocytes, and this effect is associated with the upregulation of PU.1. Plasmacytoid DCs (pDC) represent a third DC subset which can be generated from both, granulomonocyte progenitors and lymphoid progenitors. In contrast to cDCs, they lack myeloid markers and CD11a, and are PU.1 low/negative. Their lineage relationship to cDCs remains poorly characterized. We generated myeloid progenitors (MP) by expanding cord blood CD34+ cells with early acting cytokines in vitro. Using the OP9 stroma culture system, we here show that DL-1 promotes pDC development from MPs. Notch signaling promoted pDC generation from MPs at the expense of CD11b+ cells. These data suggest that pDCs and cDCs share a common myelomonocytic committed progenitor cell, and that Notch and PU.1, together with additional factors, might induce the lineage specification of pDCs versus cDCs.

**0434**

**VACCINATION WITH WT1 AND PR3-DERIVED PEPTIDES IN PATIENTS WITH AML/MDS AND MUC1-DERIVED PEPTIDES IN PATIENTS WITH MULTIPLE MYELOMA - PRELIMINARY RESULTS**


Johannes Gutenberg University Mainz, MAINZ, Germany; Evangelisches Krankenhaus Essen-Werden, ESSEN, Germany

**Background.** It has been demonstrated that the Wilms Tumor gene (WT1) is highly expressed in various types of leukemia. WT1 expression level reflects the extent of minimal residual disease and significantly increases at relapse. Proteinase 3 is an aberrantly expressed myeloid leukemia protein and T cells with specificity for both, Wt1 and Pr3-derived antigens, have been generated in vitro from healthy individuals and cancer patients and lysed myeloid leukemia blasts. MUC1(CD227) is presented on a considerable amount of multiple myeloma cell lines and plasmacytoma cells, but only some B-cells. Several lines of investigation have provided conclusive evidence that MUC1-derived HLA-class I/II epitopes do represent universal tumor antigens, which are also expressed by malignant plasmacytoma cells and could thus be attacked by MUC1-specific CTLs. **Aims.** We investigate safety and feasibility of a vaccination with WT1 and Pr3 or MUC1-derived peptides in patients suffering from AML or multiple myeloma, respectively. Thereby we assess the induction of tumor-antigen specific T-cells as well as clinical responses. **Methods.** HLA A2.1 positive patients with AML/MDS <30% blasts in the bone marrow biopsy receive 6 injections of WT1 and Pr3-derived peptides, combined with Montanide ISA51 (incomplete Freund’s adjuvants), PADRE and VaxImmune (Cpg 7909). Vaccination is given every two weeks. HLA A2.1 positive patients with multiple myeloma Stage I, stable disease or partial remission after chemotherapy receive 6 injections of two different MUC1-derived peptides, VaxImmune and Montanide with or without PADRE. Safety and feasibility as well as clinical course is reassessed every visit. Induction of immune response is assessed by ELISPOT, Cr-release-Assays and FACS-analysis (Tetramer-staining). **Results.** So far, three patients completed our ongoing AML-vaccination protocol; four patients were vaccinated in the myeloma-study. A total of 10 patients will be treated in each trial. Local inflammatory responses at the injection site, such as redness, swelling and pain were observed in all patients (Grade II). In one case, skin necrosis (Grade II) and superinfection occurs. Four out of seven patients developed a systemic reaction including influenza-like symptoms and fever (Grade I-II). One patient suffered from an anaphylactic reaction (Grade III) after vaccination. Clinical response data have so far been analysed for patients vaccinated with Pr3 and WT1-derived peptides: Peripheral platelet counts of a patient suffering from MDS RAEB-T improved while blasts detected in the bone marrow remained stable. Two out of three patients with refractory AML remained progressive even after six vaccinations. Clinical responses of patients treated with MUC1-derived vaccine as well as immunological analyses of all vaccinated patients are pending and will be presented at the meeting. **Summary / Conclusion.** Vaccination with WT1, PR1 or MUC1, PADRE, VaxImmune or Montanide is safe and feasible, even in advanced disease and after multiple previous therapies, including stem cell transplantation. Observed local as well as systemic side effects were predominantly mild to moderate. Overall clinical and immunological response data will be presented.

**0435**

**CANCER-TESTIS ANTIGENS ARE COMMONLY EXPRESSED IN MULTIPLE MYELOMA AND INDUCE SYSTEMIC IMMUNITY FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION**

D. Atanackovic, Y. Cao, J. Arfsten, A.R. Zander, C. Bokemeyer, N. Kröger

University Medical Center Hamburg, HAMBURG, Germany

**Background.** Immunotherapies using cancer-testis (CT) antigens as targets represent a potentially useful treatment in patients with multiple myeloma (MM) who commonly show recurrent disease following chemotherapy. Furthermore, CT antigens might represent targets for graft-versus-leukemia (GVL) effects following allogeneic stem cell transplantation (alloSCT). **Methods.** We analyzed the expression of 11 CT antigens in bone marrow samples from MM patients (N=107) and healthy donors (N=32). Furthermore, we analyzed 66 MM patients for antibody responses against MAGEA3, SSX2, and NY-ESO-1 in an ELISA assay. Finally, we screened a patient with a humoral responses against NY-ESO-1 for T cells against the same antigen in an ELISPOT assay using overlapping peptides. **Results.** CT antigens were frequently expressed in MM with 56% (MAGEC2), 55% (MAGEA3), 35% (SSX1), 20% (SSX4, SSX5), 16% (SSX2), 15% (BAGE), 7% (NY-ESO-1), and 6% (ADAM2, IPII) expressing the given CT antigen (see Figure). Importantly, with the exception of SSX4 none of the CT antigens were expressed in healthy bone marrow. Analyzing our patients for IgG antibodies against MAGEA3, SSX2, and NY-ESO-1, we found strong antibody responses against CT antigens in 9 patients who had received alloSCT. Antibody responses against NY-ESO-1 correlated with NY-ESO-1-specific CD4+ and CD8+ T cell responses against peptide NY-ESO-1 51-62 and CD4+ responses against peptide NY-ESO-1 121-140 in one of these patients. These allogeneic immune responses were not detectable in pre-transplant samples and in the patients’ stem cell donors indicating that CT antigens might indeed represent natural targets for graft-versus-leukemia effects. **Conclusions.** We show here for the first time that CT antigens induce spontaneous antibody and T cell responses in MM patients who received alloSCT. These immune responses induced by alloSCT could probably be boosted by active CT antigen-specific immunotherapy which might help to achieve long-lasting remissions in patients with MM.

**0436**

**PHENOTYPIC CHARACTERIZATION OF PLASMACYTOID DENDRITIC CELLS LINEAGE MATURATION PATHWAY IN NORMAL ADULT BONE MARROW: A FRAME OF REFERENCE FOR UNDERSTANDING DENDRITIC CELL MALIGNANCIES**

M.L. Martin, J. Almeida, A. Orfao

Centro de Investigación del Cáncer, SALAMANCA, Spain

**Background.** A new entity of haematological malignancy has been identified recently as arising from plasmacytoid dendritic cell (pDC) precursors, the maturation pathway of their normal counterpart in bone marrow (BM) being largely unknown. **Aims.** To analyze the immunophenotypic features and their changes associated to pDC maturation in BM samples from normal adults as a frame of reference for better identifying pDC precursor malignancies. **Methods.** A total of 25 BM samples were stained by direct immunofluorescence techniques using a large panel of monoclonal antibodies (mAbs) in 6-color combinations. Data acquisition was performed in a FACS Canto flow cytometer and the FACSDiva software program was used for data analysis. **Results.** In all cases, three pDC maturation stages were clearly identified based on the expression of CD34, HLA-DR, CD123 and CD45. Accordingly, the more immature precursors (stage I), represent-
ed 17.0±6% of all pDC and showed a CD34+/aHLA-DR+CD123+/CD45+ phenotype; intermediate stage pDC (stage II), represented 21.0±6% of all pDC and they were CD34+/HLA-DR+CD123+/CD45+ and the more mature pDC (52.0±14%) were CD34+/HLA-DR+CD123+/CD45+ (stage III). Both HLA class I and class II molecules showed a high expression in stage I pDC, which decreased in the intermediate stage to finally recover and remaining expressed at high levels in stage III pDC. Congruently, the expression of costimulatory molecules, BM pDC were negative for CD1a, CD1b, CD1c, CD209, CD275 and the TLR4 and TLR9 Toll-like receptors, while CD303 (BDCA2), CD304 (BDCA4) and CD305 appeared in the more immature stage, progressively increasing their levels along the differentiating pDC. CD4 showed an identical pattern. Expression of other molecules, such as CD11a, CD36, CD38, CD45RA, CD54, CD62L, CD184 and CD197 was high and quite stable through maturation of pDC, in contrast, expression of CD11c, CD13, CD33, CD64, CD99, CD116, CD117 and CD126 progressively decreased during pDC differentiation, becoming negative in stage III pDC. Interestingly, the CD86 co-stimulatory molecule was detected at high levels in stage I, and dropped quickly afterwards. In contrast, CD40 was only detected in stage III. CLA was heterogeneously expressed throughout the maturation. Conclusion. In summary, we show that at least three maturation stages of pDC are identifiable in normal adult BM, on the basis of their different phenotypic characteristics, representing a frame of reference for a better identification and understanding of pDC precursor malignancies.

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0437

COMBINED TYROSINE KINASE INHIBITION AND IMMUNOTHERAPY AS A STRATEGY TO IMPROVE OUTCOME AFTER REDUCED INTENSITY ALLOGENIC STEM CELL TRANSPLANTATION IN CHRONIC MYELOID LEUKAEMIA

C. Craddock,1 E. Olavarria,2 M. Griffiths,3 J.M. Arrazi,3 S. Siddique,1 A. Lennard,1 J. Byrne1

1Queen Elizabeth Hospital, BIRMINGHAM, United Kingdom; 2Hammersmith Hospital, LONDON, United Kingdom; 3West Midlands Genetics Laboratory, BIRMINGHAM, United Kingdom; 4Royal Victoria Infirmary, NEWCASTLE UPON TYNE, United Kingdom; 5Nottingham City Hospital, NOTTINGHAM, United Kingdom

Background. The graft-versus-leukaemia effect of allogeneic stem cell transplantation and donor lymphocyte infusions (DLI) is capable of producing long-term disease free survival in CML. Despite this the toxicity of the conditioning regimen and risk of graft-versus-host disease (GVHD) make allografting an unattractive therapeutic option in most patients. Recently it has been shown that reduced intensity conditioning (RIC) regimens incorporating alemtuzumab reduce both transplant related mortality and GVHD risk. However such regimens are associated with a high rate of relapse which occurs in most patients within the first year post-transplant. Whilst DLI is a safe and effective salvage therapy after a myeloablative transplant, its use is associated with a significant risk of severe GVHD in patients who relapse early after a RIC transplant. Therefore strategies which permit the effective use of DLI after RIC allografts are required if they are to fulfill their curative potential. Aim. We have studied whether the administration of leukaemia specific therapy in the form of the kinase inhibitor Imatinib for a limited period after a RIC transplant can postpone the requirement for DLI thereby reducing its toxicity in patients who eventually relapse. Methods. Patients with an available sibling donor underwent allogeneic stem cell transplantation using fludarabine 25mg/m2/day iv (days -7 to -8), Busulphan 8 mg/m2 orally (days -5, -4) and alemtuzumab 10 mg/day iv (days -7 to -5). Cyclosporin was used as GVHD prophylaxis. Imatinib was commenced on day +35 and continued until one year post transplant at 400mg daily. Minimal residual disease levels were measured by quantitation of BCR-ABL transcript numbers at three monthly intervals. Escalating dose DLI was administered in patients who relapsed after the discontinuation of Imatinib. Results. DLI in 1st relapse case was effective and patients were in first chronic phase between March 2002 and September 2005. The median age was 49 (25-57). All patients engrafted promptly and commenced Imatinib on day +35. All tolerated continuous treatment until one year post-transplant apart from 3 who it was temporarily discontinued because of gastrointestinal intolerance. The day 100 transplant related mortality was 0%. Only one patient developed acute GVHD (grade 3) and all patients achieved molecular remissions. 4 patients achieved durable molecular remissions. 4 patients developed GVHD in association with DLI. Summary. We conclude that the combination of Imatinib and a RIC allograft is remarkably well tolerated in patients with CML and may allow the subsequent delivery of DLI without compromising its ability to produce molecular remission. The use of adjunctive kinase inhibitors may enhance the immunotherapeutic potential of DLI and improve outcome after RIC allografts in other diseases in which targeted therapies are available.

0438

CYTOKINE INDUCED KILLER CELLS TRANSLUCED WITH ANTI-CD19 CHIMERIC RECEPTORS CONTAINING 4-1BB HAVE POWERFUL ANTI-LEUKEMIC ACTIVITY

V. Marin,1 H. Kakuda,2 S. Iwamoto,3 G. D’Amico,4 D. Campagna,2 A. Bianchi1

1Centro di Ricerca M.Tettamanti, MONZA, Italy; 2St Jude Children’s Research Hospital, MEMPHIS, USA

Background. CIK cells are a population of ex-vivo expanded cells with MHC-unrestricted cytotoxicity against several tumoral targets, except B-lineage Acute Lymphoblastic Leukemia (ALL). We have recently demonstrated that transduction of an anti-CD19-ζ chimeric receptor in CIK cells rendered them efficient killers of CIK-resistant ALL cells. Conceivably, the capacity to proliferate after and contact with leukemic cells and to exert prolonged anti-leukemic cytotoxicity after infusion should be important to maximize the likelihood of success of this cell therapy. It was previously shown that incorporation of costimulatory molecules into chimeric receptors markedly enhances target-cell stimulated proliferation and cytotoxicity in T lymphocytes and Natural Killer cells. Aims. to identify costimulatory molecules that increase the cytolic activity and proliferative capacity of anti-CD19-ζ receptor transduced CIK cells. Methods. CIK cells were transduced with a RD114-pseudotyped retroviral vector carrying different types of receptors: anti-CD19-ζ, anti-CD19-DAP10, anti-CD19-4-1BB-ζ and anti-CD19-CD28-ζ. A truncated form of the receptor was used as control. The cytotoxic activity of transduced CIK cells against ALL cells was evaluated by co-culture with the OP-1-1 (OP-1-1)4007. 1V. Galtseva,1 M. Lebrun,1 R. Durand,1 I.V. Galtseva,1 M. Lebrun,1 R. Durand,1 L. Guitton,1 M. Monard,1 E.L. Granjon,6 C. Debaillie,1 A. Symoens,1 P. Moiroux,1 P. Deyts,1 J.P. Chastan,1 R. Fabre,1 and E. Gobert1

1Centre de Recherche M.Tettamanti, MONZA, Italy; 2St Jude Children’s Research Hospital, MEMPHIS, USA

Background. CD107a expression is a hallmark of cellular immune activation and degranulation. Since 2000, IL-2 and IL-15 have been used in adoptive T-cell therapy. However, the cytolytic activity of CD107a expression in these T-cell therapies remains unclear. Methods. CD107a expression by flow cytometry was studied in CIK cells transduced with anti-CD19-ζ, anti-CD19-DAP10, anti-CD19-4-1BB-ζ and anti-CD19-CD28-ζ receptors. CIK cells were transduced with a RD114-pseudotyped retroviral vector carrying different types of receptors: anti-CD19-ζ, anti-CD19-DAP10, anti-CD19-4-1BB-ζ and anti-CD19-CD28-ζ. A truncated form of the receptor was used as control. The cytotoxic activity of transduced CIK cells against ALL cells was evaluated by co-culture with the OP-1-1 (OP-1-1)4007. 1V. Galtseva,1 M. Lebrun,1 R. Durand,1 I.V. Galtseva,1 M. Lebrun,1 R. Durand,1 L. Guitton,1 M. Monard,1 E.L. Granjon,6 C. Debaillie,1 A. Symoens,1 P. Moiroux,1 P. Deyts,1 J.P. Chastan,1 R. Fabre,1 and E. Gobert1

1Centre de Recherche M.Tettamanti, MONZA, Italy; 2St Jude Children’s Research Hospital, MEMPHIS, USA

0439

INTRACELLULAR EXPRESSION ANGIOTENSIN-CONVERTING ENZYME (ACE, CD143) BY LEUKEMIC DENDRITIC CELLS IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

I.V. Galtseva,1 V.G. Savchenko,2 E.N. Farovitchnikova,3 I.A. Vorobiov,1 E.M. Greтов,2 S.M. Danilov2

1National Research Center for Hematology, MOSCOW, Russian Federation; 2University of Illinois, CHICAGO, USA

Background. Dendritic cells (DC) play a key role in the induction of adaptive immune response because they are efficient in antigen presen-
We propose that the absence of surface ACE expression in LDC is generated autologous CML-DC in Immunotherapy represents a promising treatment strategy LDC derived from AML blasts did not express surface ACE against a variety of tumor cells targets through a perforin based mechanism mediated by NKG2D. We started a pilot clinical trial in patients with refrac-
tion of intracellular ACE expressing LDC was significantly higher than in DC (p<0.001 and p<0.001 for clones 1D8 and 9B9, respectively). The proportion of surface ACE positive DC was significantly lower than surface ACE positive DC (p<0.001 for clone 9B9). Conclusion: The data demonstrated that ACE transport to the cell surface of LDC and therefore, provide another evidence of the distorted differentiation capacity of AML blasts.

O440
AUTOLGOU IMMUNOTHERAPY WITH CYTOKINE INDUCED KILLER CELLS FOR HEMATOPOIETIC AND SOLID TUMORS

'Ospedale Sperone Santo, PESCARA, Italy; 'Dipartimento di Medicina Trasfusionale, Italy; 'Dipartimento di Oncologia, Italy; 'Dipartimento di Ematologia, Italy; 'UTHE per il Trapianto Emopoietico, Italy

Background. Immunotherapy represents a promising treatment strategy for many types of cancer. This approach is hampered by the difficulty in generating sufficient number of cytotoxic cells especially in patients heavily treated. CIK cells are a novel population of immune effector cells that have been shown to possess a broad spectrum of antitumor activity against a variety of tumors targets through a perforin based mechanism mediated by NKG2D. We started a pilot clinical trial in patients with refractory lymphoma and metastatic solid tumors according to GMP guidelines that is currently ongoing. The aim of this study is to assess the safety and to determine the antitumor activity of CIK cells. CIK cells were generated from PBMC and incubated in LifeCell culture bags in the presence of IFN-γ followed by IL-1b, OKT3 and IL-2. Expansion was assessed between day 21 and 28 and flow cytometric analysis was performed every week. Patients were monitored before and after treatment. Results. We enrolled 11 patients: 6 advanced lymphomas, 4 metastatic kidney carcinoma (RCC) and 1 hepatocellular carcinoma (HC). The median number of transferred cells per patient was 19×10^9 (6.3×10^9 to 1.4×10^10) and the absolute number of CD3+CD56+ cells infused ranged from 1 to 16×10^10 (median value 5×10^10). Patients affected by solid tumors received in association with IL-1b or interferon-α. Protocol adherence was excellent and the toxicity profile was favourable. Only 2 patients developed low-grade fever during the first cycle of infusions (5%), recovered without antibiotic treatment. After CIK cells infusion, in patients’ peripheral blood the absolute median count of PBLS, CD3+, CD4+ and CD8+ cells significantly increased with a p-value of 0.034, 0.025, 0.054 and 0.935, respectively. Clinical outcome appeared promising, 2 of the 7 evaluable patients achieved complete response: 1 RCC and 1 HC and 2 patients had stabilization of disease (1 NHL and 1 RCC). At the last follow-up they are still alive at 26, 14, 19 and 9 months respectively, after the start of therapy. Conclusions. These preliminary data showed that adoptive immunotherapy with CIK cells is a safe therapy with some suggestion of efficacy that significantly enhances immune functions increasing absolute numbers of effector cells without side effects. If confirmed in larger scale studies, these promising results may have a favourable impact on conventional treatment strategy of malignancies.

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of memory T cells. Aims. Based on these data, in this study we evaluated the importance of modulating the anti-leukemia response, in terms of specificity and phenotypic characteristics of CTLs, using common γ-chain cytokines (IL-2, IL-7 and IL-15) at different phases of the in vivo induction and expansion of anti-leukemia CTL lines. Methods. In particular, anti-leukemia CTL lines supported with the different cytokines were compared for i) levels of the in vivo proliferation and cytotoxic activity of the autologous LT and normal BL non-tumor cells, and ii) population and activation of T memory and effector subsets. Results. We found that, even though sizeable levels of anti-leukemia T-cell response can be obtained in all cultures, the use of different cytokines during the various phases of the induction of antileukemia CTL response allows us to modulate not only the expansion of the corresponding CTLs and their leukemia-directed cytotoxicity, but also the percentage and the absolute number of T memory and effector cells, without loss of specificity. Conclusions. In particular, we demonstrated the crucial role of IL-15, in increasing T central memory (TCM) cells, potentially able to display long-term survival and capacity to in vivo proliferate in the presence of limited amount of LT. Further experiments are in progress to confirm this phenomenon and to evaluate precisely the role of IL-7 in the maintenance of TCM cells during the expansion of anti-leukemia CTLs.

**0444**

**CROSS-COSTIMULATION BY DONOR ANTIGEN PRESENTING CELLS PLAYS A ROLE IN ACUTE XENOGENIC GRAFT-VERSUS-HOST DISEASE**

A. Bondanza, S. Kaneko, M. Ponzoni, F. Ciceri, E. Goulmy, C. Bordignon, C. Bonini

Leiden University Medical Center, LEIDEN, Netherlands; San Raffaele Scientific Institute, MILANO, Italy

Background. Graft-versus-host disease (GvHD) limits the efficacy of allogeneic cellular immunotherapy for the cure of human disease. In mouse models, acute GvHD appears to be initiated by the encounter of donor T cells with host antigen presenting cells (APC). Cross-presentation of host antigens by donor APC arising de novo from the haematopoietic cell graft also participates to GvHD. Aim is to verify if the adoptive transfer of donor APC along with T cells plays a role in acute GvHD. Methods. Adoptive transfer of human T cells in conditioned non-obese diabetic (NOD)/severe combined immunodeficient (scid) and evaluation of the requirements for acute xenogenic GvHD. Results. In vivo, human blood mononuclear cells (PBMC) proliferate in response to dendritic cells (DC) derived from NOD/scid mice. Proliferation depends on the presence of human APC. Posing costimulatory properties of human APC by chemical treatment induces proliferation, while neither blocking MHC class II-restricted antigen presentation with anti-human antibodies nor interfering with antigen processing by chloroquine does. The intraperitoneal transfer of human PBMC into sub-lethally irradiated NOD/scid mice causes acute xenogenic GvHD in a dose dependent manner. After NK cell inactivation, the intravenous route is also effective. In the latter setting, depletion of human APC from PBMC significantly reduces the incidence and the severity of xenogenic GvHD. Conclusions. The adoptive transfer of human APC along with T cells exacerbates acute GvHD possibly through costimulation provided in trans (cross-costimulation). This has important implications for the designing of novel therapeutic strategies.

**0445**

**DEVELOPMENT AND CHARACTERISATION OF GMP-GRDE CYTOMEGALOVIRUS PPRG-SPECIFIC CD8+ AND CD4+ T-CELL LINES FOR ADOPATIVE TRANSFER**


Leiden University Medical Center, LEIDEN, Netherlands

Background. Reactivation of cytomegalovirus (CMV) remains a major cause of morbidity and mortality during the period of immune deficiency following allogeneic stem cell transplantation. Antiviral pharmacotherapy is not satisfactory due to significant toxicity and moderate efficacy. It has been shown that adoptive transfer of donor-derived CMV-specific T-cells may be an effective strategy to control established CMV infection. For a persistent function in vivo the transfer of both virus-specific CD8+ and CD4+ T-cells is essential. Aims. In this study we developed a protocol for the generation of CMV pp65-specific CD8+ and CD4+ T-cell lines for adoptive transfer. The isolation and culture conditions were optimised for clinical implementation which is fully compliable with Good Manufacturing Practice (GMP) conditions. Methods. PBMCs from five CMV seropositive donors were stimulated with different concentrations (6.6-66 μg/mL) of recombinant CMV pp65 protein (Milenyi Biotech) and/or HLA-A*0201/HLA-B*0702 restricted immunodominant pp65 peptides (NIV/TFR). Peptides used were clinical grade and recombinant protein was γ-irradiated (50 Kgy, 80 °C) to eliminate possible microbiological contamination. IFNy producing cells were enriched using the IFNγ secretion assay (Milenyi Biotech) at day 1 after stimulation, and cultured with autologous feeders (10%) and low or high dose of IL-2 (10 or 50 IU IL-2/mL). At day 7-11 cells were harvested and cryopreserved. Cells lines were analysed at different time points for staining by peptide-MHC tetramer (NIVA22/TRP-B7) and phenotypic markers. In addition, pp65-specificity was evaluated by intracellular IFNy staining after restimulation with a pp65 protein spanning pool of 15-mer peptides. CMV-specific lysis was tested in a 51-chromium release assay on pp65-transduced target cells. Results. Enrichment of IFNy producing cells after pp65 protein stimulation resulted in pp65-specific cell lines consisting of both CD8+ (median 28%, range 20-74%) and CD4+ T-cells (median 48%, range 12-79%). The CD8+ compartment contained immunodominant tetramer staining cells (median 60%, range 5-75%). The majority of both CD8+ and CD4+ T-cells produced IFNγ on restimulation with the pp65 peptide-pool and cell lines showed CMV specific lysis of target cells. The phenotype of pp65-specific T-cells was predominant CD8+/CD45RO+ and CD45RA-/CCR7-/CD62L-, although CCR7 and CD62L were transiently expressed at day 4 and 7 after stimulation. Addition of higher concentrations of protein during the initial stimulation had a negative effect on enrichment.
probably due to non-specific stimulation of cells. Addition of immunodominant pp65 peptides resulted in stronger stimulation and proliferation of epitope-specific CD8+ T-cells, although isolation efficiency was not increased. Except for the enhancement of proliferation, no effect of high dose compared to low dose IL-2 was observed. Cryopreservation did not affect the composition or functionality of T-cell lines. Summary/Conclusions. Based on these results, we propose a GMP-compliant method for generation of pp65 epitope-specific T-cell lines using 6.6 µg/mL of pp65 protein for stimulation followed by isolation of specific T-cells based on IFN-γ production. Isolated T-cells will be cultured for a short period on low dose IL-2 in order to retain maximal in vivo potential. This procedure yields GMP-grade T-cell lines comprising both CD8+ and CD4+ CMV-specific T-cells, which will be assessed for their clinical efficacy.

0446

FUNCTIONAL CHARACTERIZATION OF CYTOMEGALOVIRUS (CMV)-SPECIFIC CD4 AND CD8 T CELL LINES GENERATED BY USING PROTEIN-SPANNING POOLS OF PP65 AND IE1 DERIVED PEPTIDES

E. Dander, G. Li Pira, E. Manca, I. Mazzarini, E. Biagi, A. Biondi, G. D’Amico

Research Center M. Tettamanti, MONZA (MI), Italy; G. Gaslini Institute, GENOA, Italy

Background and Aims. Reactivation of latent CMV in immunocompromised recipients of allogeneic stem cell transplantation remains a major cause of morbidity and mortality. Reconstitution of immunity by CMV-mobilized recipients of allogeneic stem cell transplantation remains a major challenge. We have developed flow cytometry methods for the study of in vivo interactions between T cells and leukemic cells. These methods allow the accurate measurement of the growth and apoptosis of leukemic cells in coculture with T lymphocytes. Our laboratory is one of the pioneers in the use of microbeads coated with monoclonal antibodies (MAbs) to study the polyclonal stimulation of T lymphocytes in vitro. Microbeads coated with Abs combinations can simultaneously stimulate the T cell antigen receptor and costimulatory receptors such as CD28 working like artificial antigen presenting cells that can be used to activate and expand antileukemic T cell clones. We are developing in vitro methodologies to break the immune tolerance to the leukemic B-CLL cells and to induce the cytotoxic T cell response against these tumor cells. The first strategy is to polyclonally stimulate (with anti-CD3, anti-CD28 and IL-2) T lymphocytes from these patients. A second and very promising strategy was pioneered by our laboratory. It uses microbeads to bridge leukemic cells and T cells. It uses one antibody against a leukemic cell antigen and other against costimulatory antigens of cytotoxic lymphocytes (anti-CD28). These microbeads link the leukemic cell and the cytotoxic T lymphocyte providing costimulatory signals for the T cell in the case it recognizes one antigen presented by the leukemic B cell. The application of methodologies of cell enumeration in coculture has demonstrated the efficacy of anti-CD28 and anti-CD28 coated microbeads in the stimulation of cytotoxic T cells to kill autologous leukemic B-CLL cells. We are now developing methods of generation of antileukemic effector T cells in vitro. We have demonstrated the efficacy of these cytotoxic T cells to kill autologous leukemic B cells in vitro. We also want to identify and select the T cell subsets with the highest potential for growth and the strongest killing activity against leukemic cells.

0448

THE IMMUNOMODULATORY EFFECTS OF HUMAN UNRESTRICTED SOMATIC STEM CELLS ON CORD BLOOD T CELLS

M. Soleimani, A. Atashi, N. Ahmadbeigi, A.A. Movassaghpour

Tarbiat Modarres University, TEHRAN, Iran

Background. unrestricted somatic stem cells (USSCs) generation was initiated from fresh and cryopreserved cord blood. Reports are indicating that USSCs have unique immunologic properties, making them ideal for cell-based therapy. Indeed, USSCs are not rejected by the immune system of the host, do not induce alloreactivity, and they escape lysis by cytotoxic T-cells and natural killer (NK)-cells. Thus, USSSCs may be transplantable than HLA-mismatched individuals without the need for host immunosuppression. Aims. To evaluate probable immunomodulatory effects of USSSCs on T cells proliferation. Methods. USSSCs were plated in 96-well plates (2,000/well), and co-cultured for 3 days with T cells isolated from cord blood. In control group, cord blood T cells did not co-culture with USSSCs. After cord blood T cells stimulated by PHA for 60 hours, T cell proliferation was assessed by MTT assay. Secretion of IFN-γ from stimulated cells was measured by ELISA kit. Expression of immunoregulatory molecules on USSSCs was analyzed by flow cytometry. Results. USSSCs expressed major histocompatibility complex (MHC) class I, lymphocyte function-associated antigen (LFA-3) constitutively and intercellular adhesion molecule (ICAM-1) antigens upon γ interferon treatment but do not express CD80, CD86, or CD40 costimulatory molecules. The results from IFN-γ measurement showed that cord blood T cell proliferation was suppressed when 2,000 USSSCs were plated on each well. Summary/Conclusions. USSSCs actively inhibit T-cell proliferation, suggesting that allogeneic USSSCs transplantation might be accomplished without the need for significant host immunosuppression. USSSCs transplantation may be use for modulation of immune system in hyper reactive and autoimmune diseases.
Myelodysplastic syndromes

VALIDATION OF THE NIJMEGEN PREDICTIVE SCORE FOR INDUCTION THERAPY IN MDS WITH HIGH-RISK MDS OR AML

U. Gernig,1 C. Strupp,1 S. Knipp,1 A. Giagounidis,1 S. Balleisen,1 B. Hildebrandt,1 A. Kuendgen,1 C. Aul,1 N. Gattermann,1 R. Haas1
1University of Düsseldorf, DUSSELDORF, Germany; 2Heinrich-Heine-Universit"at, DUSSELDORF, Germany; 3Johannes-Hospital, DUSSEBURG, Germany; 4Inst. of Human Genetics, University of DUSSELDORF, Germany

Background. Intensive Chemotherapy in high-risk MDS patients still is a matter of debate. The majority of MDS patients is too old to undergo induction chemotherapy, the relapse rate is high and the proportion of long-term survivors is with about 10-25% relatively low. Taking into account comorbidities, side effects and complications of the therapy as well as about 10% early death rates, one should try to select patients very carefully for intensive chemotherapy. Although it became clear that the initial karyotype predicts CR rate as well as long-term outcome (Knipp et al Blood, abstract 2004), it is not possible to use this as the only parameter to decide whether or not the patient should undergo induction therapy. The Nijmegen group recently proposed a predictive score that was calculated on the basis of a large amount of pa-tients (Oester-veld, Leukemia Research, abstract 2005). Besides the karyotype, WBC, age, an-tercedent haematological malignancy and number of cytopenias were rated differentially to form 3 risk groups, associated with different long-term outcome. Aims. In order to validate this predictive score with an independent number of patients, we undertook a retrospective study and attempted to select patients either with epigenetic aberrations or with age, antecedent hematological malignancy and number of cytopenias who would meet the criteria for risk groups. Methods. There were 283 patients with either high-risk MDS or MDS/AML who were treated with induction at our institution between 1988 and 2005. Median age was 57 years (16-74). The patients received Induction therapy with Ara-C and an Antracyclin and patients younger than 60 years additionally received Etoposide. 16 patients underwent allografting after achievement of CR. 58% of the patients entered CR, 9% of the patients achieved PR, 23% of the patients had no remission and 10% of the patients died within 8 weeks after induction. Results. We then retrospectively tested the Nijmegen Score using this database. 25 patients were allocated to the low risk group (9%), 129 patients to the intermediate risk group (45%) and 129 patients to the high-risk group (46%). There was a difference in early death rate (0% vs. 6% vs. 15%, p=0.002). The overall survival was 40 months in the low, 24 month in the intermediate and 12 months in the high-risk group (p<0.00005). The difference between intermediate and high-risk group was also statistically significant (p<0.00005). The percentage of patients still alive two years after induction was 75% vs. 48% vs. 27%, and after 5 years 50% vs. 25% vs. 7%. Only within the low-risk group, there are patients with a long-term survival up to 16 years. Conclusions. These data indicate that a) the Nijmegen Score in validated in a large independent patient group and b) patients aged above 55-60 years in the high-risk group should not undergo induction therapy. The Nijmegen group recently proposed a predictive score (Knipp et al Blood, abstract 2004), it is not possible to use this as the only parameter to decide whether or not the patient should undergo induction therapy. The Nijmegen group recently proposed a predictive score that was calculated on the basis of a large amount of patients (Oesterveld, Leukemia Research, abstract 2005). Besides the karyotype, WBC, age, an-tercedent haematological malignancy and number of cytopenias were rated differentially to form 3 risk groups, associated with different long-term outcome. Aims. In order to validate this predictive score with an independent number of patients, we undertook a retrospective study and attempted to select patients either with epigenetic aberrations or with age, antecedent hematological malignancy and number of cytopenias who would meet the criteria for risk groups.

LENALIDOMIDE IN DEL(5q) MDS PATIENTS: DIFFERENT PATTERNS OF RESPONSE

A.A.N. Giagounidis, S. Haase, V. Lohrbacher, M. Heinsch, C. Aul
St. Johannes Hospital, DUSSEBURG, Germany

The novel amino-substituted thalidomide analogue lenalidomide (Revlimid®) has recently been approved in the USA for the treatment of transfusion dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes associated with a del(5q) cytogenetic abnormality with or without additional chromosomal abnormalities. The decision was based on data of a phase II study on a total of 148 del(5q) patients that showed an impressive number of cytogenetic remissions and transfusion independence. Given that 15% of patients with MDS bear the del(5q) abnormality, and that 12,000 to 15,000 new MDS cases are diagnosed yearly in the European Union, up to 2,300 new patients every year will be eligible for treatment with lenalidomide. In this presentation, we give an overview of treatment approaches based on our experience gained on more than 40 patients treated with lenalidomide at a single center. An important number of patients have different patterns of response to the drug the attending physician must be familiar with to avoid under- or overtreatment and to assure the best possible care. Five response types are described: First, the uncomplicated responder who does not need drug dose reduction and goes into long-term hematological and cytogenetic remission. Second, the typical responder who does require dose reduction but still achieves long-term remission. Third, the intermittent responder needs careful long-term blood count monitoring and individual dosing. This type of responder is characterized by an initial beautiful response with both transfusion independence and cytogenetic response that suddenly Platelets <50x10⁹/L, Grade 3/4 neuropathy is common, but only the minority of patients needed granulocyte stimulating factors. Titrating the drug until neutropenia and thrombocytopenia occurs has proven effective in achieving erythroid response during regeneration of neutrophilia. We conclude that lenalidomide is a reasonably safe drug in the del(5q) patient population that is characterized by a large number of specific phenotypic and genotypic characteristics will enable the physician to safely induce remissions in an important part of patients.
those induced by alkylating agents. Since combining 2 alkylating agents causes a very high risk of tMDS/tAML, the combination of fludarabine and cyclophosphamide may be highly leukaemogenic. Our data support this hypothesis. The recognition of 50% of our cases retrospectively, is in line with emerging trial data which suggest that tMDS/tAML may be missed unless specifically looked for. In summary we have shown a high incidence of tMDS/tAML in elderly patients treated with fludarabine and cyclophosphamide, with a clear relationship to fludarabine dose. The true extent of tMDS/tAML may be under-recognised. The best approach in the predominantly palliative treatment of the elderly may be to exercise caution with the combination of fludarabine and cyclophosphamide, and to reduce total cytotoxic drug exposure.

### Table 1. Comparison between patient groups who did and did not develop tMDS/tAML.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Median age in years (range)</th>
<th>Median dose F mg/m² (range)</th>
<th>p value for difference between F dose (tMDS/tAML vs non-tMDS/tAML)</th>
<th>Median no of other chemotherapy given (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tMDS/tAML patients</td>
<td>73 (8) (alla had F and C) (62-82)</td>
<td>541 (350-703)</td>
<td>2 (1-3)</td>
<td></td>
</tr>
<tr>
<td>Non-tMDS/tAML patients</td>
<td>72 (42-83)</td>
<td>300 (64-1038)</td>
<td>p&lt;0.01</td>
<td>2 (0-4)</td>
</tr>
<tr>
<td>Subgroup 69</td>
<td>270 (46-83)</td>
<td>p&lt;0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup non-tMDS/tAML patients who had F alone (6)</td>
<td>72 (42-79)</td>
<td>375 (100-690)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup non-tMDS/tAML patients who had F alone (7)</td>
<td>72 (42-79)</td>
<td>375 (100-690)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 patient had fludarabine, mitoxantrone and dexamethasone.

### EVI-1 Gene Expression in Myelodysplastic Syndromes: Quantitative Assessment and In Vitro Modulation Induced by Arsenic Trioxide


1. University of Turin, TURIN, Italy; 2. University of Catania, CATANIA, Italy; 3. Ematologia-Ospedale di Alessandria, ALESSANDRIA, Italy; 4. Hematology Dept. University of Turin, TURIN, Italy; 5. Hematology Dept. Molinette Hospital, TURIN, Italy; 6. Hematology Dept Cuneo Hospital, CUNEO, Italy; 7. Internal Medicine Dept Ivrea Hospital, IVREA (TO), Italy; 8. University of Eastern Piedmont, NOVARA, Italy

**Background.** The EVI-1 gene is located in chromosome 3q26 and codes for a zinc finger protein which acts as transcriptional repressor. Rearrangements of the EVI-1 locus in chromosome band 3q26 are associated with poor prognosis in myeloid malignancies. The overexpression of the gene has been described in a subset of patients without evidence of rearrangements. Moreover it was suggested that the overexpression of EVI-1 gene conveys a high degree of sensitivity to Arsenic trioxide therapy. Aims. The aim of the study was to analyze the expression level of EVI1 in a large number of different subtypes of myelodysplastic syndromes and to study the in vitro effects of arsenic trioxide. Methods. We analyzed the expression levels of EVI-1 in 250 BM samples and 162 PB collected from 345 MDS patients. 126 were refractory anaemia (RA), 144 refractory anaemia with excess of blasts (RAEB) and 75 RAEB-T or secondary AML (s-AML). Moreover we tested 37 de novo AML patients and 22 BM and 35 PB samples obtained from healthy volunteers. 4 AML and 1 MDS patients showed the 3q26 rearrangement detected by cytogenetic analysis. The expression level of EVI-1 was established using quantitative Real-Time PCR based on a primer and probe set (Applied Biosystems). The values obtained were normalized using ABL as housekeeping gene and the final results were expressed using the DeltaDeltaCt method. 80 BM MNCs were incubated with 1 micromolar arsenic trioxide and proliferation and colony growth were evaluated. Results. We detected very low levels of EVI-1 expression in BM (mean value of 2-DeltaDeltaCt = range 0-11) and undetectable levels in PB. By contrast, in 134 patients out of 345 (39%) abnormal levels of EVI-1 were detected. Significantly higher levels were found in patients with 3q26 rearrangements (range 294-35120). The patients expressing high levels of EVI-1 were distributed as follow: 58 RA (mean value of Delta Delta Ct = 49; range 11-64), 62 RAEB (mean value = 128; range 60-264) and 34 RAEB-T (mean = 2196; range 162-6653). 5 out of 37 de novo AML showed abnormal expression of EVI-1 (mean value 468 range 56- 530). EVI-1 expression was evaluated during follow-up of twelve patients who converted into overt leukaemia and in all the cases EVI-1 levels increased during progression. in vitro treatment with arsenic trioxide induces in 22 out of 80 samples (28%) a significant increase of BFU-E colony number, and this was observed mainly in patients characterized by high EVI-1 levels (17 out of 22). Moreover a significant reduction of EVI-1 gene expression was observed after arsenic trioxide incubation (p=0.005) as compared to controls. Conclusions. These data allow to establish that the overexpression of EVI-1 gene is present in 39% of MDS patients, regardless of the presence of the 3q26 rearrangement. The overexpression seems to be more frequent in RAEB and s-AML respect to RA and it increases during disease progression. The arsenic trioxide treatment induces reduction of EVI-1 transcript amount and a significant increase of BFU-E growth in patients overexpressing EVI-1.
Cytogenetic Evolution in del(5q) Myelodysplastic Syndrome

A.A.N. Giagounidis,1 U. Germing,2 B. Schlegelberger,2 H. Hildebrandt,2 S. Haase,3 V. Lohrbacher,1 G. Göhring,1 L. Wilkens,1 M. Heinisch,1 C. Aul3
1St. Johannes Hospital, DUISBURG, Germany; Heinrich-Heine-Universität, DÜSSELDORF, Germany; 2Medizinische Hochschule, HANNOVER, Germany; 3Institut für Humangenetik, DÜSSELDORF, Germany

Myelodysplastic syndromes are clonal hematopoietic stem cell disorders that are characterized by a wide prognostic heterogeneity. While some MDS like the pure sideroblastic anemia tend to remain stable for years, others evolve to higher risk MDS or acute myeloid leukemia. During disease progression, cytogenetic evolution occurs and is an independent prognostic risk factor. To assess cytogenetic evolution in MDS with del(5q), we analyzed data of 33 patients with a novo del(5q) MDS. Inclusion criteria were del(5q)(11.1) MDS at initial cytogenetic investigation in at least two metaphases irrespective of International Prognostic Scoring System (IPSS) rating. Cytogenetic evolution was defined as appearance of additional chromosomal abnormalities within the initial del(5q) clone. Gain of chromosomes was accepted if recorded in at least two metaphases, loss of chromosomal material had to be evident in at least three metaphases according to ISCN rules, or if single cell abnormalities were confirmed by FISH analyses. Additional clones with chromosome aberrations other than del(5q) were regarded as unrelated clones but not as cytogenetic evolution within the del(5q) clone. Initial cytogenetics were performed between 1989 and 2005. The last follow-up investigation was done in 2004 by the reference cytogenetic center of the German MDS study group (BS). Median age of the 33 patients was 62.2 years (range, 32 to 85). All patients had at least two cytogenetic evaluations (range, 2 to 9) with a median time between first and last examination of 36 months (range, 3 to 172). 19 patients had RA according to FAB, 4 had RARS, 8 RAEB, and 2 were not classifiable. 2 patients (6%) acquired additional cytogenetic lesions within the initial del(5q) clone. These were t(1;3)(p35;p14) after 25 months, and inv(3)(q13q25) after 43 months. One patient had a trisomy 8 in a different clone in a previous examination in 8 metaphases that was inapparent at follow-up in 20 metaphases after 32 months. One patient with RAEB who had initially additional trisome 8 in the del(5q) clone acquired a second clone with a del(18;21). All true cytogenetic evolutions occurred in 5q-syndrome patients. One of the two patients with cytogenetic evolution did not respond to lenalidomide treatment. However, karyotype complexity in del(5q) MDS does not seem to impact on response to lenalidomide therapy as there is increasing evidence that del(5q) patients with complex karyotype have the same amount of cytogenetic remission rates as 5q- syndrome patients. As a conclusion, del(5q) MDS display long-term karyotype stability, with cytogenetic evolution being evidenced in less than 10% of patients. Clonal evolution is not necessarily linked to advanced MDS at initial presentation.

A Role for the Endoplasmic Reticulum and the Mitochondrion in Erythroid Cell Apoptosis that Characterizes Low Grade Myelodysplastic Syndromes

E.G. Emmanuel,1 J.C.D. Deschêmen,1 C.G. Garrido,2 C.R. Randamamapita,3 A.D.K. Dubart-Kupperschmitt,1 O.B.R. Beyne-Rauzy,1 F.D. Dreyfus,1 M.G. Guesn,1 C.L. Lacambre,1 M.P. Mayeux,1 E.S. Solary,2 M.F. Fontenay1
1Inserm U5455, PARIS, France; 2CHU Toulouse, TOULOUSE, France; 3Hôpital Cochin AP-HP, PARIS, France

Cell death by apoptosis was shown to be accounted for the ineffective erythropoiesis that characterises low grade myelodysplastic syndromes (MDS). We have shown previously that the death receptor Fas was overexpressed at the surface of MDS erythroid precursors. Using an ex vivo liquid culture to analyse the differentiation of low grade MDS CD34+ cells into red cells, we demonstrated that apoptosis of MDS erythroid precursors could be prevented by either Fas-Fc or the ectopic expression of a dominant negative mutant of the adapter molecule FADD (Fas-associated death domain), a component of the death-inducing signalling complex that mediates death receptor-induced apoptosis. We and others also showed that the release of mitochondrial cytochrome c participated to this process, suggesting a connection of the extrinsic to the intrinsic pathway of apoptosis. To further address this latter question, we over-expressed the anti-apoptotic Bcl-2 protein in CD34+ bone-marrow cells before inducing their erythroid differentiation. For that purpose, we used lentiviral constructs including CDNA encoding either wild-type Flag-Bcl-2 (WT) or a Flag-Bcl-2 targeted to endoplasmic reticulum (ER) by a cytochrome b6 sequence. These constructs, which also encoded eGFP under the control of an IRES, were used to infect either control (n=10) or low grade MDS (n=15) CD34+ bone-marrow cells. These cells were subsequently induced to go along the erythroid lineage during 14 days. Phosphatidyserine exposure at the cell surface and mitochondrial membrane permeability (MMP) were increased in MDS compared to control erythroid precursors. The increase in MMP was associated with enhanced cytochrome c release and caspase-9 activation. Infection of CD34+ cells with the two Bcl-2 encoding lentiviral constructs delayed or inhibited both erythroid differentiation in more than 80% of control and MDS erythroid precursors, compared to less than 10% of those infected with the control vector. Specific targeting of Bcl-2 to the ER was confirmed by both immunofluorescence analyses and the lack of inhibition of lonidamine-induced cell death. Overexpression of Bcl-2 WT which is located in mitochondria and ER, or overexpression of Bcl-2 ER delayed the erythroid differentiation of both MDS and normal cells. In contrast, the two Bcl-2 encoding viruses specifically inhibited phosphatidyserine externalisation, MMP decrease and caspase-9 and -3 activation associated with erythroid differentiation of MDS bone-mar...
row CD34+ cells. Interestingly, over-expressed Bid-2, either wild-type or ER-targeted, failed to affect the truncation of the BID-only protein BID. This suggests that the mitochondrial pathway of apoptosis is activated downstream of Fas through BID. Over-expressed Bc-2 decreased also the release of ER calcium in response to thapsigargin, reflecting lower intracellular calcium stores and a less apoptosis. Altogether, these results confirm the involvement of mitochondria in erythroid cell death that characterizes low grade MDS and strongly argue for a participation of ER in this apoptotic pathway, both organelles acting downstream of the death receptors.

0457
ISSUES AFFECTING QUALITY-OF-LIFE IN PATIENTS LIVING WITH MYELODYSPLASTIC SYNDROMES: RESULTS OF PATIENT FORUM DISCUSSIONS IN EUROPE

K.V. Heptinstall,1 D. Bowen,2 F. Fenaux,3 S. Killick,4 T. Hamblin,5 G.J. Muird6

1Myelodysplastic Syndromes, Inc., CROSSWICKS, USA; 2The Leeds Teaching Hospitals, DUNDEE, United Kingdom; 3Hôpital Avicenne, BOBIGNY, France; 4Royal Bournemouth Hospital, BOURNEMOUTH, United Kingdom; 5King’s College London, LONDON, United Kingdom

Background. Patients living with MDS experience significant deterioration in their quality-of-life. New treatments (approved and under development) for MDS have provided patients with hope that this deterioration in quality-of-life can be limited or eliminated in the near future. Aims. Patient forum discussions were conducted with the goals of determining the key issues regarding quality-of-life (QoL) in patients living with MDS including: feelings about the attitude of, and support offered, by health care providers, the patient’s depth of knowledge about MDS, and effect of treatment on QoL. Educational programming will be developed based on the information derived from these forums. Methods. Nine MDS Foundation Centers of Excellence volunteered to participate and 4 forums convened to date. Questionnaires were developed, vetted, and translated by participating sites. Questionnaires were consistent in all locations. Discussion focus varied due to the free-flowing nature of the forums. These forums were conducted in Edinburgh, Paris, Bournemouth (England), and London. Results. A total of 67 patients and 92 caregivers participated. Participant sample was Caucasian (100%); male (52%); female (48%); Age range <50 (10%), 50-75 (67%), >75 (33%), 5 have less than 6 years of education, 30 had 10-12 years, and 32 had >12 years. 50 are married (75%) and the 95% live with other people. 21% are employed full or part time and 50% are retired with 29% unknown. Patient QoL experiences were similar between sites and reflected substantial feelings of life disruption due to MDS and time required for disease management. Physician visits, testing, transfusions, treatment, travel time, and symptom/adverse event management contributed to feelings of loss of life control. Fatigue is the issue affecting QoL most often - impacting patient’s ability to perform activities of daily living, work, and participation in social and family life. Emotional well being is significantly decreased and described as waiting for something to happen. Physician relationships at the COEs were viewed positively by the majority while relationships with community physicians were viewed in a negative context due to physician’s lack of knowledge. Patients described time spent educating the doctor. Nurses were viewed as key to patient’s knowledge and well being. Patients expressed overall satisfaction with current treatment however 65% felt that new drugs were not being made available quickly enough within the EU. Transfusions, in tandem with chelation therapy, were viewed as impacting QoL significantly second only to fatigue. Patients viewed transfusions as a necessary evil to deal with their fatigue. Caregivers expressed a need for information to assist them in dealing with family/friends with MDS. Conclusion. MDS has a substantial impact on patients QoL including interactions with family and friends. Physicians and other healthcare professionals should be aware of this impact and attempt to provide patients with information and options to lessen the burden of this disease and minimize its impact. New treatment options should be explored with patients, including participation in clinical trials, with the goal of improving QoL and lessening fatigue, oral medications for chelation therapy.

0458
FOUR DIFFERENT TYPES OF MDS PATIENTS WITH 5Q- ANOMALIES

U. Germing,1 A. Giagounidis,2 B. Hildebrandt,3 C. Aul,4 A. Kuendgen,5 N. Gattermann,6 R. Haas7

1University of Düsseldorf, DÜSSELDORF, Germany; 2Johannes-Hospital, DUISBURG, Germany; 3Heinrich-Heine-University, DÜSSELDORF, Germany

Background. About 50% of all MDS patients show karyotype aberrations at the time of diagnosis. The most frequent chromosomal anomaly is the del(5q) aberration. The WHO recognized the 5q− Syndrome as a separate entity within the group of myelodysplastic disorders. However, a high number of MDS patients show 5q− aberrations together with other abnormalities, or a complex karyotype. Aims. A better description of the different types of 5q− aberrations in warranted. Methods. We screened the German MDS registry Düsseldorf for patients presenting with 5q− aberrations, regardless of WHO type and other chromosomal aberrations. Results. Out of 2897 patients in the registry, 1068 patients have been karyotyped at diagnosis (56%). 180 of them (17%) showed a 5q− anomaly either alone or in combination with other aberrations. We then separated the patients into 4 groups: 5q− Syndrome (del(5q) as a single anomaly, medullary blast count <4, Group A), del(5q) as a single anomaly with elevated blasts (Group B), del(5q) with an additional chromosomal abnormality (Group C) and del(5q)-MDS within a complex karyotype (group D). We then examined haematological data and prognosis of the 4 groups. For calculating the prognosis, patients who underwent Induction therapy, allogeneic stem cell transplantation or Revlimid treatment were censored. Per definition, all patients in group A had a medullary blast count of <5%, and all patients in group B had RAEB or RAEB-T. Group C consisted of RA and RARS in 85%. 68% of the patients in group D had RAEB or RAEB-T. The degree of hematopoietic insufficiency was more pronounced from in B, C and D and the prognosis was adverse in group C and D. Median survival of group A was 65 months as compared to 68, 31 and 7 months respectively (p<0.05). The cumulative risk of AML was 13% in the group A, as compared to 33%, 42% and 55% (p<0.05). Conclusions. These data show that the prognostic impact of 5q− anomaly as well as its pathophysiologic impact is heavily influenced by other factors, such as medullary blast count and additional aberrations. This should be taken into account, when assessing the prognosis is planning treatment for those patients.

0459
THE PROGNOSTIC MEANING OF INVOLVEMENT OF 5Q- ABERRATIONS IN PATIENTS WITH MDS AND COMPLEX KARYOTYPE

U. Germing1, A. Giagounidis2, B. Hildebrandt3, C. Aul4, C. Strupp5, A. Kuendgen5, N. Gattermann6, R. Haas7

1University of Düsseldorf, DÜSSELDORF, Germany; 2Johannes-Hospital, DUISBURG, Germany; 3Heinrich-Heine-University, DÜSSELDORF, Germany

Background. It is well known, that patients with Myelodysplastic syndromes with a complex karyotype have a very poor prognosis, facing a median survival of less than 1 year. There is ample evidence on the efficacy of lenalidomide in patients with del(5q), not only in 5q− Syndrome but also in patients with del(5q) as part of a complex karyotype. Aims. In order to examine the prognostic role of del(5q) involvement within complex karyotypes, we screened the German MDS registry of Düsseldorf. Methods. A complex karyotype was defined according to the IPSS definitions. 155 patients were diagnosed with a complex karyotype. Median age was 66 years (18-89). There were 3 patients with RA, 25 with RCMD, 18 with RCMD/DS, 27 with RAEB 1, 38 with RAEB II, 7 with CMML, and 37 with RAEB-T. 45 patients have been treated either with induction chemotherapy or underwent allogeneic stem cell transplantation. These patients were ex-cluded from calculations of prognosis. Results. We then separated the patients into a group with involvement of del(5q) (n=58) and a group, in which del(5q) was not part of the complex karyotype (n=102). The distribution of both groups to WHO types didn’t differ significantly (p=0.45). Clinical characteristics (haemoglobin, platelet count, WBC, ANC, LDH) were not different between the groups; median age at diagnosis was 63 years in the non-del(5q) group and 66 years in the del(5q) group. The median survival of the del(5q) group was 7 months as compared to 14 months in the patients without 5q-involvement (p=0.006). 12 months after diagnosis, 22% of the del(5q) patients were alive, as compared to 49% of the non-del(5q) patients. 77% of the del(5q) pa-tients and 75% of the non del(5q)

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patients died disease-related (AML, infections, bleeding). The risk of AML was increased 1 and 2 years after diagnosis was 60 and 54% in the del(5q-) group and 44 and 50% in the non del(5q) group (p=n.s.). The overall percentage of patients that developed AML was not different (53% vs 55%, p=n.s.). Conclusions. Del(5q) is associated with an extreme poor prognosis when diagnosed within a complex karyotype. Because only a minority of these patients can undergo a curative ap-proach, the efficacy of lenalidomide should be studied in this patients group the near future.

M. Tanner, A. Lopez, M. Boogaerts, T.J. Hamblin, M. Delforge
‘University Hospital Leuven, LEUVEN, Belgium; Immunology, University Hospi-tal, LEUVEN, Belgium; Biocers NV, LEIDEN, Netherlands; Hematology, University Hospital, LEUVEN, Belgium

Background. Myelodysplastic syndromes (MDS) form a heterogeneous group of clonal haematopoietic disorders charac-terized by peripheral cytopenia, marrow dysplasia and an increased risk to develop AML. There is increasing evidence that the cytopenias in early phase MDS are partially caused by immunological mechanisms. The aim of this study was to investigate if the CD40-CD40L interaction plays a role in the pathogenesis of MDS-related bone marrow failure. Our hypothesis is based upon the knowledge that the interaction between CD40 and its natural ligand CD40L (CD154) is involved in normal immune responses, but also in the pathogenesis of several non-hematological disorders. Methods. 1/ With FACS we measured the expression levels of CD40 on CD14+ monocytes and CD40L on CD4+/CD4+ lymphocytes in PB samples of 18 untreated and non-trans-fused MDS patients (10 RA, 8 RARS) and 12 controls. 2/ CD4+ cells were isolated from PB from 17 patients (14 RA, 2 RARS, 1 RAEB) and 19 controls and MACS columns and used for 7 days in IMDM + 15% fetal bovine serum. They were subsequently stimulated for 24h with lipopolysaccharide (LPS) 1.0 µg/mL or agonist monoclonal anti CD40 antibody (clone 64, Biocers NV, Netherlands) at a concentration of 10 µg/mL. TNF-α concentrations in supernatants were measured with ELISA. 3/ Bone marrow MNCs of 11 patients (5 RA, 2 RARS, 3 RAEB and 1 RAEB-t) were cultured in methylcellulose + growth-factors (M4434, Stem Cell Technologies) in the presence or absence of 10 µg/mL 5D12 (antagonist chimeric monoclonal anti-human CD40 antibody, Biocers NV, The Netherlands). Results. 1/ MyD5 patients had a significantly high-er percentage of circulating CD40+-CD4+ (9.40% ± 2.05 vs. 1.39% ± 0.55, p=0.0125), and CD40L+CD4+CD3+ cells (5.65 ± 2.76 vs. 5.99 ± 1.34, p=0.049) compared to controls. 2/ CD40-ligation, but not LPS, induced a significantly higher TNF-α production in patients compared to controls (588 ± 516 vs. 83 ± 24 pg/ml, p=0.0065). In patients, TNF-α production after CD40 stimulation was also significantly higher than after stimulation with LPS (588 ± 516 vs. 64 ± 159 pg/ml, p=0.016). 3/ In controls, TNF-α levels after LPS or CD40 stimulation were comparable. 3/ Co-culture of MDS bone marrow MNCs with 5D12 increased in vitro colony formation (148 ± 48 vs. 116 ± 43, p=0.067), an effect not observed in controls. Conclusion. We conclude from these observations that CD40-CD40L interactions might play a role in the pathogenesis of MDS-related bone marrow failure. This is supported by the observation of an increased number of circulating CD40+/CD414+ monocytes and CD40L+/CD4+/CD3+ lymphocytes in MDS patients. We have also shown that CD40-ligation induces a significantly higher TNF-α produc-tion by monocytes from patients compared to healthy volunteers. Final-ly, we have preliminary evidence that blocking the CD40 receptor can increase colony formation in vitro. These results mark a possible new tar-get to treat cytopenias in MDS.

IMMUNOPHENOTYPIC ANALYSIS OF CD34+ CELL SUBSETS IN BONE MARROW SAM-PLES FROM MYELODYSPLASTIC SYNDROME PATIENTS

‘University of Salamanca, SALAMANCA, Spain; Hospital Miguel Servet, ZARAGOZA, Spain

Introduction. Current investigations regarding the presence of phenotypic aberrations among CD34+ cells in patients with myelodysplastic syndrome (MDS) have provided limited information about their frequency and subtypes due to the use of limited monoclonal antibody panels and/or an evaluation of insufficient amounts of CD34+ bone mar-row (BM) cells. Objective. Our aim was to phenotypically characterize the myeloid and lymphoid CD34+ BM cell compartments in patients with MDS using a large panel of monoclonal antibodies in order to identify phenotypic alterations that may be useful for the diagnosis and classification of the disease. Material and Methods. Over 60 BM samples corresponding to 11 normal BM (NBM) and 52 BM from patients with MDS (including 19 low risk MDS patients (LR-MDS) and 33 high risk MDS cases (HR-MDS). CD34+ cells were identified and sub-
All three CD34+ cell MDS patients displayed normal number of TLR-4 is over-expressed. As shown by recent studies, BMMCs in the controls; (infliximab; 10 µg/mL), TNFa (200-IU/mL), anti-TNFα (10 µg/mL), anti-TLR-4 (10 µg/mL), and anti-ICAM.1 (positive control for the TLR expression. All cell lines studied were treated with 10 ng/mL and evaluating the cell doubling time (2^n=cells counted/cells plated) in each passage. Results. MDS patients displayed impaired CFU-F potential time-course (p<0.001; P1-P7). Summary-Conclusion. Patients with MDS display normal number and differentiation potential of BM MSCs. The clonogenic and proliferative potential of patient MSCs, however, is defective compared to the respective of the healthy controls. Analysis of the production of growth factors and inhibitors at protein level, evaluation of the telomeric length as well as cytogenetic analysis of patient MSCs is currently under investigation to elucidate further the pathophysiological basis of the observed MSC abnormalities in MDS patients.
0465
A RANDOMIZED STUDY OF DASATINIB VERSUS ESCALATED DOSE OF IMATINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA RESISTANT TO IMATINIB: RESULTS OF THE CAL180013 START-R STUDY

P. Rousselot,1 N. Khoroshko,2 N. Shah,3 To demonstrate the activity of dasatinib in patients (pts) with CP-CML resistant to primary or acquired resistance, or detection of BCRABL mutations. Aims. To demonstrate the activity of dasatinib in patients (pts) with CP-CML resistant to primary or acquired resistance, or detection of BCR-ABL mutations. Methods. START-R is a multicenter randomized (2:1 ratio) trial of D 70 mg twice daily (BID) and IM 500 mg/day in pts with CP-CML resistant to prior IM 400 to 600 mg/day. Cross-over was allowed for lack of response or intolerance (grade 3-4 non hematologic toxicity). D dose escalation to 90 mg BID was allowed for inadequate response at 12 wks, and dose reduction to 50 or 40 mg BID for drug toxicity. Dose reduction to 600 mg/day was allowed for IM. Evaluations consisted of weekly blood counts for the first 12 wks, bone marrow and cytogenetics every 3 months, molecular monitoring of BCR-ABL transcript levels by real-time quantitative polymerase chain reaction (RT-PCR) every 4 wks for the first 12 wks, and then every 12 wks, and mutation status at baseline and end of treatment. Results. From February 2005 to November 2005, a total of 150 pts were randomized (101 to D and 49 to IM). There were 75 (50%) males; median age was 51 yrs (range 24’85). Median time from diagnosis was 59 months and 34% had IM resistant mutations. Prior therapy included interferon in 107 (71%) pts, chemotherapy in 57 (38%) and stem cell transplant in 9 (6%). All pts received prior IM; 96 (64%) had 600 mg/day, 60 (40%) were treated >3 years and 42 (28%) achieved major cytogenetic response (MCyR) at 12 months. An interim analysis was conducted on the first 56 randomized pts (22 D and 14 IM). Confirmed complete hematologic response was documented in 21 D (96%) pts and 13 (93%) IM pts. The MCyR rate at 12 wks was 45% for D and 21% for IM with 7 complete CyR on D and 1 on IM. Best MCyR rate at any time was 11/22 (10 complete) for D and 3/14 (1 complete) for IM. Two (9%) D and 12 (50%) IM pts crossed over. Among pts who crossed to the alternate treatment, best MCyR rate was 6/12 (5 complete) for D and 0/2 for IM. Grade 3-4 neutropenia or thrombocytopenia were reported in 11 and 9 D pts and 8 and 2 IM pts. Most common grade 1-2 non-hematologic toxicities in D and IM groups were diarrhea (11 and 2), nausea/vomiting (11 and 10), edema (9 and 5), and pleural effusion (3 and 0). Conclusion. Dasatinib was effective in pts with CP-CML resistant to IM 400 to 600 mg/day. An updated analysis on all 150 patients will be presented including molecular response.

0466
LONG-TERM BENEFITS OF IMATINIB FOR PATIENTS NEWLY DIAGNOSED WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE: THE 5-YEAR UPDATE FROM THE IRIS STUDY

F.G. Guilhot,1 R.A. Larson,2 S.G. O’Brien,3 B.J. Druker,4 on behalf of IRIS Study Group
1Univeristy Hospital, PERTERS, France; 2University of Chicago, CHICAGO, USA; 3University of Newcastle, NEWCASTLE, United Kingdom; 4Howard Hughes Medical Institute, PORTLAND, USA; 5IRIS Study Group, France

Background. IM was proven to be superior to IFN+Ara-C for newly diagnosed patients (pts) with CML-CP (O’Brien et al, NEJM 2003). 1106 pts were randomized between June 2000 and Jan 2001 to either IM 400 mg or IFN+Ara-C with 553 pts to each treatment. This abstract is based on data collected up to 54 months after last patient had been recruited on IM. 60 months (5-year) data will be available for presentation. Methods. Evaluations included complete hematologic response (CHR), complete/partial cytogenetic response (CCyR/PCyR - defined as 0% / 1-35% Ph+ metaphases respectively), major cytogenetic response (MCyR=CCyR+PCyR), major molecular response (MMR) defined as ≥3 log reduction of BCR-ABL transcript levels from the standardized baseline, time to progression - defined as loss of CHR/MMR, evolution to accelerated phase/blast crisis (AP/BC), or death due to any cause during treatment, and overall survival. Results. With a median follow-up of 54 months, 72% of the 553 randomized pts remain on initial IM treatment (5% of pts discontinued due to adverse events, 9.5% due to unsatisfactory therapeutic effect and 11% due to other reasons; another 2.5% crossed over to IFN+Ara-C). Overall, the cumulative best response rates of CHR, MCyR and CCyR are 97%, 88% and 82%, respectively. The overall estimated survival was 90% (95% when censored at bone marrow transplant). An estimated 84% of pts have not progressed on treatment and 93% of pts were free from progression to AP/BC. The annual rate of progression to AP/BC of <1% in the fourth year was lower than each of the first three years (1.5, 2.8, 1.6% respectively). Of the pts with MCyR at 12 months (n=486), an estimated 96% were free of progression to AP/BC at 54 months whereas it was only 81% for the 73 pts who did not achieve a MCyR at 12 months (p<0.001). No patient with a MMR at 12 months progressed to AP/BC within 54 months. Conclusions. This analysis confirms the high rates and durability of responses to IM. Encouragingly, the rate of progression in the fourth year was lower than in each of the preceding three years. Results further demonstrate the beneficial effect of cytogenetic and molecular responses on long-term outcomes.

Figure 1. Progression-free survival and survival without AP/BC on first-line imatinib.

0467
A PHASE II STUDY OF DASATINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA WHO ARE RESISTANT OR INTOLERANT TO IMATINIB: RESULTS OF THE CAL180013 START-C STUDY

A. Hochhaus,1 H. Kantarjian,2 N. Shah,3 G. Roati,4 E. Cervantes,5 T. Facon,6 A. Countouriotis,7 R. Ezzeddine,8 S. Branford,9 B. Druker10
1University of Heidelberg, MANNHEIM, Germany; 2MD Anderson Cancer Center, HOUSTON, USA; 3UCLA, LOS ANGELES, USA; 4Universita Di Bologna, BOLOGNA, Italy; 5University of Barcelona, BARCELONA, Spain; 6Service Des Maladies du Sang, LILLE, France; 7Mattel Children’s Hospital, LOS ANGELES, USA; 8Bristol-Myers Squibb, WALLINGFORD, USA; 9IMVS, ADELAIDE, Australia; 10Oregon Health and Science University, PORTLAND, USA

Background. Dasatinib (BMS-586825) is a novel, oral, multi-targeted kinase inhibitor of BCR-ABL and SRC kinases with preclinical and clinical activity against imatinib resistant BCR-ABL mutations. Aims. To demonstrate the activity of dasatinib in patients (pts) with CP-CML who are resistant to (primary or acquired resistance, or detection of BCR-ABL mutations highly associated with imatinib resistance) or intolerant (grade 3-4 non hematologic or persistent hematologic toxicity) of imatinib. Methods. START-C is an open-label Phase II study of dasatinib in imatinib-resistant (IM-R) or intolerant (IM-I) pts with CP-CML. Between February-August 2005, 387 pts were recruited at 75 centers worldwide. Dasatinib was given at 70 mg twice daily (BID) with dose escalation to 90 mg BID in pts lacking response, and dose reductions to 50 and 40 mg BID for intolerance. Evaluations were weekly blood counts

Figure 1. Progression-free survival and survival without AP/BC on first-line imatinib.
for the first 12 weeks, bone marrow cytology and cytogenetics every 3 months; and molecular monitoring of BCR-ABL transcript levels by real-time quantitative polymerase chain reaction (RT-PCR) every 4 weeks for the first 12 weeks, and then every 12 weeks while on study. The primary endpoint was major cytogenetic response (MCyR) rate. Results. Of the 387 pts, 271 were IM-R and 116 were IM-I pts. Median age was 58 yrs (range 21-85); 49% of pts had a complete hematologic response (CHR), and 45% (45%) pts had a MCyR; 40% (31%) of IM-R pts, and 43% (73%) of IM-I pts. Rate of MCyR was 37% among the 65 pts with BCR-ABL mutations. Grade 3/4 neutropenia or thrombocytopenia was reported in 35 (45%) pts and 65 (46%) pts with onset after 4.8 weeks of therapy in most pts. Dose interruptions occurred in 46 (78%), and dose reductions in 96 (52%) pts with an average daily dose of 108 (range 19-169) mg. Non-hematologic toxicity consisted mainly of Grade 1/2 diarrhea, headache, superficial edema, and pleural effusion, with 52% Grade 3/4. There was no cross-intolerance between dasatinib and IM. Conclusions. Dasatinib demonstrated substantial hematologic and cytogenetic activity in IM-R and IM-I pts with CP-CML. An updated analysis of 387 pts with 6 months of follow up, in addition to the molecular response analysis, will be presented.

0469
Predictive Value of BCR-ABL Transcript Levels After 12 Months of Imatinib Monotherapy for the Outcome of CML Patients After 5 Years


Background. Despite most chronic myelogenous leukemia (CML) patients (pts) treated with imatinib achieve a complete cytogenetic remission (CCR), about 4% of pts per year will relapse. The achievement of an at least 3 log reduction of BCR-ABL transcript levels compared with a predefined baseline has been associated with a favorable long term outcome. Aims. We sought to establish a relationship between 12 month quantitative PCR data and relapse free survival based on the determination of ratios BCR-ABL/ABL. Methods. Serial peripheral blood (n=874) and bone marrow (n=685) samples from 68 pts randomized for first line imatinib therapy within the international IRIS trial have been investigated employing qualitative and quantitative RT-PCR and conventional cytogenetics. Degree of molecular response was classified retrospectively using a direct comparison of the log reduction terminology and the ratio BCR-ABL/ABL. Results. 0.01%, 0.12%, and 1.4% represent a 4, 5, and 2-log-reduction, respectively. Results. After 12 months of imatinib therapy, ratios <0.01% were achieved in 4 pts (cohort 1, 6%), ratios of 0.01-0.12% in 22 cases (cohort 2, 32%), >0.12-1.4% in 27 pts (cohort 3, 40%), and >1.4% in 15 pts (cohort 4, 22%). Overall median observation time was 57 mo and not different between the 4 cohorts. The most recent analysis showed CCR in 4/4 pts in cohort 1 (100%), 21/22 pts in cohort 2 (95%), 24/27 pts (89%) in cohort 3, and 7/15 pts in cohort 4 (47%, p=0.0006). Most recent Q-PCR values differ significantly between cohorts (cohort 1 0.0091%, cohort 2 0.0063%, cohort 3 0.057%, cohort 4 1.1%, p=0.0002). The same applies to overall best Q-PCR results (cohort 1 0.0067%, cohort 2 0.0095%, cohort 3 0.026%, cohort 4 1.1%, p<0.0001). In 10/26 (36%) pts of cohorts 1-2 BCR-ABL was not detectable by a sensitive nested PCR at the most recent analysis whereas none of 42 pts demonstrated undetectable BCR-ABL in cohorts 3 and 4 (p<0.0001). Five pts have relapsed with reappearance of Ph+ metaphases after a median of 6 mo (range 3-9) post first CCR. These pts belonged to cohorts 2 (n=1), 3 (n=2), and 4 (n=2) after 12 mo of therapy. There was a trend towards higher ratios BCR-ABL/ABL at mo 12 in pts with subsequent relapse compared to those in continuous CCR (0.40 vs 0.15%, p=0.29). Two pts progressed to accelerated phase/blast crisis after 16 and 54 months, these pts had 70 and 100% Ph+ metaphases at mo 12, respectively. Conclusions. BCR-ABL transcript levels after 12 mo of imatinib therapy are predictive for long term cytogenetic and molecular remission. Overall rate of CCR parallels the degree of early molecular response. A ratio BCR-ABL/ABL <0.12 is predictive for a better chance to achieve CCR accompanied by a >95% probability of CCR as well as a 38% chance of becoming nested PCR negative after 5 years. The in vivo data confirm the equivalence of the definition of a major molecular response as a 3-log reduction compared to a predefined baseline vs the ratio BCR-ABL/ABL of 0.12%.
Acute myeloid leukemia

0470
DOSAGE-DEPENDENT COMPARISON BETWEEN NPM LEUKEMIC MUTANTS AND ARF PROTEIN FOR SUBCELLULAR DISTRIBUTION: A NUCLEAR TUG-OF-WAR

Università di Perugia, PERUGIA, Italy

Acute myeloid leukemia (AML) is frequently targeted by mutations at exon-12 of the nucleophosmin (NPM) gene (Falini et al., NEJM, 352:254, 2005), which 1) disrupt either tryptophan 290 or tryptophans 288 and 290, constituting the nucleolar localization signal (NoLS), and 2) create a new carboxy-terminal Nuclear Export Signal (NES) motif, with 6 variations observed to date. Both phenomena lead to a deficient NPM accumulation in leukemic cell cytoplasm (NPMc+ AML). In NPM mutants, the new NES motif non-randomly correlates with NoLS disruption at the C-terminus. The most common NES motif (LxxxVxxVxL) always associates with mutations of tryptophans (W) 288 and 290, e.g. mutant A; a NES variant sequence, such as LxxxLxxVxL, always associates with W288 retention in rare NPM mutants, e.g. mutant E (Falini et al., Blood, pub-ahead, February 2, 2006). These findings suggest diverse sequences of mutant NES motifs function differently. Mutated NPM downregulates Arf from nucleoli, shortens its half-life and blunts its function (Den Besten W et al., Cell Cycle, 4:1595, 2006). In different NPM mutants this study addressed: 1) the role of variations in NES motifs; and 2) interactions with Arf. 1) Role of different NES motifs in altered NPM nucleo-cytoplasmic traffic: Arf-negative NIH-3T3 cells were transfected with eGFP-tagged NPM mutant A in which W288 had been artificially inserted by site-directed mutagenesis (eGFP-NPMMa, C288W). Unlike mutant E, which is unaffected by the presence of W288, this protein displayed greatly reduced cytoplasmic export. Additionally, in NPM mutant E, replacing the LxxxLxxVxL NES sequence with LxxxVxxVxL (eGFP-NPMMaE_IVVVL) partially relocated mutant E to the nucleus. These results demonstrate efficiency differences between NES: LxxxVxxVxL is weaker than LxxxLxxVxL. The former is strong enough to export the NPM mutants only if both tryptophans are mutated whilst LxxxLxxVxL is needed if W288 is retained. 2) Interactions of NPM mutants with Arf: We investigated how changes at the NPM mutant C-terminus influence NPM-Arf binding, and NPM and Arf subcellular distribution. Arf-negative NIH-3T3 cells were co-transfected with DSRed-tagged Arf (DSRed-monomer-Arf) and eGFP-tagged NPM mutants. Arf partially relocated NPM mutants A and E from cytoplasm to nucleus in a dose-related manner. In turn, NPM mutants partially relocated Arf from the nucleolus to nucleoplasm and cytoplasm. These results demonstrate a reciprocal interaction between Arf and NPM mutants. Moreover lower doses of Arf completely relocated artificial mutants eGFP-NPMMaA, C288W and eGFP-NPMMaE, C288W and eGFP-NPMMaE, IVVVL to the nucleus, suggesting these artificial mutants have a stronger affinity for Arf than NPM mutants A and E. Co-immunoprecipitation studies showed mutants A and E bind less Arf than wild-type NPM or eGFP-NPMMaA, C288W and eGFP-NPMMaE, IVVVL. Conclusions. The non-random correlation between NPM NoLS disruption and NES sequence variants is feasibly explained by need for 1) efficient cytoplasmic accumulation of mutated NPM, and 2) less efficient binding of mutant NPM to Arf as compared to wild-type NPM. Both mechanisms may contribute to Arf dislocation/degradation, thus having the same functional consequences as NPM silencing. These findings may be relevant to the pathogenesis of NPMc+ AML.

0471
A NOVEL MOLECULAR MECHANISM LEADING TO PRIMARY RESISTANCE TO FLT3-TYROSINE KINASE INHIBITORS IN AML BY FORMATION OF FLT3-ITD627E

1 University Hospital Mainz, MAINZ, Germany; 2 ALL Münchner Leukemiela-
bor GmbH, MINCHEN, Germany; 3 Children’s Hospital Research Building, BOSTON, USA; 4 MD Anderson Cancer Center, HOUSTON, USA; 5 Cornell University Cancer Center, NEW YORK, USA; 6 Novartis Oncology, FLORHAM PARK, USA; 7 University Carl Gustav Carus, DRESDEN, Ger-
many; 8 UCLA Medical Center, LOS ANGELES, USA; 9 Memorial Sloan Ket-
tering Cancer Center, NEW YORK, USA; 10 Dana Farber Cancer Center, BOSTON, USA

Background. Activating mutations in the FLT3 receptor tyrosine kinase are detected in approximately 35% of AML patients. Currently, several small molecule FLT3 tyrosine kinase inhibitors (TKI) are being tested in clinical trials. These studies showed that monotherapy using FLT3-TKI results in measurable clinical responses including significant reductions in PB and BM blasts. Most of these responses are transient, however, in a subset of AML patients blasts recur. We recently described mechanisms of primary resistance to PKC412-treatment in a subset of AML patients relapsing after various durations of remission and identified a resistance mutation (N676K) in the tyrosine kinase domain of FLT3 (Heidel et al., 2006). Aims. Here, we investigate molecular mechanisms leading to primary clinical resistance toward PKC412-therapy. Methods. In AML patients showing primary resistance to PKC412 within a clinical phase II study, molecular analysis of clinical material was performed. In addition, 753 unsel ected AML cases were screened for genomic localization of ITD integration sites. Results. Ex vivo analysis of primary AML-blasts at the time of clinical resistance to PKC412 in an index AML patient showed persistent tyrosine phosphorylation of FLT3 despite sufficient PKC412-plasma levels. FLT3 sequence analysis revealed an ITD-allele integrating in exon 15 at codon 627 and thereby leading to an aa-exchange at codon 627 (A627E). This particular position has previously been implicated in FLT3 TKI-resistance in vitro. FLT3 cases analyzed by cloning and expression of this ITD-allele (FLT3-ITD627E) in 32D cells led to IL-3 independent growth as well as inherent high level resistance toward PKC412-treatment in apoptosis assays. Moreover, FLT3-ITD627E expressing 32D cells were also resistant toward treatment with alternative FLT3-TKIs (SU5614 and K-252a (similar to CEP-701)). This demonstrates that the ITD627E allele is sufficient to confer resistance to these compounds in combination with chemotherapy. Interestingly, additional analysis revealed that this particular ITD-allele was already present before start of treatment with PKC412. Thus, this well explains the clinical course of the patient being refractory to PKC412-therapy. To investigate the hypothesis, that integration of ITDs in combination with FLT3-TKI, experiments are currently underway, and will be presented, to determine the relevance of the position of ITD-inte-
gration for resistance towards FLT3-TKIs. Specifically, we are investigat- ing the role of ITD integration at codon 627 without mutation of aa 627. To estimate the prevalence of ITD integration in exon 15 and in codon 627, in particular, we screened FLT3-ITD sequences of 753 unsel ected AML cases using suitable techniques (de-novo AML, secondary AML after MDS, chemotherapy-related AML). This analysis showed that 35 (4.6%) of these patients had ITDs integrating in exon 15 but only 1 additional patient with ITD integration in codon 627 was identified. Conclusions. Our results present evidence for a nov- el molecular mechanism leading to primary resistance to FLT3-TKIs. However, analysis of a large cohort of AML cases suggests that this is a rare event and does not represent a therapeutic obstacle for FLT3-TKIs in the vast majority of patients carrying FLT3-ITDs.

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Microarray-Based Characterization of AML with Complex Karyotypes

Décloître Novel Genomic Imbalances Harboring New Candidate Genes


'University of Ulm, Ulm, Germany; 'Department of Internal Medicine III, Ulm, Germany; 'Division of Neuroinformatics, Ulm, Germany; 'PO San Gennaro, NAPLES, Italy

Approximately 10 to 15% of acute myeloid leukemia (AML) cases exhibit complex karyotypes, i.e., three or more chromosome abnormalities without presence of a specific fusion transcript. 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. Therefore, we designed a 2.8k-microarray consisting of 2799 different BAC- or PAC-vectors with an average resolution of approximately 2 Mb. Using this microarray platform, 85 AML cases with complex karyotypes were analyzed. Genomic losses were found more frequently than gains; the most frequent losses were deletions of 5q (71%), 17p (55%), 7q (48%); followed by deletions of 18q (30%), 16q (28%), 3p and 12q (20% each), 12p (18%), 20q (17%), and 11q (12%). The most frequent genomic gains were trisomies of 11q (59%) and 8q (51%); followed by trisomies of 1p (22%), 8p (20%), 9p (14%), 22q (15%), 13q (12%), and 6p (10%). In part, some critical segments were delineated to genomic fragments of 0.8 to a few megabase pairs in size. Furthermore, 47 high-level DNA amplifications in 19 different regions were identified; amplifications occurring in at least two cases mapped to (candidate genes in the amplicon) 11q23.3 (n=10; ETS, FLI1), 11q23.3-25.3 (n=3; MLL, DDX6), 21q22 (n=5; ERG, ETS2), 15q12 (n=5; CDX2, FLT1, FLT3, PAN2), 8q24 (n=5; C8FW, MYC); 9p24 (n=2; AK2); 12p13 (n=2; FG6, CCND2); and 20q11 (n=2; ID1, BCL2L1). For better characterization of the amplicons, we applied array-CGH using a 6.0k-microarray with an average resolution of approximately 1 Mb revealing highly complex amplicon structures. Furthermore, in a subset of cases we profiled global gene expression for detecting a gene dosage effect with significant lower/higher average gene expression levels across the genes located in the lost/gained regions as compared to unaltered cases. Additionally, parallel analysis displayed overexpressed candidate genes in critical amplified region, e.g., C8FW and MYC in 8q24 as well as FLT3 and CDX2 in 13q12. In conclusion, using high-resolution genome-wide screening tools such as array-CGH allows to unravel the enormous genetic diversity of AML cases with complex karyotypes, and correlation with global gene expression studies facilitates the delineation of disease-related candidate genes located in the critical regions.

Gene Expression Based Characterization of NPM1-Mutated/FLT3 ITD-Negative Acute Myeloid Leukemia with Normal Karyotype


'University of Ulm, Ulm, Germany; 'Università di Genova, GENOA, Italy; 'Stanford University, STANFORD, USA

Background. Acute myeloid leukemia (AML) with normal karyotype encompasses a large number of molecularly distinct variants. While the presence of internal tandem duplications (ITDs) of the FLT3 (fms-related tyrosine kinase 3) gene is associated with poor outcome, recently mutations of the NPM1 (nucleophosmin) gene have been shown to be prognostically favorable. However, this effect is mainly attributed to the NPM1-mutated/FLT3 ITD-negative AML cases. While NPM1-mutated cases are characterized by a distinct gene expression pattern, it remains unclear whether NPM1-mutated/FLT3 ITD-negative cases also display a characteristic signature, which might provide additional insights into the molecular basis for the good clinical outcome. Aims. Having demonstrated the presence of a signature correlated with NPM1-mutational status, we sought to define a molecular profile for AML cases with NPM1-mutated/FLT3 ITD-negatve normal karyotype disease. Methods. Towards this goal, we have profiled gene expression of 138 samples of adult AML patients with normal karyotype using DNA microarray technology. All samples analyzed were derived from AML patients entered within the randomized multicenter treatment trial HD-98A of the German AML Study Group. Results. Based on a genome-wide microarray data analysis using SAM (Significance Analysis of Microarrays), we were able to identify a 116-gene comprising expression pattern correlated with NPM1-mutated and FLT3 ITD-negative AML cases. In accordance with previous findings in NPM1-mutated cases (Alcalay et al. 2005, Verhaak et al. 2005), the NPM1-mutated/FLT3 ITD-negative pattern was also in part characterized by a prominent HOX gene cluster, which clearly separated the NPM1-wildtype from the NPM1-mutated cases. Similarly, the expression levels of BAALC and MN1 showed a correlation with the NPM1 mutational status, with NPM1-unmutated cases displaying higher expression in our data set. However, as expected the newly defined signature also defined a NPM1-mutated group that did not contain many FLT3 ITD-positive samples. This group was characterized by several interesting genes including for example TLE1, which encodes a Groucho/TLE family protein. Groucho/TLE family proteins are transcriptional co-repressors, which mediate repression essential in embryonic development and are involved in regulation of Wnt signaling in adult tissue. Moreover, we identified several other genes of potential pathogenic relevance which also have been previously shown to be predictive in normal karyotype AML. Conclusions. Our findings support a distinct molecular mechanism associated with the favorable outcome of NPM1-mutated/FLT3 ITD-negative AML cases. Furthermore, the reported signature might contribute to improved risk stratification and clinical management of AML patients with normal karyotype disease.

Effect of MDR1 Single Nucleotide Polymorphisms (SNPs) C1236T, G2677T and C3435T on MDR1 Function and Expression in Leukemic Blasts, and on Treatment Outcome in Elderly Patients with AML


'Erasmus MC-Daniel den Hoed Cancer Center, ROTTERDAM, Netherlands; 'Erasmus MC-Sophia Childrens Hospital, ROTTERDAM, Netherlands; 'Erasmus MC, ROTTERDAM, Netherlands

Background. The classical multidrug resistance (MDR) gene MDR1 (ABCB1) encodes for the drug efflux pump P-glycoprotein (P-gp). MDR1 expression is an adverse prognostic factor for treatment outcome in acute myeloid leukemia (AML), and is more frequently observed in older patients. Single nucleotide polymorphisms (SNPs) of the MDR1 gene, C1236T, G2677T and C3435T, have been associated with altered drug metabolism and treatment outcome. Aims. We prospectively determined these SNPs in a cohort of patients with AML of 60 years and older, and evaluated their relevance for MDR1 function and expression, MDR1 mRNA expression and clinical outcome. Methods. We have analyzed purified bone marrow derived leukemic blasts of 150 patients treated within the multicenter, randomized phase 3 trial HOVON 51 AML (Novartis FSC C 302-E-00) (Van der Holt et al. Blood. 2005;106:2646-2654). In that trial, 419 eligible Caucasian patients aged 60 years and older with previously untreated de novo and secondary AML (FAB classification MO-M2 and M4-M7) were randomized to receive standard induction chemotherapy with or without the MDR1 inhibitor PSC-833 (Valspodar®, AMD71, Novartis Pharmaceuticals, Basle, Switzerland). The 150 patients genotyped patients were selected for MDR1 analysis based on availability of blast samples in our cell bank. The significance of the allelic MDR1 variants of C1236T, G2677T and C3435T was evaluated with respect to P-gp expression and function in leukemic blasts, and MDR1 mRNA expression levels. The relationship between each of these genetic polymorphisms of MDR1 with clinical outcome, i.e. complete response (CR) rate, event-free survival (EFS), disease free survival (DFS) and overall survival (OS) was also assessed.

Figure 1. OS of elderly AML patients, by genotype. (A) C1236T. (B) G2677T. (C) C3435T. (D) Patients with the same variant for the 3 SNPs.

Results. Each of the 3 SNPs was in Hardy-Weinberg disequilibrium
(p<0.001), contrary to other published results. Each combination of two SNPs was in linkage disequilibrium (p<0.001), which confirms results reported by Imler et al (Cancer Res. 2002;62:955-4962). Patient baseline characteristics were not significantly different between wild-type, heterozygous or homozygous mutant patients, neither for the 3 genetic polymorphisms, nor for the patients with the same allelic variant of all 3 SNPs. β2-gp efflux and expression data in purified AML blasts and in the CD34-positive subpopulation, as well as the MDRI mRNA expression levels of AML patients did not vary significantly among any of the allelic variants of MDRI. All functional and expression data were highly correlated (p<0.001). The median follow up of 24 patients still alive was 57 months (range, 8-81). No statistically significant differences in CR rate and survival endpoints were observed between the allelic subgroups (Figure 1), neither unadjusted nor adjusted for treatment arm, nor was there any apparent interaction between the allelic variants of each SNP and treatment arm with respect to outcome. Summary/Conclusions. In AML patients aged 60+, allelic MDRI variations of C1236T, G2677T or C3435T are not associated with altered MDRI function, nor with MDRI expression at the transcriptional or translational level in leukemic blasts, and they do not significantly impact on clinical prognosis, suggesting that they do not exert a major impact on drug resistance in elderly patients with AML.

**Stem cell biology**

0475

THE MOLECULAR SIGNATURE OF PURIFIED CANCER STEM CELLS REVEALS A STEM CELL ORIGIN OF 5Q- SYNDROME

L. Nilsson,1 P. Edén,1 E. Olsson,1 R. Månsson,1 I. Astrand-Grundström,1 B. Strömbeck,1 K. Theilgaard-Mönch,1 R. Hast,1 E. Hellström-Lindborg,1 J. Samuelsson,2 G. Bergh1, B. Johansson,1 M. Sigvardsson,1 A. Borg,1 S.E.W. Jacobsen1

‘Lund Stem Cell Center, LUND, Sweden;1 Complex Systems Division, LUND, Sweden;1 DNA Microarray Resource Center, LUND, Sweden;1 Department of Clinical Genetics, LUND, Sweden;1 Granuloocyte Research Laboratories, COPENHAGEN, Denmark;1 Hamatology Center, Karolinska University, STOCKHOLM, Sweden;2 Department of Medicine, Division of Hema, STOCKHOLM, Sweden;2 South Hospital, STOCKHOLM, Sweden;2 Helsingborg Hospital, Helsingborg, Sweden;2

Background. Although it has been postulated that leukemic and other cancer stem cells frequently may originate in the corresponding rare multipotent stem cell population, conclusive evidence for such a model has only been obtained for Philadelphia chromosome-positive chronic myeloid leukemia. Aim. To identify the origin of cancer stem cells by applying global gene expression profiling and to uncover MDS stem cell specific gene expressions. Methods. Fluorescence activated cell sorting (FACS) for enrichment of candidate MDS 5q- syndrome stem cells, Fluorescence in situ hybridization (FISH) to show clonal (5q-) involvement, long-term culture initiating-cell (LTC-IC) assay to show stem cell function and oligonucleotide microarrays to evaluate the global gene expression profile of candidate MDS stem cells. Results. Global gene expression profiles of candidate MDS 5q- stem cells (CD34+CD38-Thy-1+) and normal stem cells (CD34+CD38-Thy-1+) are more similar than candidate MDS stem cells and normal progenitors (CD34+CD38-Thy-1+) are. However, BMI1 and CEBPalfa are up-regulated in MDS stem cells from most patients. Furthermore, these differences are specific for MDS stem cells since CEBPalfa is down-regulated and BMI1 is unaffected in MDS progenitor cells. Conclusions. Global gene expression profiling supports that MDS 5q- syndrome originates in normal stem cells. BMI1, a critical regulator of self-renewal, is up-regulated in MDS stem cells, as is the myeloid transcription factor CEBPalfa. These changes are specific for MDS stem cells and could potentially be involved in defining the MDS 5q- syndrome stem cell clonal advantage and defective differentiation process. We have demonstrated the importance of identifying the specific cancer stem cell population to uncover potential gene expression changes contributing to unique cancer stem cell properties.

0476

PROTEIN KINASE B: A MOLECULAR SWITCH IN REGULATION OF LINEAGE CHOICE DECISIONS DURING MYELOPOIESIS

M. Buitenhuis,1 L. Verhagen,1 H.W.M. van Deutekom,1 A. Castor,2 S. Verploegen,1 L. Koenderman,1 S.E. Jacobsen,1 P.J. Coffer1

‘UMC Utrecht, UTRECHT, Netherlands;1 Lund Stem Cell Center, LUND, Sweden

Introduction. Hematopoiesis is a highly regulated process resulting in the formation of all blood lineages. The specific signal transduction pathways involved in lineage choices during hematopoiesis remain largely unsolved. The PI3K/PKB pathway has been reported to play a critical role in proliferation and survival of cells, however, a role in regulating hematopoiesis is largely unknown. Aim. The aim of this project is to investigate whether the PI3K signaling module plays a role in regulation of myelopoiesis. Methods. Human umbilical cord blood derived CD34+ cells cultured in presence of IL-5 or G-CSF resulting in eosinophil or neutrophil differentiation, respectively, were either treated with pharmacological inhibitors to block the PI3K signaling pathway or retrovirally transduced to ectopically express constitutively active PKB, a downstream target of PKB. Results. Inhibition of PKB blocked progenitor proliferation without affecting cell survival. Interestingly, inhibition of PKB abrogated neutrophil differentiation, but conversely, dramatically enhanced eosinophil differentiation. Retroviral transduction of CD34+ cells with constitutively active PKB (myrPKB) resulted in enhanced neutrophil differentiation and monocyte development, whereas eosinophil differentiation was blocked. In contrast, dominant-negative PKB (PKBcaax) induced eosinophil differentiation and inhibited neutrophil maturation. Transplantation of β2-microglobulin (-/-) NOD/SCID mice with CD34+
cells ectopically expressing myrPKB resulted in enhanced neutrophil and monocyte development, whereas ectopic expression of PKBcaax induced eosinophil development. Inhibitory phosphorylation of C/EBPα, a transcription factor known to play a critical role in regulation of myelopoiesis, was abrogated upon PKB activation in hematopoietic progenitors. Conclusion. These results demonstrate that PKB activity plays a critical role in regulation of cell fate choices during myeloid lineage commitment. High PKB activity promotes neutrophil differentiation and monocyte development, while reduction of PKB activity is required to induce eosinophil differentiation.

CONSTITUTIVE EXPRESSION OF THE ‘LYMPHOID ENHANCER FACTOR 1’ (LEF-1) PERTURBS HEMATOPOIETIC DEVELOPMENT AND INDUCES LEUKAEMIA IN A SUBSET OF TRANSPLANTED MICE

LMU University, GSF-Hematologikum, MUNICH, Germany; Max Planck Institute of Immunobiology, FREIBURG, Germany.

Background. LEF-1 is a key transcription factor of the Wnt/β-catenin signalling pathway and is crucially linked to normal B- and T-cell development. Recently its aberrant expression has been associated with different types of leukemia. Aims. Aims of this project were to elucidate the expression pattern of LEF-1 in different hematopoietic subpopulations and to test whether constitutive expression of this transcription factor affects early hematopoietic development. Methods. Expression Analysis was performed by using sem-quantitative RT-PCR and Real-Time PCR. Functional relevance was demonstrated by induction of constitutive expression of wild type LEF-1 (WT) and of a constitutive active LEF-1 mutant (CA) in primary murine bone marrow cells by retroviral gene transfer, using a MSCV based retroviral construct with an IRES-GFP cassette. Results. Analysis of LEF-1 expression showed expression in both lymphoid and myeloid subpopulations, but also in highly purified hematopoietic stem cells. In vitro, at the level of clonogenic progenitor cells, the colony forming potential of progenitors was increased more than 2-fold by both WT and CA compared to the empty vector control (n=4; WT: p<0.02; CA: p<0.05). At the level of the short-term repopulating stem cell, both LEF-1 constructs remarkably increased the number of spleen colonies, resulting in a 46-fold and 7-fold increase in the CFU-S frequency in WT and CA compared to the GFP control, respectively (median 50 (WT) and 135 (CA) CFU-S/1x10^6 cells versus 20 CFU-S/1x10^6 cells, respectively; p<0.001; WT n=7, CA n=6, control n=19). To assess the impact of LEF-1 on long-term repopulating stem cells mice were transplanted with BM cells transduced either with WT LEF-1 or CA-LEF-1. In vivo, normal hematopoietic development was severely perturbed in transplanted mice. Both constructs induced a reduction of lymphoid cells as well as a dramatic increase of myeloid cells with an inversion of the lymphoid/myeloid ratio (WT: ratio 0.43, p<0.01; CA: ratio 0.10, p<0.001; vs. 1.02 in control mice). Engrafted mice succumbed to a lethal myeloproliferative syndrome that resulted in or was secondary to acute leukemia, which were readily transplantable into secondary recipients and showed indefinite IL-3 dependent cell growth in vitro. Conclusions. These data show that balanced expression of LEF-1 plays a key role in early hematopoietic development and that deregulation of this transcription factor favours the development of myeloid malignancies.

ALTERED PROLIFERATION/DIFFERENTIATION POTENTIAL OF COMMON MEGAKARYOCYTIC-ERYTHROID PROGENITORS FROM MICE CARRYING THE HYPOMORPHIC GATA1 LOW MUTATION

Istituto Superiore Sanità, ROME, Italy; University of Chieti-Pescara, CHIETI, Italy; Children Hospital, Harvard, BOSTON, USA.

Background. Several recent evidences suggest that, in addition to its function at late stages of maturation, Gata1 also controls the proliferation/differentiation potential of hematopoietic progenitor cells. We have previously shown that the hematopoietic tissues from mice carrying the hypomorphic Gata1 low mutation contain numerous (~10–20% of all the cells) unique tri-lineage progenitor cells, committed toward the erythroid, megakaryocytic and mastocytic lineage (Migliaccio et al. J. Exp. Med. 2003,197:281). Although predicted by the stochastic model of hematopoietic commitment, the nature of these cells is unclear because progenitors with such a biological function have not been prospective-ly isolated from mouse tissues as yet. Aims. To clarify the effect of the Gata1 low mutation at the level of the hematopoietic progenitor cells by prospectively identifying the tri-lineage progenitors present in the tissues of these mutants. Methods. The number and function of mast cells from Gata1 low mice, normal littermates (positive controls) and W/Wv mutants (negative controls) was compared in bone marrow derived mast cells and in non-adherent cell suspensions of the common myeloid (CMP), granulocytic-monocytic (GMP), megakaryocyte-erythroid (MEP) and mast cell (MCP) progenitors in the marrow and spleen from wild type and Gata1 low littermates was compared on the basis of specific antigenic profiles. The biological functions of these cells was investigated in single cell cultures followed by single cell replating experiments. Results. BMMC from Gata1 low mice generated different types of hematopoietic cell lines like mast cells that proliferated 10-fold more than their normal counterparts and consistently gave rise to growth factor dependent tri-lineage (erythroid, megakaryocytic and mastocytic) cell lines. Although the frequency of CMP, GMP and MEP was normal, MCP were not detectable in the tissues from Gata1 low mice. On the other hand, MEP isolated from mutant mice, in contrast to those isolated from wild type controls, generated, after seven days of culture, not only erythroblasts and megakaryocytes, but also mast cells and their precursors. Many (40%) of the mutant CMP and MEP generated in culture, at the single cell level, high numbers of cells that could be sequentially recloned for up to 40 days. By this time, almost all the cells present in each of these single cell cultures had an inverted lymphoid/myeloid ratio, and their progeny resembled in morphology the cell lines obtained from Gata1 low mice in BMCC. Conclusions. These results indicate that CMP, GMP and MEP, but not MCP, are present in the tissues of the Gata1 low mice. However, in these mutants, the mast cell generating activity is abnormally retained by MEP, that represents the ‘unique’ tri-lineage progenitor previously identified in the tissues from these mutants. Therefore, Gata1 low MEP are antigenically but not functionally, equivalent to MEP from wild-type animals. These results indicate as new target for Gata1 mutation the restriction point when CMP became committed toward MFC or MEF.

CD97 IS DIFFERENTIATED EXPRESSED ON MURINE HEMATOPOIETIC STEM CELLS AND PROGENITOR CELLS

M. van Pel, H. Hagoort, R. Willemze, J. Hamann, W.E. Fibbe
Leiden University Medical Center, LEIDEN, Netherlands; Academic Medical Center, AMSTERDAM, Netherlands.

CD97 is a member of the EGF-TM7 family of class II seven-span transmembrane receptors and is broadly expressed on hematopoietic cells including lymphocytes, granulocytes, and monocytes. We have recently demonstrated that CD97 is involved in IL-8-induced hematopoietic stem cell (HSC) mobilization (Blood 2003;102:455a). To determine a possible role of HSC in this process, we studied the expression of CD97 on HSC. Murine HSC are characterized as c-Kit^POS thy-1^LOLin^NEG/LO cells that could be sequentially recloned for up to 40 days. These results indicate that CD97 is differentially expressed on murine hematopoietic stem cells and progenitor cells using a CD97 specific monoclonal antibody (clone 1B2) and FACS analysis. Based on CD97 expression levels, BM cells were then sorted into different fractions and further characterized in colony-forming assays, CAFC assays and in situ vivo transplantation model. FACS analysis of BM cells showed three major populations i.e. CD97^HI, CD97^INT and CD97^NEG cells (71.5%, 24.4% and 4.4% of total BM cells respectively). Analysis of CFU-GM colony forming capacity of these BM subsets revealed that the majority of colony-forming cells were present in the CD97^NEG (714±506 CFU-GM) population compared to CD97^HI (178±170 CFU-GM) and CD97^INT (50±47 CFU-GM per 10^6 BM cells). Analysis of CAFC frequencies of CD97^HI, CD97^INT and CD97^NEG BM cells showed that CAFC-day 35 activity was found in CD97^INT BM population (8.0±1.2 vs. 9.0±1.1 CAFC-day 35 per 10^5 BM cells for CD97^INT and total BM respectively), whereas no CAFC-day 35 activity was found in CD97^HI BM fractions. In addition, FACS analysis revealed that the majority (82.6%) of c-Kit^POS thy-1^LOLin^NEG/LO cells were present in the CD97^INT BM population. To investigate the in vivo repopulating ability of the different CD97 BM subsets, lethally irradiated (9.5 Gy) BALB/c recipient mice (n=10 per group) were reconstituted with 105 syngeneic BALB/c total BM with CD97 sorted BM cells. Repopulating capacity entirely resided in the CD97^INT subset (repopulation rate 90% versus 0% in the CD97^HI and CD97^NEG subset). These data indicate that 1) CD97 is differentially expressed on HPC and HSC and that 2) CD97 expression can be used to separate colony forming cells from repopulating hematopoietic cells.
Dendritic cells, vaccination and cellular immunotherapy

0480

CLINICAL BENEFIT ASSOCIATED WITH IDIOTYPIC VACCINATION IN FOLLICULAR LYMPHOMA

M. Bendandi
University Clinic of Navarra, PAMPLONA, Spain

Background. So far, no human tumor vaccine has proved beneficial to any cancer patients. Aims. The formal demonstration of such an efficacy is currently widely sought, particularly in the setting of idiotype vaccination for follicular lymphoma (FL). However, standard randomized trials struggle with the major flaw of experimental arms in which each and every patient ultimately undergoes a different, customized treatment. Methods. For this reason, we have instead conducted a phase II study of RHAMM/CD168+ AML blasts in a MHC class I-restricted and epitope-specific manner. Aims. We therefore initiated a phase II R3 peptide vaccination to induce immunological and hematological responses for patients with AML, MDS or MM overexpressing RHAMM/CD168 to induce immunological and clinical responses. Methods. Patients were included with positive RHAMM/CD168 expression but with a limited tumour load. At a biweekly interval, 300 mcg RHAMM R3 peptide emulsified with the incomplete Freund’s adjuvant (day 0) and GM-CSF (100 mcg, days 1-5) was administrated four times subcutaneously. The primary aim of the study is safety and feasibility of this peptide vaccination, secondary aims the evaluation of a specific T cell immune response to RHAMM/CD168 R3 peptide and the assessment of the influence of the R3 peptide vaccination on the remission status. Since January 2005, twelve patients were enrolled in this study. Results. The first ten patients (2 AML, 4 MDS, 4 MM) have completed the course of four vaccinations and four patients have been evaluated. The only side effects observed under R3-peptide vaccination were erythema and induration of the skin at the site of injection (CTC I°). In 7/10 patients, we found in the peripheral blood a significant increase of specific CD8+ T cells (from 0.01% to 0.8%) recognizing the R3 peptide in ELISPOT analysis and seven-color flow cytometry including tetramer staining, two patients showed already initially a high number of HLA-A2/R3 tetramer+ WT1 tetramer-CCR7-CD27-CD28-CD45RA+ effector T cells and main-tained this level of T cell response. Clinical responses have been assessed by the examination of peripheral blood and bone-marrow samples before and after vaccination. Patients showed a reduction of the tumor-specific expressed antigen RHAMM/CD168 in real-time RT-PCR analysis after vaccination. 8/7 patients with myeloid disorders (1 AML, 2 MDS/RAEB1) showed a reduction of CD33+ cells in FACs analysis of the bone-marrow after four vaccinations from 10 and 7% to 1-2 and <1%, respectively. Two patients with MM showed a reduction of plasma cells in bone-marrow and a stable quantity of light chains in peripheral blood, one patient with AML showed a progressive disease. Conclusion. 70% of immunological and 40% of hematological responses were observed. RHAMM/CD168 is therefore a promising target antigen for immunotherapies in patients with hematological malignancies.

0482

TRIGGERING OF P38 MAPK BY CONDITIONAL MKK6 INDUCTION IS SUFFICIENT FOR DENDRITIC CELL MATURATION, AN EFFECT FURTHER ENHANCED BY INHIBITION OF NUCLEAR RELB

A. Jorgl, B. Platter, S. Taschner, B. Höcher, E. Göbel, H. Strobl
Medical University of Vienna, VIENNA, Austria

Activation of Langerhans cells (LCs) by diverse signals involves p38 MAPK phosphorylation. Whether p38 is sufficient to trigger LC activation remains unknown. We show that conditional induction of a dominant active form of MAPK kinase 6 (d.a.MKK6), a direct upstream kinase of p38, in LCs is sufficient to induce the upregulation of co-stimulatory molecules and to enhance their T cell stimulatory capacity. These immediate effects showed no or only a minor requirement for classical NF-kB signaling. Concomitant with LC activation, d.a.MKK6 strongly induced the alternative NF-kB member RelB, whose nuclear localization marks mature DCs. Specific inhibition of nuclear RelB during MKK6-induced LC activation further enhanced their maturation state, thus suggesting a novel LC intrinsic control mechanism regulated by RelB.

0481

RHAMM/CD168-R3 PEPTIDE VACCINATION OF HLA-A2+ PATIENTS WITH ACUTE MYELOID LEUKEMIA, MYELODYSPLASTIC SYNDROME AND MULTIPLE MYELOMA ELICITS IMMUNOLOGICAL AND HEMATOLOGICAL RESPONSES

J. Greiner,1 A. Schmitt,2 K. Giannopoulos,1 L. Li,1 P. Liebisch,1 C. Wendt,1 J. Chen,2 M. Ringhoff,1 F. Guillaume,1 G. Ritte,1 H. Döhner1 M. Schmitt1
1University of Ulm, ULM, Germany; 2Medical School of Southeast University, NANJING, China; 3Ludwig Institute of Cancer Research, LAUSANNE, Switzerland

Background. The receptor for hyaluronic acid mediated motility (RHAMM/CD168) is expressed in more than 80% of acute myeloid leukemia (AML) or multiple myeloma (MM). Recently, we characterized RHAMM/CD168 as a leukemia-asso-
Chronic myeloid leukaemia (CML) is characterised by the BCR-ABL oncoprotein. The peptide sequences spanning the junctional region are completely leukaemia-specific. Vaccination with these peptides could therefore elicit/augment an immune response directed to CML cells. Entry requirements to our ongoing Evaluation of Peptide Immunisation in CML (EPIC) study were all of the following: first chronic phase of CML, expression of the e14a2 (b3a2) BCR-ABL transcript, and prior treatment with imatinib at a stable dose of at least 400 mg daily for at least 8 months. Each patient received intradermally 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 peptides were administered at either 100 (5 patients), 300 (5 patients), 600 (5 patients), or 1000 µg (3 patients) with sargramostim on 6 occasions over 2 months. Immune responses to the vaccine were monitored by IFNγ and IL-5 ELISPOT assays on peripheral blood mononuclear cells. Currently, 18 patients are evaluable at 6 months of follow-up. At entry, no patient showed a detectable immune response to the PADRE peptide, but all 18 patients had detectable T cell responses within 3 months of commencing vaccination. These typically persisted at 5 and 6 months (i.e. 3 and 4 months after completion) of vaccination. These anti-PADRE responses were carried out by CD4+ T cells as demonstrated by flow cytometry analysis of IFN-γ-producing cells, and indicated that the vaccination protocol was capable of stimulating T cells in all 18 patients. Immune responses to BCR-ABL junctional peptides were monitored using the 9-mer sequence used in the vaccine and an 18-mer spanning the whole junctional region. In all but one case, there was no evidence of T cell responses to these peptides pre-vaccination. Upon vaccination, IFN-γ-producing cells to the 9-mer peptides were detected in 11/18 patients, and these cells were demonstrated to be CD8+ T cells by flow cytometry analysis. Moreover, CD4+ T cells specific for the 18-mer junctional peptide were detected in 14/18 patients. Interestingly, immunophenotyping indicated that these BCR-ABL-specific T cells were of a memory phenotype (CD45RO+). However, the anti-BCR-ABL responses were typically transient, disappearing by 5 months (i.e. 3 months after completing vaccination) in all but one case, in sharp contrast to the responses to PADRE. A good correlation was observed between the presence of BCR-ABL-specific T cells and a decrease in the level of BCR-ABL transcripts. These data demonstrate that peptide vaccination can elicit anti-BCR-ABL peptide responses in CD8+ and CD4+ T cells, but these are less frequent and less durable than those to the novel antigen PADRE. This suggests that BCR-ABL is either a weak antigen or that patients may be tolerised to it. Further functional characterisation (e.g. granzyme B production) of these BCR-ABL specific T cells is currently ongoing.

Minor Histocompatibility antigens (mHag) play an important role in both graft versus tumor effects and graft versus host disease (GVHD) after allogeneic stem cell transplantation (SCT). From a patient with multiple myeloma after allogeneic SCT entering complete remission after donor lymphocyte infusion (DLI) coinciding with mild GVHD, several T cell reactivities were isolated at the time of the clinical response. The most dominant T cell reactivity showed HLA-A2 restriction and strongly recognized tumor cells and activated B and T cells (PHA-blasts), whereas resting T cells were only moderately recognized. From mHag positive EBV-LCL peptides were isolated, tested for reactivity and subjected to sequence analysis. One candidate peptide reconstituted CTL reactivity. This peptide was identical to an alternatively translated protein sequence derived from the human ADIR gene. A SNP in this gene resulting in an aminoacid change in the candidate peptide was shown to be present in patient cells. Synthetic peptides of both patient and donor SNP variants were synthesized and specific recognition of the identified patient derived peptide was demonstrated. Transfection experiments with plasmids containing patient or donor ADIR gene constructs confirmed involvement of this SNP in T cell recognition. A population study revealed 100% correlation of the presence of the relevant SNP with recognition of PHA-blasts in 51Cr release assays. The SNP was present in 43 out of 76 individuals tested. We designated the epitope as LB-ADIR-1F. Tetramer analysis of patient samples that were taken after DLI showed up to 2.6% LB-ADIR-1F specific T cells coinciding with conversion to remission. It was previously shown that IFNα could upregulate ADIR gene expression, and the patient was treated with IFNα during DLI. Therefore, SNP positive MNC were cultured with IFNα prior to addition of the LB-ADIR-1F CTL. IFNα increased both susceptibility to lysis and stimulatory capacity of pretreated MNC. Quantitative PCR showed increased ADIR mRNA levels in IFNα stimulated cells thus supporting the role of IFNα in ADIR gene expression. Recognition of SNP positive mesenchymal stem cells as a representative of non hematopoietic cells was low and growth arrest further decreased recognition. Analysis of the immunological response in this patient also revealed T cell reactivities designated to the mHag LB-EGF-1H and HA1. The sum of the percentages of these circulating mHag specific T cells at the time of the clinical response was 4.3%, approaching the total number of activated circulating CD8+ T cells in the patient as measured by HLA-DR expression suggesting that these 3 reactivities were responsible for the clinical course. Whereas expression of HA1 and ECGF is relatively restricted to hematopoietic cells, ADIR gene expression is more broad. LB-ADIR-1F specific T cells were shown to be highly cytotoxic for multiple myeloma cells and other hematological malignancies. Since only mild acute GVHD was observed which rapidly disappeared after discontinuation of the IFNα treatment and administration of corticosteroids, we hypothesise that the activation status of GVHD target tissues determines the clinical outcome of treatment with LB-ADIR-1F specific T cells in adoptive immunotherapy.
Vascular biology and granulocytes

0485
INHIBITION OF HIF-1α BY A POTENT RNA ANTAGONIST IS ASSOCIATED WITH MULTIPLE MECHANISMS OF ANTI-TUMOUR ACTIVITY

Santaris Pharma, COPENHAGEN, Denmark

Substantial evidence has accumulated to demonstrate that over-expression of Hif-1α is associated with tumour angiogenesis, tumour progression and poor prognosis in a broad range of cancers. It thus represents a potential point of intervention for targeted therapeutics for this group of cancers. The unique properties of Locked Nucleic Acid (LNA) chemistry have been used to generate a single stranded RNA antagonist to the Hif-1α mRNA (SPC2968), which exhibits increased stability, improved resistance to nucleases and much higher binding affinity to the target, than other second and third generation oligonucleotides. We demonstrate that SPC2968 potently inhibits Hif-1α expression in vitro (IC50 < 1nM) and that this is correlated with parallel inhibition of Hif-1α regulated genes in vitro under hypoxic and hypoxia-responsive conditions. Hif-1α knock down in cancer cell lines was also correlated with increased induction of apoptosis and cell death. Following administration of a single dose of SPC2968 to wild type mice, liver Hif-1α mRNA levels were substantially reduced for periods up to 5 days. Hif-1α inhibition was also correlated with reduced expression of genes regulated by Hif-1α, namely MMP2 and VEGF. Ex vivo assays of endothelial tube formation and aorta ring outgrowth demonstrated that SPC2968 administration was also associated with impaired ability of endothelial cells to form capillaries and sprout. Potent anti-tumour effects of the drug were also observed in murine xenograft models, both when tumour cells were transfected with SPC2968 prior to implantation and when pre-treated tumours were subsequently treated in the host during the study, suggesting that both initial and later phases of tumour growth can be impeded by Hif-1α down-regulation. The bio-distribution of SPC2968 following intravenous administration, as assessed by whole body autoradiography using tritium-labelled SPC2968, showed extensive tissue distribution with the LNA oligo still detectable in the kidney 21 days after the injection. Fluorescent-labelled SPC2968 distribution and cellular localisation were additionally investigated in several organs including skin, tumor, liver, kidney and bone marrow. All cell lineages tested were found to be positive for the label. Correlation between uptake of SPC2968 and Hif-1α expression was also addressed by HPLC and QPCR analysis in different points in tumour development.

0486
T-CELL RECEPTOR VB REPertoire ANALYSIS IN CHRONIC IDIOPATHIC NEUTROPENIA: EVIDENCE FOR PRESENCE OF PROMINENT T-CELL CLONES WITH PATHOGENETIC SIGNIFICANCE

M. Spanoudakis, H. Koutala, C. Gemetzis, G. Eliopoulos, H.A. Papadaki
University of Crete School of Medicine, HERAKLION, Greece

Background. Chronic idiopathic neutropenia (CIN) is an acquired underproduction neutropenia syndrome characterized by hypplastic and left-shifted granulocytic series in the bone marrow (BM). We have previously shown that CIN patients display increased number of activated T-lymphocytes with myelosuppressive properties in the BM and peripheral blood (PB) that induce Fas-mediated apoptotic death of the myeloid progenitor cells by producing interferon-γ and Fas-ligand. Epidemiological data have also shown an association of CIN with HLA-DRB1*1302 genetic background. Aims. To characterise T-cell receptor (TCR) Vb repertoire of patients with CIN seeking for dominant T-cell expansions with possible pathogenetic significance. Methods. Fifty-nine patients with CIN were studied. All patients had neutrophil counts below 1.5x10^9/L (mean 1.41±0.58x10^9/microliter, range 1.00-1.7999 neutrophils/microliter) and were satisfying the previously reported diagnostic criteria for the disease. PB samples from the patients were subjected to flow-cytometric analysis for the quantification of the TCR Vb repertoire of the CD3+ cells (IO Test β Mark kit, Beckman-Coulter). Vb family expansions were defined as above of 2SD (standard deviation) from the mean in 85 healthy controls. Blood DNA samples were also subjected to multiplex PCR using the BIOMED2 protocol that covers all Vb TCR gene rearrangements. Results. Forty-four of the patients, i.e. a proportion of 74.6% displayed expanded Vb sets, where Vb16 and Vb12 representing the most frequent expanded clones. Specifically, the patients as a group displayed statistically significant increased proportion of Vb16 and Vb12 expressing T-cells (2.17%±1.40% and 2.62%±2.83%, respectively) compared to controls (0.90%±0.29% and 1.66%±0.54%, respectively) (p<0.001 and p<0.01, respectively). These TCR-Vb over-representations were associated with a parallel under-representation of Vb5.3 (p<0.05), Vb7.1 (p<0.001), Vb9 (p<0.01), Vb17 (p<0.001), Vb18 (p<0.01), Vb5.1 (p<0.01), Vb13.1 (p<0.01), Vb13.6 (p<0.02), Vb5.2 (p<0.01), Vb2 (p<0.001), Vb14 (p<0.02), Vb11 (p<0.05), Vb22 (p<0.01), Vb4 (p<0.01) expressing T-cells compared to controls. Blood samples from patients displaying a significant TCR-Vb expansion in multiplex PCR analysis. Summary-Conclusions. in vivo dominant T-cell responses are identified in the majority of patients with CIN. These data substantiate further the hypothesis for the immune nature of CIN providing therefore novel insights in the pathophysiology of the disease with possible therapeutic implications, i.e. immunosuppressive therapy in severely neutropenic patients. The cloning and sequencing of the complementarity-determining region 3 (CDR3) of the expanded Vb subsets is under investigation to identify, if any, specific antigen-driven T-cell responses in CIN patients.

0487
CONCURRENT MUTATIONS IN NEUTROPHIL ELASTASE AND GRANULOCYTE SIMULATING FACTOR RECEPTOR GENES IN A CASE OF SEVERE CONGENITAL NEUTROPENIA

C. Ward,3 A.C. Ward,3 R.S. Lewis,3 F. Majeed,3 S. Shigdar,1 A.A. Aprikyan,3 D.C. Dale,3 Y. Dror3
1. Deskin University, BURWOOD, Australia; 2. The University of Toronto, TORONTO, Canada; 3. University of Washington, SEATTLE, USA

Background. Severe congenital neutropenia (SCN) is a heterogeneous disorder characterized by extremely low levels of circulating neutrophils, and a propensity for myelodysplastic syndrome and acute myeloblastic leukemia. Germine mutations in the ELA2 gene, encoding neutrophil elastase, are the cause of the disease in 65-80% of the cases. In contrast, mutations in the CSF3R gene, encoding granulocyte colony-stimulating factor receptor (G-CSF-R), are found in approximately 20% of SCN patients and are almost universally acquired. They typically lead to truncation of the intracellular domain of the receptor and result in extended signaling, particularly of SCF, that may play a role in the predisposition of SCN patients to leukemia. However, we have previously described an SCN patient with a constitutive mutation in the G-CSF-R extracellular domain that results in hyporesponsiveness to SCF and suppresses STAT5 signaling. Aims. To further our understanding of SCN etiology through a re-examination of this patient with respect to the status of the ELA2 and CSF3R genes. Methods. Genomic DNA and hematopoietic cell-derived cDNA were analysed for the presence of mutations in the ELA2 and CSF3R genes. Compound G-CSF-R mutants were then examined for their ability to activate STAT5. Results. A novel germline ELA2 mutation was identified in this patient, causing a frameshift after P205 and a premature stop. In addition, two independent truncating mutations within the G-CSF-R intracellular domain, R710X and Q718X, were detected at different times in this patient. Ex vivo studies demonstrated that such intracellular truncations could partially restore the STAT5 response in the context of the extracellular P206H mutation. Summary-Conclusions. These data add to our understanding of the etiology of SCN adding to the evidence that ELA2 mutations are a likely primary cause. These may be exacerbated by CSF3R mutations, particularly those in the extracellular domain that affect signal transduction to G-CSF-R, and that the neutrophil environment conducive to the subsequent expansion of cells expressing truncating G-CSF-R mutations. In addition, these results further attest to the importance of STAT5 in mediating responses to G-CSF.
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 microvascular ECs in B-cell lymphomas 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 clarify if, in different hematologic malignancies with known cytogenetic aberrations, CECs are tumor-derived. Methods. We studied 21 patients with different hematologic malignancies (6 MM, 2 CML, 5 AML, 1 ALL and 7 CLL). To isolated CECs, we used a dual step immunomagnetic sorting by means of CD146 and CD45 antibodies. By using immunomagnetic sorting in combination with CD45, we first eliminated all hematopoietic cells, which are CD45 positive, without affecting the EC component, which is characteristically CD45 negative. We then sorted CECs by means of CD146, an antigen expressed almost exclusively on ECs and absent on hematopoietic cells. To confirm the EC commitment, we then performed additional phenotypic studies with antibodies recognizing endothelial and neoplastic cells. FISH analysis was finally performed on sorted CECs with different commercially available probes in dual colour experiments. Results. In all experiments more than 95% of immunomagnetically sorted cells were of EC origin as demonstrated by phenotypic analyses. After immunomagnetic selection less than 0.5% of cells were CD45+ while CD14 was expressed in 0.1% of all immunomagnetically sorted CECs. More than 95% of immunomagnetically sorted CECs expressed VEGFR2, vWF, CD144 and UEA-1 lectin. Very few immunomagnetically sorted CECs expressed antigens expressed on neoplastic cells (CD138, CD38, CD35, CD19, CD5). FISH analysis showed that a significant proportion of CECs was tumor-derived because they harbored the same genetic lesion as observed in neoplastic cells. The fraction of CECs showing the cytogenetic aberration averaged 20% (range, 11-34%, 200 cells observed in each case). The majority (>85% of CECs presented features of EPCs because they expressed CD133, a marker gradually lost during EC differentiation and absent in mature ECs. Overall, 98.0% of CECs with genetic lesions were CD133 positive. Conclusions. These findings suggest that in many hematologic malignancies CECs are in part tumor related and with EPC features. These CECs may contribute to tumor neovasculogenesis and possibly to the spreading and progression of the disease. It is possible to speculate that neoplastic CECs may have arisen from a common hemangioblast precursor that can give rise to both neoplastic cells and ECs or alternatively through a process of dedifferentiation of a already committed cell into a cell with EPC characteristics followed by a redifferentiation into a terminally differentiated EC. Disguised plasma cells may then mimic functional CECs and contribute to tumor neovasculogenesis.
**Multiple Myeloma - Clinical**

**0490**

**A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL OF ORAL MELPHALAN, PREDNISONE, THALIDOMIDE VERSUS ORAL MELPHALAN, PREDNISONE IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS**

A. Palumbo,1, S. Bringhen,1 T. Caravita,2 V. Callea,3 R. Zamibello,1 P. Pregno,1 A. Carubelli,1 A. Baraldi,1 C. Cellini,1 F. Dore,1 E. Piro,1 M.T. Ambrosini,1 P. Musto,1 A.M. Liberati,1 M. Boccadoro1

1Divisione di Ematologia Univ. Torino, TORINO, Italy; 2Italian Myeloma Network, GIMEMA, Italy

**Background.** For patients older than 65 years of age oral melphalan and prednisone (MP) has remained the treatment of choice since 1960. So far no major improvement in outcome from the original combination MP has been achieved in these elderly patients and new treatments are urgently needed. In this multicentre randomised trial we compared oral melphalan and prednisone plus thalidomide (MPT) with MP alone in 60 to 85 years old patients. Aims. The primary objective was to compare the clinical response rates and the event-free survival in the two treatment groups. Secondary end points included overall survival, prognostic factors, time to the first evidence of response and incidence of any grade 3 or higher adverse events. Methods. The trial was conducted at 54 centres in Italy. Patients with newly diagnosed multiple myeloma were randomly assigned to receive oral MP (N=129) for four six-week cycles plus thalidomide 100 mg per day continuously until any sign of relapse or progressive disease (Pharmion LTD, Windsor, UK) or MP alone (N=126). 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. No anticoagulation prophylaxis was administered until December 2003 when the protocol was amended and enoxaparin at 40 mg per day was delivered subcutaneously during the first four cycles of therapy. Results. Patients treated in MPT arm experienced higher response rates and a longer event-free survival than patients who were not. In intention-to-treat analysis, the complete and partial response rates were 76.0% for MP and 47.6% for MP alone (absolute difference +28.3%, 95% CI 16.5 to 39.1), and the near complete and complete response rates were 27.9% and 7.2%, respectively. The two-year event-free survival rate was 54% in patients receiving MPT and 27% in patients receiving MP. The hazard ratio (HR) for MP was 0.51 (95% CI 0.35 to 0.75), p=0.001. This is a 49% decrease in the risk of events in the MPT group. The three-year survival rate was 80% in patients taking MPT and 64% in patients taking MP, the HR for MPT was 0.66 (95% CI 0.38 to 1.22), p=0.19. Grade 3-4 adverse events were 48% in MPT patients and 25% in MP patients (p=0.001). In the MPT group, the most frequent grade 3-4 adverse events were haematological, thromboembolism, infections and peripheral neuropathy. The introduction of enoxaparin prophylaxis significantly reduced the incidence of thromboembolism from 20% to 3% (p=0.005). Conclusion. Oral MPT is superior to MP as first-line treatment for elderly patients with multiple myeloma. Anticoagulant prophylaxis reduced the frequency of thrombosis. Longer follow-up is needed to assess the effect on overall survival.

**0492**

**HAEMATOLOGICAL PROFILES WITH BORTEZOMIB OR HIGH-DOSE DEXAMETHASONE TREATMENT IN RELAPSED MULTIPLE MYELOMA: PHASE 3 APEX TRIAL**

S. Lonial,1 P. Richardson,2 P. Sonneveld,3 M. Schuster,4 D. Irwin,5 E. Stadtmaurer,6 T. Faccon,7 J.L. Harousseau,8 D. Ben-Yehuda,9 H. Goldschmidt,10 D. Reece,11 J. San Miguel,12 J. Blad6,13 M. Boccadoro,14 J. Caveagh,15 K. Anderson1

1Winship Cancer Institute, ATLANTA, USA; 2Dana-Farber Cancer Institute, BOSTON, USA; 3University Hospital Rotterdam, ROTTERDAM, Netherlands; 4NY-Presbyterian Hospital, NEW YORK, USA; 5Altas Bates Cancer Center, CALIFORNIA, USA; 6University of Pennsylvania Cancer Center, PENNSYLVANIA, USA; 7Hospital Claude Huriez, LILLE, France; 8Hospital Dieu Hospital, NANTES, France; 9Hadassah University Hospital, JERUSALEM, Israel; 10University of Heidelberg, HEIDELBERG, Germany; 11Princess Margaret Hospital, OTTAWA, Canada; 12Hospital University of Salamanca, SALAMANCA, Spain; 13University of Barcelona, BARCELONA, Spain; 14University of Torino, TORINO, Italy; 15St. Bartholomew’s Hospital, LONDON, United Kingdom

**Background.** Bortezomib (VELCADE®) is a novel proteasome inhibitor that has demonstrated safety and efficacy for patients with relapsed and/or refractory multiple myeloma in phase 2 and trials. Bortezomib was associated with thrombocytopenia and neutropenia in SUMMIT (NEJM 2003;348:2609) and CREST (BJH 2004;127:165) and both were transient and cyclical. Aims. This analysis characterised the haematological profiles of patients treated with bortezomib or high-dose dexamethasone in APEX, the largest phase 3 trial in patients with relapsed multiple myeloma following 1-3 prior therapies (NEJM 2005;352:2487).

**Methods.** 669 patients with relapsed multiple myeloma were randomised to bortezomib 1.3 mg/m², d 1, 4, 8, 11 q5wk for 8 cycles, then 3 cycles on d 1, 8, 15, 22 q5wk, or dexamethasone 40 mg, d 1-4, 9-12, 17-20 q5wk for 4 cycles, then 5 cycles on d 1-4 q6d. Data on adverse events, laboratory values, and transfusion experience were collected at baseline and regularly through therapy. RESULTS. Anaemia, neutropenia, and thrombocytopenia were reported as adverse events are shown (Table). The incidence of anaemia was similar in both treatment arms. 53% of patients on bortezomib and 20% of those on dexamethasone received blood transfusions for anaemia. Patients on bortezomib experienced a steady increase in haemoglobin over time and the requirement for blood transfusions decreased over time to 0% after cycle 4. Bortezomib-associated neutropenia was also transient and cyclical, and febrile neutropo-
nia was rare. G-CSF or GM-CSF was used at a low rate to manage neutropenia. Thrombocytopenia was cyclical, with recovery towards baseline during the rest period of each cycle. Overall, 15% of patients on bortezomib and 1% of those on dexamethasone received platelet transfusions for thrombocytopenia. Preclinical study of the effect of bortezomib on megakaryocytes indicates a shorter recovery time than with cytotoxic marrow injury, an absence of a lethal cytotoxic effect, and no cumulative or persistent thrombocytopenia. The number of patients requiring platelet and blood transfusions peaked within the first 2 cycles in both treatment arms. Although the number of platelet transfusions was higher with bortezomib, the number of significant bleeding events (including any grade 3/4, any with an intensity reported as serious, and cerebral haemorrhage regardless of intensity and serious effect) was similar in the 2 arms. No difference was observed in response rate or duration of response in patients who received platelet transfusions compared with patients who did not need platelet transfusion. Median duration of therapy for platelet-transfused patients was 5.8 and 3.4 mo in the bortezomib and dexamethasone arms, respectively. Conclusions. Haematological adverse events with bortezomib are predictable and manageable. The kinetics and mechanism appear different from those observed with standard cytotoxic therapy. Neutropenia was transient and rapidly recovered to baseline during the rest period of each bortezomib treatment cycle, with few patients requiring growth factor support. Thrombocytopenia was also transient and reversible. When clinically indicated, platelet transfusion rather than dose reduction or treatment interruption may be warranted to maximise the benefit of bortezomib therapy.

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**Table 1.**

<table>
<thead>
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<th>Event</th>
<th>Bortezomib (n=331)</th>
<th>Dexamethasone (n=332)</th>
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<tr>
<td>Anaemia, n (%)</td>
<td>87 (23)</td>
<td>74 (22)</td>
</tr>
<tr>
<td>Neutropenia, n (%)</td>
<td>33 (10)</td>
<td>35 (11)</td>
</tr>
<tr>
<td>Thrombocytopenia, n (%)</td>
<td>62 (19)</td>
<td>5 (2)</td>
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<td></td>
<td>48 (14)</td>
<td>4 (1)</td>
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<td>All grades</td>
<td>115 (35)</td>
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<tr>
<td>G3/4</td>
<td>91 (30)</td>
<td>22 (6)</td>
</tr>
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</table>

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**0493**

**LENALIDOMIDE (REVLIMID) IN COMBINATION WITH DEXAMETHASONE IS MORE EFFECTIVE THAN DEX ALONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA WHO HAVE RECEIVED PRIOR THALIDOMIDE THERAPY**

J. Blad, 1 D. Weber, 1 J. Zeldis, 2 Z. Yu, 3 A. Dmoszynska, 4 M. Attal, 5 J. Harousseau, 5 A. Hellmann, 6 M. Prince, 7 J. San Miguel, 8 A. Spencer, 9 T. Cibeira, 9 M. Dimopoulos, 9 R. Knight 1

1 DIBAPS, Hospital Clinic, BARCELONA, Spain; 2 M.D. Anderson Cancer Center, HOUSTON, TX, USA; 3 Celgene Corporation, SUMMIT, NJ, USA; 4 University School of Medicine, LUBLIN, Poland; 5 CHU Purpan, TOULOUSE CEDEX, France; 6 Centre Hospitaller Hotel-Dieu, NANTES CEDEX, France; 7 Institute of Internal Diseases, University, GDANSK, Poland; 8 Peter MacCallum Cancer Institute, MELBOURNE, Australia; 9 Hospital Universitaria de Salamanca, SALAMANCA, Spain; 10 The Alfred Hospital, MELBOURNE, Australia; 11 University General Alexandras Hospital, ATHENS, Greece

Background. Lenalidomide is a novel, oral, immunomodulatory drug that is effective against relapsed or refractory multiple myeloma. In the prospective, randomized, placebo-controlled phase III trial MM-010, lenalidomide with dexamethasone induced a significantly higher response rate and complete remission rate, as well as longer Time-to-Progression (TTP) in comparison with dexamethasone alone. The trial included patients who had more than 1 previous unsuccessful regimen. Aim. To further investigate whether one or more prior therapies influence TTP between refractory multiple myeloma in patients treated with lenalidomide and dexamethasone alone. Methods. This post hoc analyses included 351 patients who had received 1 to 3 prior treatments and were not refractory to dexamethasone. The patients were randomized to receive either 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 4 cycles, then 40 mg on days 1-4 every subsequent cycle) (Len/Dex) or placebo plus dexamethasone (Dex alone). Standard criteria were used to evaluate the median TTP. Confidence intervals (based on Kaplan-Meier estimates), hazard ratios (HR; proportional hazards model), and differences between treatment groups (one-tailed log-rank test of survival curve differences between the treatment groups) were calculated. Results. Of the 351 patients, 4% had previously received bortezomib, 34% had received thalidomide, 67% had received dexamethasone, and 55% of patients had received stem cell transplantation treatment. A total of 65 patients had 1 prior treatment (n = 30 Len/Dex, n = 35 Dex), 150 patients had 2 prior treatments (n = 65 Len/Dex, n = 65 Dex), and 156 patients had at least 3 prior treatments (n = 81 Len/Dex, n = 77 Dex). Median TTP for patients with 1 prior regimen was not yet reached (NE) (95% CI, 24.1-NE) in Len/Dex patients vs 20.1 weeks (95% CI, 12.9-39.9) in Dex alone patients (HR, 2.8; p<0.005). Median TTP for patients with 2 prior regimens was 7.8 weeks (95% CI, 4.2-11.4) in Len/Dex patients vs 20.1 weeks (95% CI, 13.3-24.1) in Dex alone patients (HR, 3.7; p<0.001). Median TTP for patients with 3+ prior regimens was 40.9 weeks (95% CI, 32.1-52.4) in Len/Dex patients vs 20.1 weeks (95% CI, 16.1-20.9) in Dex alone patients (HR, 2.5; p<0.001). Conclusion. Lenalidomide in combination with dexamethasone is more effective than dexamethasone alone in patients with relapsed or refractory multiple myeloma irrespective of the number of prior unsuccessful therapies. TTP after Len/Dex appears to be longer when this combination is used as second line treatment than in later phases of the disease. However, further studies are needed to confirm this observation and to determine whether lenalidomide should be considered sooner in the treatment of multiple myeloma.

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**0494**

**LENALIDOMIDE (REVLIMID) COMBINATION WITH DEXAMETHASONE IS MORE EFFECTIVE THAN DEX ALONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND INDEPENDENT OF NUMBER OF PREVIOUS TREATMENTS**

M.A. Dimopoulos, 1 A. Anagnostopoulos, 2 M. Prince, 3 A. Lopez-Guillermo, 4 J.L. Harousseau, 5 A. Dmoszynska, 4 M. Prince, 7 Z. Maslik, 9 M. Lazzarino, 8 T. Facon, 9 R. Foà, 9 J. Zeldis, 9 Z. Yu, 9 M. Olesnyckyj, 11 H. Faleck 6

1 University of Athens School of Medicine, ATHENS, Greece; 2 University of Athens, School of Medicine, ATHENS, Greece; 3 Hospital Clinic, BARCELONA, Spain; 4 Centre Hospitaller Hotel-Dieu, NANTES CEDEX, France; 5 University School of Medicine, LUBLIN, Poland; 6 Institute of Internal Diseases, UNIVERSITARI, GDANSK, Poland; 7 Inst Blood Pathology & Transfusion UAMS, LVOV, Ukraine; 8 Policlinico San Matteo, PAVIA, Italy; 9 Hospital Claude Huriez, LILLE CEDEX, France; 10 Universita La Sapienza, ROME, Italy; 11 Celgene Corporation, SUMMIT, NJ, USA

Background. Lenalidomide is a novel, oral, immunomodulatory drug that is effective against relapsed or refractory multiple myeloma. In the prospective, randomized, placebo-controlled phase III trial MM-010, lenalidomide with dexamethasone induced a significantly higher response rate and complete remission rate, as well as longer Time-to-Progression (TTP) in comparison with dexamethasone alone. The trial included patients who had more than 1 previous unsuccessful regime. Aim. To further investigate whether one or more prior therapies influence TTP between refractory multiple myeloma in patients treated with lenalidomide and dexamethasone alone. Methods. A total of 692 patients were enrolled in the study. Patients were randomized to receive either 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 4 cycles, then 40 mg on days 1-4 every subsequent cycle) (Len/Dex) or placebo plus dexamethasone (Dex alone). Standard criteria were used to evaluate the median TTP. Confidence intervals (based on Kaplan-Meier estimates), hazard ratios (HR; proportional hazards model), and differences between treatment groups (one-tailed log-rank test of survival curve differences between the treatment groups) were calculated. Results. Of the 692 patients, 4% had previously received bortezomib, 1% had received thalidomide, 8.1% had received lenalidomide, 1.4% had received placebo plus dexamethasone (Dex alone). Complete response rates were also higher with Len/Dex than with dexamethasone in patients who had received prior thalidomide (8.1% vs. 1.4%, p<0.05). After a median follow-up of 11 months for all patients, there was a trend for Len/Dex to provide improved overall survival (hazard ratio 1.53, p=0.0713) versus Dex alone. Conclusions. Lenalidomide in combination with dexamethasone is more effective than dexamethasone alone in patients with relapsed or refractory multiple myeloma who have received prior thalidomide therapy.
**Chronic myeloproliferative disorders**

0495

EVIDENCE THAT THE JAK2 V617F MUTATION AND MITOTIC RECOMBINATION OCCUR IN A LYMPHO-MYELOID PROGENITOR IN POLYCYTHEMIA VERA AND IDIOPATHIC MYELOFIBROSIS


1. INSERM, VILLEJUIF, France; 2. APHP, Hôpital H. Mondor, CREteil, France; 3. Institut Gustave Roussy, VILLEJUIF, France; 4. APHP, Hôpital Htel Dieu, PARIS, France

**Background.** The JAK2 V617F mutation has recently been described as an essential oncogenic event associated with Polycythemia Vera (PV), Idiopathic Myelofibrosis (IMF) and Essential Thrombocythemia (ET). This mutation has been detected in myeloid lineages but has not yet been detected in lymphoid cells. This raises the question whether this molecular event occurs in a lymphoid/myeloid progenitor cell, as it has already been shown that at least some of these myeloproliferative disorders (MPD) derive from a multipotent stem/progenitor cell. **Aims.** Our aim was to study the presence of the mutation in both myeloid and lymphoid lineages in JAK2 V617F positive MPD. We therefore looked for the mutation first in mature myeloid and lymphoid cells and second in lymphoid/myeloid progenitor cells after CD34+ cell isolation from peripheral blood or bone marrow aspiration. **Methods.** Ten IMF, 12 PV and 6 ET patients harbouring the mutation were enrolled in the study after informed consent. Peripheral blood granulocytes and platelets were purified by standard methods and B, T, NK and monocytes were isolated by combined immunomagnetic and flow cytometric procedures. The same techniques were used to sort CD34+ and CD34+CD38- cells from peripheral blood (IMF patients) or from bone marrow mononuclear cells (PV and ET patients). Clonal B/NK/Myeloid differentiation from CD34+CD38- cells and T cell differentiation from CD34+ cells were performed respectively on a M5S layer in the presence of SCF, FLT3L, IL2, IL3, IL7, IL15, TPO and in murine Fetal Thymic Organ Cultures (FTOC). Genotyping of mature cell populations, B/NK/Myeloid clones and CD34+ derived T cells were performed by sequencing and/or Taqman real time allele specific PCR using competitive probes. **Results.** The JAK2 V617F mutation was present in granulocytes and platelets from all patients, and in monocytes from PV and IMF patients. We detected the mutation in B and NK cells from approximately half IMF patients (respectively 4/7 and 5/8 patients), a minority of PV patients (respectively 1/10 and 1/10 patients), and none of the ET patients. Moreover, 2/8 IMF patients had mutated peripheral T cells whereas none of the PV and ET patients did. The JAK2 V617F mutation could be subsequently detected in CD34+ cells and in B/NK/Myeloid and/or NK/Myeloid CD34+CD38- derived clones from all IMF patients (n=5), PV (n=5) and ET (n=1) patients, with a much higher frequency in clones derived from IMF. Interestingly, a bi-allelic (homozygous) JAK2 V617F mutation was detected in B/NK/Myeloid and/or NK/Myeloid clones from 2 IMF and 3 PV patients, demonstrating the occurrence of the mitotic recombination in a lymphoid/myeloid progenitor cell. Using the FTOC assay, the mutation was also detected in all T cell fractions derived from CD34+ cells from IMF and PV patients. **Conclusions.** These results demonstrate that the mutation and the subsequent mitotic recombination leading to a homzygous subclone occur in a lymphoid/myeloid progenitor cell in JAK2 V617F positive MPD. Thus, the phenotype of these MPD arising from a true lymphoid/myeloid progenitor cell may be related to a downstream selective proliferative advantage of the myeloid lineages.

0496

INDOLENT MYELOFIBROSIS WITH MYELOID METAPLASIA: SPECIFIC CLINICAL FEATURES AND PROGNOSIS


IRCCS Polinicino San Matteo, PAVIA, Italy; 2. DPT.Bioinformatics, University of Pavia, PAVIA, Italy; 3. Italian Registry of Myelofibrosis with Myeloid Metaplasia, PAVIA, Italy

**Background.** Patients with myelofibrosis with myeloid metaplasia (MMM) have a heterogeneous prognosis, only partially predicted by published scores and parameters. **Aims.** To identify MMM patients with good or poor prognosis, we corroborated a previously published score confirmed from intensive chemotherapy regimens. **Methods.** A prospective national cohort of 871 consecutive patients with MMM was considered. Cluster analysis (EM-algorithm) allowed to classify patients based on their clinical parameters at diagnosis: age, hemoglobin, leukocyte and platelet count, spleen size. Kaplan-Meier survival analysis was applied to the clusters. **Results.** Five clusters were identified: all showed a significantly different clinical phenotype and survival (p<0.0001). Twenty-nine percent (n=250) of the patients were assigned to the cluster with the highest survival, e.g. indolent MMM. Their median age was 61 years and 83% were females. Twenty-eight percent of the patients with indolent MMM were absolutely asymptomatic, 19% reported a previous essential thrombocythemia and a few polycythaemia vera. All the patients with indolent MMM showed at least one of the following features at diagnosis: hemoglobin values >11 g/dl, platelet count >350 Taqman/10^11, spleen size <4 cm from costal arc, CD34 count <100/mcl. No patient showed circulating blasts and 90% of the patients showed <2% circulating erythroblasts and <10% circulating immature myeloid cells. A specific rule for selecting patients with indolent MMM includes the presence of a limited splenomegaly (<6 cm from costal arc) associated with: a high platelet count (>600x10^11/L) and/or a normal hemoglobin value and/or an age lower than 68 years. A lower frequency of homozygote JAK2 V617F mutation was detected in patients with indolent MMM as compared with the rest of the patients (4% vs 31%; p=0.01), irrespectively of previous polycythaemia vera. A few patients died of causes directly related to MMM and five-year survival of patients with indolent MMM was 78%. Five-year survival of patients with indolent MMM was higher than overall patients with a low-risk disease, according to the Lille score (p=0.009). Survival did not depend on sex or comorbidity, but depended on age at diagnosis (p=0.0001), five-year survival being >95% in patients aged <40 years, but <75% in patients aged >70 years. Absolute excess mortality, as compared with age-adjusted general population, was 7%, but among patients aged >70 years, absolute excess mortality increased up to 30%. Among patients aged <70 years, females incurred a twice a high excess mortality than males. Percent circulating immature cells was the only clinical parameter that independently predicted survival (p=0.03). Survival was not significantly different in patients followed by a hematology unit as compared with patients followed by a internal medicine unit. **Conclusions.** The MS5 layer analysis on a high number of MMM patients disclosed a population with a very good prognosis. Patients with indolent MMM should not be assigned to front-line intensive therapies.

0497

COEXPRESSION OF JAK2 V617F AND TYPE I CYTOKINE RECEPTORS IS NOT SUFFICIENT FOR CYTOKINE-INDEPENDENT CELL GROWTH

H. Quentinmeier, C. Fischer, J. Reinhardt, M. Zabolinski, H.G. Drexler

DSMZ, BRAUNSCHWEIG, Germany

**Background.** An activating point mutation in the JH2 domain of Janus kinase 2 (JAK2) was recently described in chronic myeloproliferative disorders (MPD). The majority of patients with polycythemia vera, and substantial numbers of patients with essential thrombocythemia and idiopathic myelofibrosis carry the JAK2 V617F mutation. **Aims.** We set out to find cell lines with the JAK2 V617F mutation, which may be used as suitable tools to analyze basic aspects of the cell biology of these tumors. It has recently been reported that coexpression of type I cytokine receptors with JAK2 V617F proteins leads to cytokine-independence in BA/F3 cells. Our aim was to confirm or refute this correlation in JAK2 V617F positive cell lines. **Methods.** Cell lines were tested for the JAK2 V617F mutation applying the PCR-based ARMS assay, confirmed by sequencing, and restriction analysis applying the JAK2 wild-type specific restriction enzyme BsaXI.
Expression and phosphorylation status of JAK2 proteins was checked by immunoprecipitation and Western blot analysis. Cytokine-dependency and influence of JAK kinase inhibitors on cell growth was assayed monitoring 3H-thymidine uptake. Apoptotic cells were detected and quantitated with the annexin-V / propidium iodide method. Results: Five / 79 acute myeloid leukemia-derived cell lines tested expressed the JAK2 V617F mutation. While several clones expressed both mutant (mu) and wild-type (wt) JAK2, the remaining positive cell lines carried homo-hemizygous JAK2 mutations. Microsatellite analysis confirmed losses of heterozygosity (LOH) affecting the JAK2 region on chromosome 9p in the homozygously JAK2mu cell lines HEL, MB-02, MUTZ-6 and UKE-1. Confirming the importance of the mutated JAK2 protein for growth and prevention of apoptosis, JAK2mu cell lines displayed higher sensitivities to JAK2 inhibition than JAK2wt cell lines. It has recently been reported that JAK2 V617F proteins require coexpression of type I cytokine receptors to secure cytokine-independent activation of the JAK2 and STAT5 pathways and to cause cytokine-independent growth of BA/FS cells. However, 2/5 JAK2mu human AML cell lines described by us were cytokine-dependent, growing after stimulation of type I cytokine receptors: cell line MB-02 responded to erythropoetin and cell line MUTZ-8 responded to G-CSF. Therefore, coexpression of JAK2 V617F and type I cytokine receptors alone is not sufficient for cytokine-independent cell growth. Immuno-precipitation and Western blot analysis showed that HEL, SK-1 and MUTZ-6 cell lines (HEL, SK-1, SKN-1, HEL, and HEL:SKN-1), but no JAK2wt cell lines (SKM-1, SKN-1), exhibited constitutive phosphorylation of JAK2. Also the cytokine-dependent JAK2mu cell line MUTZ-8 showed constitutive JAK2 phosphorylation. However, short-term stimulation with G-CSF induced phosphorylation of JAK2 in cytokine-starved JAK2mu cells, demonstrating that the JAK2 V617F protein was still responsive to G-CSF stimulation. Summary/Conclusions. In summary, our results show (i) that coexpression of JAK2 V617F and type I cytokine receptors is not sufficient for cytokine-independent cell growth and (ii) that JAK2 V617F protein regulates the cytokine receptors.

0498

ASSSESSMENT OF MYELOID CLONALITY IN FEMALE PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS BY JAK2V617F MUTATION AND HUMARA ASSAY


1Hospital del Mar, BARCELONA, Spain; 2University Hospital of Cologne, COLOGNE, Germany

Background. Essential thrombocythemia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) are chronic myeloproliferative disorders (MPDs) arising from clonal hematopoietic stem cells. The detection of JAK2V617F mutation and myeloid clonality by analysis of the human androgen receptor gene (HUMARA), are useful tools to demonstrate clonality in these MPDs. Aim. The aim of this study was to compare JAK2V617F mutational status with myeloid clonality determined by HUMARA assay in a series of female patients with Ph-negative MPDs. Methods. DNA was extracted from peripheral blood samples of 65 female ET patients, 37 PV patients and 7 AMM patients. JAK2V617F mutation was identified in 1/6 (16.7%) AMM patients.

0499

ABERRANT GENE EXPRESSION PROFILE OF CD34+ CELLS IN IDIOPATHIC MYELOFIBROSIS IDENTIFIES A SUBSET OF DISEASE-ASSOCIATED GENES WITH CLINICAL CORRELATIONS

P. Guglielmelli, 1R. Zini, 1C. Bogani, 1S. Salati, 1A. Pancrazzzi, 1E. Bianchi, 1F. Mannelli, 1S. Ferrani, 1M.C. Le Bousse-Kerdiles, 1A.R. Migliaccio, 1A. Bosi, 1G. Baron, 1R. Manfredini, 1A.M. Vannucchi 2

1AZ. Ospedaliero Universitaria Careggi, FLORENCE, Italy; 2Department of Biomedical Sciences, MODENA, Italy; 3Azienda Ospedaliera Universitaria Careggi, FLORENCE, Italy; 4INSERM U 661, Institut Andrey Lwoff, VILLEJUIF, CEDEX, France; 5Dept Hematol. Oncol.Ist.Superiore Sani, ROME, Italy; 6IRCCS Policlinico S. Matteo, PAVIA, Italy

Background. Idiopathic myelofibrosis (IM) is a chronic myeloproliferative disorder (MPD) characterized by bone marrow fibrosis, myeloid metaplasia usually accompanied by leukoerythroblastic blood smear, variable degree of pancytopenia or leucocytosis, splenomegaly, and increased number of CD34+ cells in the peripheral blood (PB). A part for the recently described mutation in JAK2 exon 12 in about half of patients, as well as in other MPD, no recurrent chromosomal abnormality nor molecular defect specific for IM has been described to date. Aims. As an approach to identify possibly aberrantly regulated genes in IM, we performed a comprehensive transcriptome comparative microarray analysis of normal and IM CD34+ cells. Methods. For this purpose, we prepared three pools of >98% pure CD34+ cells from the PB of IM subjects, and two pools from the BM of normal donors, each comprising five subjects. The cDNA was hybridized to an Affymetrix HG-U133A oligonucleotide microarray chip representing 22,283 transcripts. Results. Two hundred eighteen differentially expressed genes were identified; among these, 50 genes that we considered as potentially involved in the pathophysiology of IM were further validated by quantitative RT-PCR. By using class prediction analysis, a set of eight gene markers (CD9, GAS2, DLK1, CDH1, WTI, NFE2, HMG2 and CXC4) was employed to successfully recognize normal from IM CD34+ cells. These genes were aberrantly regulated also in the granulocytes of IM, polycythemia vera (PV) and essential thrombocythemia (ET) patients, with some unique patterns; class prediction analysis differentiated IM from normal granulocytes in 100% of cases, while a correct class attribution was obtained in 95% of IM, PV, or ET patients. Altered gene expression was corroborated by the detection of abnormally high CD9 and CD164, and low CXC4, protein content in CD34+ cells, that characterized IM patients when compared to either normal subjects, PV or ET patients. We speculate that the significant down-regulation of CXC4 on IM CD34+ cells might suppress CD34 cell function and phagocytic activity in vivo.

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Antibodies in the treatment of non-Hodgkin’s lymphoma

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HUMAX-CD20, A NOVEL FULLY HUMAN ANTI-CD20 MONOCLONAL ANTIBODY: RESULTS OF A PHASE I/II TRIAL IN RELAPSED OR REFRACTORY FOLLICULAR NON-HODGKINS LYMPHOMA


Academic Medical Center, AMSTERDAM, Netherlands; Veje Sygehus, VEJLE, Denmark; Southhampton General Hospital, SOUTHHAMPTON, United Kingdom; KAS Herlev, HERLEV, Denmark; Klinika Noworowu Uklada Chlonnego, WARSAWA, Poland; Klinika Hematologii Akademii Medycznej, GDANSK, Poland; Holden Comprehensive Cancer Center, IOWA CITY, USA; Klinika Hematologii, Instytut, LODZ, Poland; Oddzial Chemiot., Bialostocki Osrzodek, BIALYSTOK, Poland; Universitatskliniken des Saarlandes, HOMBURG / SAAR, Germany; Universitatsklinikum Schleswig-Holstein, KIEL, Germany; University Hospital of Cologne, COLOGNE, Germany; Erasmus Medical Center, Dijkzigt Hospital, ROTTERDAM, Netherlands; Genmab AS, COPENHAGEN, Denmark; Christie Hospital, MANCHESTER, United Kingdom

Background. The fully human monoclonal IgG1 antibody HuMax-CD20 targets a novel epitope of the CD20 molecule on B-cells. HuMax-CD20 stops growth of engrafted B-cell tumors in SCID mice more efficiently and exerts stronger complement activation than Rituximab. HuMax-CD20 kills Rituximab-resistant cells expressing low levels of CD20. Aims. The objective of the present trial is to establish the safety, efficacy and the pharmacokinetics of HuMax-CD20 in patients with relapsed or refractory follicular lymphoma grade 1-2. Methods. Data are presented from a recently completed, open label, dose-escalation, multicenter clinical trial in patients with relapsed or refractory CD20+ follicular non-Hodgkin’s lymphoma. Four cohorts of 10 patients were treated with 4 weekly i.v. infusions of 300, 500, 700 or 1000 mg. The endpoints include adverse events, centrally reviewed CT verified tumor response according to the Cheson criteria, B-cell depletion, pharmacokinetics and progression free survival. Results. Forty patients have been treated. Mean age was 57 years; median number of prior treatment regimens was 2; 15 patients were previously treated with Rituximab. Rapid, efficient and sustained peripheral B-cell depletion was observed in all dose groups. No dose limiting toxicity has been reported. Only 8 short lasting episodes of grade 3 CT C were observed. Hematological toxicity was low and confined to 6 events of grade 1 neutropenia; no cases of thrombocytopenia were reported. The following pharmacokinetic parameters were derived (medians per dose group): Cmax 129, 185, 380 and 610 μg/mL, T½ 447, 245, 322 and 621 hr, CI 9, 19, 10 and 7 mL/hr/kg and AUC 75000, 51000, 185000 and 326000 hr μg/mL, for the 300, 500, 700 and 1000 mg dose groups, respectively. No correlation between pharmacokinetics and response was found. Objective responses (CR, CRu, PR) have been evaluated in 37 patients and were obtained in all 4 dose groups; 4 CR + 1 CRu/8 (300 mg), 1 CR + 2 PR/9 (500 mg), 2PR/10 (700 mg) and 1 CRu + 4 PR/10 (1000 mg). Objective responses were achieved in 9 of 14 (64%) evaluable patients previously treated with Rituximab, i.e. 3CR, 1 CRu and 5 PR. In total 18 patients showed stable disease; progression was observed in only 3 patients. Based on Kaplan Meier estimates, the median time to progression for all patients was 267 days (95% CI 135-372 days). The median time to progression for responders and the median duration of response have not yet been reached. Conclusions. This final analysis demonstrates a favourable safety profile and encouraging efficacy of HuMax-CD20 in patients with follicular NHL. Objective responses were achieved in all dose groups with response rates up to 63%, including a 64% response rate in patients previously treated with Rituximab. The median time to progression for responders has not yet been reached.
Background. Peripheral (mature) T-cell lymphomas (PTCL) represent a group of lymphoma entities with an unfavorable outcome after treatment with CHOP or CHOP-like regimens. Aims. The purpose of the study was to investigate the feasibility of a combination of the monoclonal antibody alemtuzumab with chemotherapy consisting of fludarabine, cyclophosphamide and doxorubicin. Methods. Patients were treated with alemtuzumab 3, 10, 30, 30 mg, days 1-4, fludarabine 25 mg/m² days 2-4, cyclophosphamide 600 mg/m² day 3, and doxorubicin 50 mg/m² day 4. Included were patients with primary diagnosis, with first relapse, or with primary refractory disease, excluded were patients with primary cutaneous T-cell lymphomas and ALK-positive large cell anaplastic T-cell lymphomas. Results. So far, 58 patients have been included, 26% of patients responded to chemotherapy and 12 patients were PTCL-NOS, 9 with AILD, two with ALK negative ALCI, one with enteropathy-associated T-cell lymphoma, one with NK-cell lymphoma, and one with T-PLL. 15 patients were enrolled with primary diagnosis and 11 patients in relapse. The median age was 58 years (range 21-77); 71% of the patients had an international prognostic index intermediate high or high. In patients with primary diagnosis the CR rate was 67% (10/15), three patients were primary progressive, and two patients dropped out because of treatment associated complications. 9 of the responding patients are in ongoing CR at 2+, 5+, 6+, 13+, 14+, 17+, 20+, 26+, and 28+ months, respectively. The patient with T-PLL relapsed after being in CR for 25 months. In the group of relapsed or refractory patients two CR and two PR (36% overall response) were observed. The main toxicity was leukopenia (64% grade III and IV of all evaluable treatment cycles), other grade III and IV toxicities included anemia (17%), thrombocytopenia (5%), infections (14%), pruritus/skin reactions (9%), nausea/emesis (6%), mucositis, and cardiac toxicity (5%), two patients with relapse disease after pre-treatment with CHOP-like regimens developed severe heart failure). 11 (42%) patients reactivated CMV, however, 9 without developing CMV-related disease. Conclusions. The combination is an effective first-line regimen for peripheral T-cell lymphoma, however, regarding the general outcome a longer follow-up period of a larger population is required. Here we report final results from the Phase II part of the study. Methods. Patients received galiximab (500 mg/m² qwk x 4) with a standard course of rituximab (375 mg/m² qwk x 4). Rituximab refractory or response for a response with TTP 6 months was excluded. International Workshop Response Criteria were used to evaluate response. Results. Sixty-four patients received treatment. The median follow-up is 20.4 months. Median age at study entry was 59 yrs. Eighty-eight percent of patients were Stage III/IV, with FLIPI low (27%), intermediate (39%), or high (34%) risk groups. All patients had received at least 1 prior lymphoma therapy; 42% were rituximab naïve. Galiximab infusions were delivered over 1 hr and were well tolerated. No DLTs were reported. Sixty one (95%) patients experienced study related AEs; the most common were lymphopenia (44%), leucopenia (36%), fatigue (38%), neutropenia (23%), and chills (25%). An ORR of 64% was observed: 17% CR, 16% CRu, and 31% PR. The median PFS was 12.1 months. Combination therapy did not appear to alter pharmacokinetics. The mean serum half life was 25.7 days for galiximab and 24 days for rituximab. These results were retrospectively compared with 3 historical studies of follicular NHL patients treated with a standard course of rituximab monotherapy. Baseline characteristics were similar; however, there was a higher incidence of rituximab-naïve patients in the rituximab monotherapy group (77%) compared with galiximab + rituximab (42%). The toxicity profile of the combination regimen was similar to that observed in the single agent rituximab studies. However, the median PFS was longer in the galiximab + rituximab group (12.1 months) than in the rituximab monotherapy group (9.4 months). In a subset analysis of rituximab-naïve patients, the difference in PFS was even more pronounced: 15.4 months in the galiximab + rituximab group vs. 9.4 months in the rituximab monotherapy group. Conclusions. These results suggest that galiximab can be safely combined with a standard course of rituximab, produce promising response rates, and may potentially extend PFS in patients with relapsed or refractory, follicular NHL. A Phase III, randomized, double-blind study is planned.
Cancer genetics and cytogenetics in myeloid diseases

GENE EXPRESSION PROFILING FOR MOLECULAR SUBCLASSIFICATION OF LEUKEMIA: INTERIM ANALYSIS OF THE INTERNATIONAL MULTI-CENTER STUDY (MILE) ON 1437 PATIENTS

T. Haferlach,1 K. Mills,2 W.K. Hofmann,3 T. Kronrie,4 J. Hernandez Rivas,5 J. Downing,4 J. De Vos,5 C. Preudhomme,1 M.C. Béné,5 E. Macintyre,4 T. Kipps6,7 L. Wieczorek,1 A.E. Yeoh,1 R. Foà,1 A. Kohlmann1

1MIL, Munich Leukemia Laboratory, MUNICH, Germany;2ROCHE Molecular Systems, PLEASANTON, USA;3Department of Haematology, CARDIFF, United Kingdom;4Charité, BERLIN, Germany;5Oncohematology, PADOVA, Italy;6Hematologica, SALAMANCA, Spain;7St.Jude Children’s Research Hospital, MEMPHIS, USA;8Institut de Recherche en Biotherapie, MONTPELLIER, France;9Service Hematolagie. Hosp. Miguel Servet, ZARAGOZA, Spain;10Hematology, LILLE, France;11Hematologie, PARIS, France;12University of California, SAN DIEGO, USA;13Roche Molecular Systems, PLEASANTON, USA;14Department of Paediatrics, SINGAPORE, Singapore;15Università La Sapienza, ROME, Italy

Background. Microarray analysis can identify differentially expressed genes associated with distinct clinical and therapeutically relevant classes of both pediatric and adult leukemias. Aims. The MILE (Microarray Innovations in Leukemia) study has started in 11 centers in Europe. The MILE compares the accuracy of gene expression profiles of 16 acute and chronic leukemia subclasses, MDS, and non-leukemia as control group to current routine diagnostic workup. Methods. In a pre-phase each center was trained on the identical microarray protocol and used the same laboratory equipment and reagents for target preparation and analysis on Affymetrix HG-U133 Plus 2.0 microarrays. Two cell lines (MCF-7, HEPG2) were tested. In parallel, each center prepared total RNA from cell lysates of three leukemia patients (AML with t(8;21), CML, and CLL) and processed them with replicates. After successfully passing proficiency testing, the centers started to analyze prospectively 2000 leukemia samples as next step in MILE. Results. The pre-phase demonstrated a very high intra- and inter-laboratory comparability among the participating centers. In detail, unsupervised analyses of a total of n=175 leukemia analyses accordingly grouped each sample type in unique clusters. Remarkably, the replicates of the leukemia samples demonstrated squared correlation coefficients of gene expression ranging between 0.950 and 0.997 (median=0.990) for the CML, between 0.940 and 0.999 (median=0.980) for the CLL, and between 0.940 and 0.999 (median=0.980) for the AML with t(8;21) sample. Here we present for the first time classification results of a first series of n=1437 tested patients from the 11 centers that were included in a training data set to form linear classifiers for all 18x(18 - 1) / 2 = 153 class pairs. The average cross-validation accuracy of this training data set is 89.4%. This classifier was further tested on two independent patient cohorts. In a first independent cohort (HG-U133 Plus 2.0, n=105) 89.5% classification accuracy were achieved. In a second independent cohort (n=1,094), analyzed previously in two centers on HG-U133A/B microarrays, 83.5% classification accuracy were achieved. In detail, 136 out of 159 (97.8%) chronic leukemia samples (CML or CLL) are classified fully in agreement with standard diagnostic procedures. For acute leukemia subtypes 767/904 (84.8%) are classified correctly. In the MDS group (n=81) miscalls occur both in the distinction between MDS and AML with normal karyotype or cytogenetically so-called other aberrations as well as between MDS and non-leukemia. Interestingly, an AML-like signature can be found in MDS samples correlating with IPSS >1.5. These samples are currently further investigated regarding a potential later progression into full-blown AML. Conclusions. This international multi-center study demonstrates a very high inter- and intralaboratory reproducibility of microarray analyses. Moreover, a first series of 1437 leukemia patients was successfully analyzed and classified with high accuracy. Data will be used to design a new custom format microarray dedicated to further develop the application of gene expression profiling for diagnosis and subclassification of leukemia.
TRISOMY 8 IS ASSOCIATED WITH A HIGHER EXPRESSION OF A SUBSET OF GENES LOCATED ON CHROMOSOME 8 DETERMINED BY THE ACCOMPANYING GENETIC ABNORMALITIES


Background. Trisomy 8 is the most frequently observed trisomy in AML occurring as a sole karyotypic abnormality or in addition to other chromosome aberrations. Aims. It was the aim of this study to analyze the impact of trisomy 8 on the expression of genes located on chromosome 8 in different AML subgroups. Methods. Gene expression analyses were performed in a total of 567 AML cases. The following 14 subgroups were analyzed: +8 sole (n=19), +8 within a complex aberrant karyotype (n=11), +8 with t(15;17) (n=7), +8 and inv(16) (n=6), +8 with t(8;21) (n=3), +8 and 11q23/MLL (n=8), and +8 with other abnormalities (n=10). These were compared to 200 AML with normal karyotype and the following subgroups without trisomy 8: complex aberrant karyotype (n=73), t(15;17) (n=86), inv(16) (n=46), t(8;21) (n=57), 11q23/MLL (n=57), and other abnormalities (n=77). Results. A significant higher mean expression of genes located on chromosome 8 was observed in subgroups with +8 in comparison to their respective control groups. A varying number of significantly higher expressed genes was identified in all comparisons. No gene was significantly overexpressed in all comparisons. No distinct gene expression pattern was identified allowing the identification of cases with trisomy 8. Therefore, the gain of chromosome 8 may represent a higher expression of genes located on chromosome 8. However, no consistent pattern of genes was identified which shows a higher expression in all AML subtypes with trisomy 8. Summary / Conclusions. This data suggests that trisomy 8 rather provides a platform to relate cytogenetic characteristics to mutation of classification of t-AML, but with or without abnormalities of chromosome 7. In this pathway 77% presented p53 mutations often combined with deletions of 17p, complex karyotypes and complicated chromosome rearrangements. Sometimes amplification of 11q23 or 21q22 was observed. Most patients in pathway I and II had received alkylating agents. Pathway III included patients with t-AML and translocations involving 11q23, RAS or AML1, frequently with mutation of the last two genes in combination. Pathway III and IV included patients with balanced chromosome aberrations involving 21q22 or 16q22. Except for cases with t(3;21) these patients presented as t-AML. Patients with involvement of 21q22 often had additional 7q-> and occasionally c-KIT / FLT3 mutations. Pathway V included patients with t-AML and translocations to 17q12. They sometimes presented FLT3-ITD. Pathway VI included cases of t-MDS and 7q/-7 but normal chromosomes 5 and 7. Pathway VII included patients with normal karyotype, 11q22 and rearrangement of the NUP98 gene. Most patients in pathway III-VI had previously received toposomerase II inhibitors. Pathway VII included patients with a normal karyotype often presenting as t-AML and 50% had mutations of FMS-like tyrosine kinase-3 (FLT3), RAS or AML1. Frequently with mutation of the last two genes in combination. Mutation of JAK2 was observed in only two atypical cases of MDS in this pathway. Pathway VIII included patients with atypical chromosome arrangements and only 2/20 in this pathway presented RAS mutations. A significant association was observed between mutations of genes in the JAK/STAT-RAS-BRAF signalling pathway (n=58) and mutation or rearrangement of genes for putative transcription factors (n=49), so called class I and II mutations, as 18 patients, 15 belonging to pathways III-V and VII, presented both types of mutation (p=0.012, Fishers exact test, two sided). Conclusions. Classification of t-MDS/t-AML in different genetic pathways is supported and an association between class I and II mutations is confirmed.

NEW STRATEGY TO IDENTIFY TUMOR SUPPRESSOR GENES IN ACUTE MYELOID LEUKEMIA

R. Beekman, M. Valkhof, S.J. Erkelder, I.P. Touw

Erasmus MC, ROTTERDAM, Netherlands

Background. Retroviral integration mutagenesis in mice is a powerful tool to discover novel genes involved in the development of leukemia. Using the murine leukemia virus (MLV) (G4-MuLV) we identified candidate disease genes of acute myeloid leukemia (AML). Erkeland et al. (J Virol. 2004: 78; 1971-80). Recently, we reported that genes adjacent to the virus integration site (VIS), so-called VIS genes, contribute significantly to gene expression profiles of distinct subgroups of human AML, supporting the importance of deregulation of VIS genes in the pathogen-
methylation, which could form the initiating event for silencing of AML. (Erkeland et al., Cancer Res. 2006: 66; 622-6). Because MuLV preferentially, albeit not exclusively, integrate in the 5' promoter region of genes, it is generally assumed that expression of VIS genes is most frequently increased due to the transcription enhancing activities of the viral LTR. However, CpG islands within the LTR are potential target for de novo methylation, which could form the initiating event for silencing due to methylation spreading. This would imply that retroviral mutagenesis screens could also be used for identification of potential tumor suppressor or haplo-insufficient genes. Aims. To determine whether and to what extent proviral LTR sequences are methylated in Gr-1.4 MuLV-induced myeloid leukemia; (2) To investigate whether methylated LTR sequences can be used to identify (new) potential tumor suppressor genes; (3) To establish the significance of these genes for human AML. Methods. A methylation sensitive quantitative PCR (Q-PCR) was developed to determine the ratio between methylated and unmethylated LTR in the different tumor samples (n=81). Samples that showed high levels of methylated LTR were used to identify methylated VIS flanking genes. To this end, we developed a novel method in which methylated DNA immunoprecipitation (MeDIP) with anti-5-methylcytidine (a5-mC) antibody is combined with inverse PCR (iPCR). Results. The methylation sensitive Q-PCR showed a marked heterogeneity of LTR methylation status among different tumor samples. Distinct methylation categories were defined: high (n=7), medium-high (n=15), medium (n=12), low (n=20) and none (n=27). Enrichment of LTRs after MeDIP with a5-mC was found in 25/34 samples. As expected, MeDIP on normal hematopoietic tissues was negative for LTR, but positive for the methylation imprinted gene H19. MeDIP/iPCR resulted in 1 to 7 bands per tumor sample. These gene products include known suppressor genes such as Smad1 and Mad1-like, as well as a number of genes with as yet poorly characterized roles in cancer. Summary/conclusions. We present a new strategy to identify tumor suppressor genes in AML. The marked variability in DNA methylation of VIS in different tumor samples indicates that most viral integrations occur in non-methylated parts of the DNA, otherwise the DNA methylation of VIS in different tumor samples would be more equal. The potential tumor suppressor genes in these regions, which may be silenced through methylation spreading, will be identified by direct PCR strategies in combination with bisulfite treatment. To test the relevance of these genes for clinical disease, their expression will be analyzed in a large cohort of AML patients, of which gene expression profiles are already available.

0510

ORAL CONTRACEPTIVE USE, THROMBOPHILIA AND THEIR INTERACTION FOR ISCHEMIC STROKE IN YOUNG WOMEN

T. Battaglioni, I. Martinelli, I. Burgo, S. Di Domenico, P.M. Mannucci
IRCCS Maggiore Hospital, MILAN, Italy

Background. Oral contraceptive use is a risk factor for ischemic stroke in young women. While hyperhomocysteinemia increases the risk of the disease, the role of inherited thrombophilia is still uncertain. Little data exists on the interaction between such risk factors and the risk of ischemic stroke in young women. Aims. To assess the interaction between thrombophilia and oral contraceptive intake in determining the risk of ischemic stroke in young women. Methods. One-hundred and five women with a first ischemic stroke at an age less than 45 years and 293 healthy controls were investigated for the presence of thrombophilia due to factor V Leiden, prothrombin G20210A mutation, antithrombin, protein C and protein S deficiency, and hyperhomocysteinemia. The presence of oral contraceptive use was recorded. Fifty-five women had a stroke of undetermined origin. Results. Oral contraceptives were associated with an increased risk of ischemic stroke (odds ratio 2.3, 95%CI 1.4-3.8) in the first 6-18 months of use. The risk of ischemic stroke was also higher in patients with hyperhomocysteinemia (odds ratio 3.5, 95%CI 1.9-6.4), in those with factor V Leiden (odds ratio 2.6, 95%CI 0.8-8.0), but not in those with prothrombin G20210A (odds ratio 0.9, 95%CI 0.1-11.2). After stratification for the presence of oral contraceptive use and thrombophilia due to factor V Leiden or hyperhomocysteinemia, the odds ratio for ischemic stroke in women with both risk factors was 12.9 (95%CI 1.3-133.7) for factor V Leiden and 9.2 (95%CI 2.5-33.5) for hyperhomocysteinemia. No increased risk was observed when oral contraceptive use and prothrombin G20210A were present together. The risk associated with oral contraceptive use, hyperhomocysteinemia, or both, was more pronounced for stroke of undetermined than determined etiology. Conclusions. The use of oral contraceptives is associated with a 2-fold increased risk of ischemic stroke in high risk patients, who suffered from objectively confirmed recurrent VTE. This risk quintuples in the presence of hyperhomocysteinemia or factor V Leiden. These findings should be taken into account for thrombophilia screening after an ischemic stroke and for individual decision making when prescribing oral contraceptives.

0511

VENOUS THROMBOEMBOLISM A METABOLIC DISEASE?

C. Ay, T. Tengler, R. Simanek, R. Vormittag, T. Vukovich, I. Fabinger
AKH Wien, Internal Medicine I, VIENNA, Austria ; AKH Wien, VIENNA, Austria ; Institute of Medical and Laboratory Diag, VIENNA, Austria

Background. Deep venous thrombosis (DVT) and pulmonary embolism (PE) are amongst the most common disorders in developed countries. Only in about 50% of patients with a history of unprovoked venous thromboembolism (VTE) a genetic or acquired risk factor can be specified. Experimental, epidemiological and clinical studies indicate an association between serum lipids and VTE. Hyperlipidemia and overweight may play a role in the development of VTE by influencing the homeostasis of the clotting and fibrinolytic system and could thereby induce a hypercoagulable state. Aims. The aim of our present study was to elucidate a possible association of lipids and overweight with VTE in high risk patients, who suffered from objectively confirmed recurrent VTE. Methods. We conducted a case-control study to analyse the relationship between serum lipids and the risk of VTE. Outpatients with a history of objectively confirmed recurrent VTE, who had at least one event of an unprovoked DVT or PE, were recruited from 01/2005 to 11/2005. Age and sex-matched healthy individuals served as controls. Venous blood samples were obtained after overnight fasting for serum lipid determinations (total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides). Height (m) and weight (kg) were recorded and body mass index (BMI) was calculated (kg/m²). Hyperlipidemia was diagnosed, when serum cholesterol level was over 200 mg/dL, triglyceride level over 172 mg/dL or cholesterol/HDL-quotient > 4. A BMI above 24.99 kg/m² characterized overweight. Mann-Whitney-U test was carried out to compare the groups. Univariate logistic regression analyses were applied to calculate odds ratios and the 95% confidence interval. Results. Hundred-sixteen patients (58 female / 63 male)
male; mean age 56 ±12 yrs) with a history of recurrent VTE and 129 age and sex-matched controls (66 female / 63 male; mean age 53 ±11 yrs) were enrolled. Patients showed a significantly higher BMI than controls (median (Md) 27.45 kg/m² vs 25.78 kg/m², p=0.032). Total cholesterol (Md = 233 mg/dL vs. 230 mg/dL, p=0.22) and LDL (Md = 147 mg/dL vs. 141 mg/dL, p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.035) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). Estimated odds ratios for VTE were 1.86 (95% CI, 1.08-3.19; p=0.024) for overweight, 1.32 for hypercholesterolemia (95% CI, 0.72-2.40; p=0.57), 1.85 (95% CI, 1.64-2.28; p=0.067) for hypertriglyceridemia and 2.12 for cholesterol/HDL-quotients > 4 (95% CI, 1.25-3.60; p=0.006). Summary/Conclusion. Overweight, hypertriglyceridemia and a cholesterol/HDL-quotient > 4 increase the risk for VTE by almost doubling it. Therefore, besides the well-known genetic factors, we suspect a possible relationship between environmental factors such as nutrition or physical activity and development of VTE.

**0512**

**THE PERSISTENCE OF RESIDUAL VEIN THROMBOSIS, AFTER AN EPISODE OF DEEP VEIN THROMBOSIS, AND THE RISK OF NEW OVERT CANCER AND CARDIOVASCULAR DISEASE**

S. Siragusa, A. Malato, R. Anastasio, M. Sciacca, V. Cigna, I. Abbene, L. Lo Coco, C. Arcara, F. Fulfaro, A. Casuccio

**University of Palermo, PALERMO, Italy**

**Background.** We have recently demonstrated that the presence of Residual Vein Thrombosis (RVT), UltraSonography (US)-detected at the 3rd month after an episode of Deep Vein Thrombosis (DVT) of the lower limbs, is an independent risk factor for developing recurrent Venous Thromboembolism (VTE). The management of DVT patients by detection of RVT may, therefore, represent a simple and reproducible method for establishing the individual risk of recurrence and for tailoring the optimal duration of Oral Anticoagulants (OA) (Siragusa S et al. Blood 2003;102(11):OC183a).

At the present, it is unknown whether RVT may also identify patients at increased risk for cancer and/or cardiovascular disease (CD). Objective of the study. In patients with DVT of the lower limbs, we conducted a prospective study for evaluating the correlation between RVT and the risk of new overt cancer and/or CD. Materials and Methods. Consecutive patients, with an episode of idiopathic or provoked DVT, were evaluated after 3 months from the index DVT. The presence/absence of RVT was detected and patients managed consequently; in those with RVT, OA was continued for 1 year while in those without, RVT, OA was discontinued. The incidence of VTE recurrence, overt cancer and new CD was evaluated over a period of almost 3 years after the index DVT. Survival curves (Kaplan-Mayer) and related Breslow test have been used for statistics. Results. Three-hundred forty-five patients were included in the analysis. The results are listed in the figures. The incidence of recurrent VTE and new overt cancer was statistically lower in patients without RVT than in those with RVT; no significant differences were found in the incidence of new CD. These data are applicable in patients with idiopathic or provoked DVT. Patients with RVT, the advantage of prolonging anticoagulation for 12 months is lost at the end of the treatment. Conclusions. This is the first study evaluating the relationship between US-detected RVT and the risk of developing cancer and CD; RVT presence, at 3rd month from the index DVT, is an independent risk factor for recurrent VTE and indicates patients at risk for new overt cancer. This risk remains over a period of almost 3 years, independently whether index DVT was idiopathic or provoked.

In these patients, the advantage of indefinite anticoagulation should be assessed in properly designed study.

**0513**

**REGULATION OF PROTEIN S EXPRESSION BY SEX HORMONES**

O.H. Hughes, J.S. Staton, M.W. Watson, V.C. Cole, M.S. Sayer, R.B. Baker

*University of Western Australia, PERTH, Australia; Royal Perth Hospital, PERTH, Australia*

**Background.** The anticoagulant Protein S (PS) is coded for by the PROS1 gene and serves as a co-factor to APC inactivation of FVα and FVIIIa. Previous studies have shown a reduction in circulating PS levels in response to increasing oestrogen (E2) levels resulting in an increased thrombotic risk. This relationship is evident in women who are pregnant or are using oral contraceptives (OCs). To date, a mechanism to describe this relationship at the molecular level has not been elucidated. We have identified a potential oestrogen response element (ERE) spanning nucleotides -850 to -867 within the promoter region of PROS1. We have further demonstrated that the presence of excess PRB, but not PRA.

**Results.** Reflecting clinical observations the expression of the PROS1 promoter fragment decreased in response to E2 and was further reduced in the presence of excess ERα. Interestingly, the opposite was seen in response to P4. Up-regulation via P4 was further increased in the presence of excess PRB, but not PRA. Summary/Conclusions. These results show that PROS1 promoter expression is reduced in the presence of E2. Down-regulation is enhanced by ERα, suggesting that the effect is mediated via an ERα-dependent mechanism. However, the promoter region is also responsive to P4 which up-regulates expression in what appears to be a mechanism involving PRB. The opposing effects seemingly counterbalance each other. Based on these results the attention given to the oestrogen component of OCs may not be as important as the progestin component. It is the progestin that varies between different OC preparations and not the oestrogen which is predominantly ethinyl oestradiol. Thus, the increasing evidence that 3rd generation OCs represent a greater thrombotic risk when compared to 2nd generation formulations, could be more about the types of progestins used in the 3rd index DVT. Survival curves (Kaplan-Mayer) and related Breslow test have been used for statistics. Results. Three-hundred forty-five patients were included in the analysis. The results are listed in the figures. The incidence of recurrent VTE and new overt cancer was statistically lower in patients without RVT than in those with RVT; no significant differences were found in the incidence of new CD. These data are applicable in patients with idiopathic or provoked DVT. Patients with RVT, the advantage of prolonging anticoagulation for 12 months is lost at the end of the treatment. Conclusions. This is the first study evaluating the relationship between US-detected RVT and the risk of developing cancer and CD; RVT presence, at 3rd month from the index DVT, is an independent risk factor for recurrent VTE and indicates patients at risk for new overt cancer. This risk remains over a period of almost 3 years, independently whether index DVT was idiopathic or provoked.
R. Liesner, Congress of the European Hematology Association

We treated 28 patients; 26 were treated for acute refractory and 2 for chronic relapsing TTP. 22 cases were female and 6 male. 75% had a history of neurological symptoms and 11% had cardiac TTP. Rituximab therapy consisted of 2-8 weekly doses of 375mg/m². All acute patients attained normal laboratory parameters and clinical remission within a median of 11 (range 0-33) days as defined by the number of PEX post the 1st Rituximab treatment. 26 patients had normal ADAMTS 13 activities (<5% normal range (NR) 66-126%) by collagen binding assay and 23 patients had normal ADAMTS 13 activity (median 78%) following Rituximab. IgG antibodies to ADAMTS 13 pre Rituximab were present in 15 patients (53%) and 23 patients had normal ADAMTS 13 activity (median 78%) following Rituximab. IgG antibodies to ADAMTS 13 pre Rituximab were detected in 27 patients and were undetectable in all patients post Rituximab. The median number of PEX pre Rituximab was 13 (2-35) and following the first Rituximab treatment, 11 (0-33), including weaning with alternate day regime. There was a significant reduction in the number of PEX's following the first dose of Rituximab (p=0.03 students paired t-test). To date, 6 patients required more than 4 Rituximab treatments as determined by ADAMTS 13 activity and IgG antibody to ADAMTS 13. Follow-up, 1-34 months, there have been 1 relapse in a patient lost to follow up. She was successfully re-treated with Rituximab. A further patient, treated with Rituximab 24 months previously, had a decrease in ADAMTS 13 activity to 10%. Elective retreatment with 4 weekly Rituximab resulted in normal ADAMTS 13 levels. Normalisation of CD 19 levels, between 6-15 months has not been associated with relapse. Conclusion. The data suggests patients with TTP respond promptly to Rituximab, with a significant reduction in the requirement for PEX. Our results suggest patients with TTP respond promptly to Rituximab and require significantly fewer PEX. In addition, Rituximab causes a reduction in IgG antibodies to ADAMTS 13 and appear to be strongly associated with disease remission. Rituximab therefore appears to be a safe, effective, targeted therapy for TTP.

H. Cohen, S. Benjamin

Associated complications. Activity and IgG antibodies to ADAMTS 13, to avoid previous TTP associated complications.

Background. Thrombotic thrombocytopenic purpura (TTP) is a life threatening disorder associated with ADAMTS 13 deficiency. Plasma exchange (PEX) remains the primary treatment modality in acute TTP, with various immunosuppressive agents (e.g. methylprednisolone (MP), vincristine, cyclosporine (CSA)) and other disease modifying agents (e.g. defibrotide), added in refractory and chronic relapsing cases. Mortality remains at 15-20%. Methods. We treated 28 patients; 26 were treated for acute refractory and 2 for chronic relapsing TTP. An additional case was a 4 year old presenting with acute acquired TTP. She was successfully treated with BPL 8Y and Rituximab. Of the 2 cases treated electively, Rituximab therapy was dictated by ADAMTS 13 activity and IgG antibodies to ADAMTS 13, to avoid previous TTP associated complications. Results. 22 cases were female and 6 male. 75% had a history of neurological symptoms and 11% had cardiac TTP. Rituximab therapy consisted of 2-8 weekly doses of 375mg/m². All acute patients attained normal laboratory parameters and clinical remission within a median of 11 (range 0-33) days as defined by the number of PEX post the 1st Rituximab treatment. 26 patients had normal ADAMTS 13 activities (<5% normal range (NR) 66-126%) by collagen binding assay and 23 patients had normal ADAMTS 13 activity (median 78%) following Rituximab. IgG antibodies to ADAMTS 13 pre Rituximab were present in 15 patients (53%) and 23 patients had normal ADAMTS 13 activity (median 78%) following Rituximab. IgG antibodies to ADAMTS 13 pre Rituximab were detected in 27 patients and were undetectable in all patients post Rituximab. The median number of PEX pre Rituximab was 13 (2-35) and following the first Rituximab treatment, 11 (0-33), including weaning with alternate day regime. There was a significant reduction in the number of PEX's following the first dose of Rituximab (p=0.03 students paired t-test). To date, 6 patients required more than 4 Rituximab treatments as determined by ADAMTS 13 activity and IgG antibody to ADAMTS 13. Follow-up, 1-34 months, there have been 1 relapse in a patient lost to follow up. She was successfully re-treated with Rituximab. A further patient, treated with Rituximab 24 months previously, had a decrease in ADAMTS 13 activity to 10%. Elective retreatment with 4 weekly Rituximab resulted in normal ADAMTS 13 levels. Normalisation of CD 19 levels, between 6-15 months has not been associated with relapse. Conclusion. The data suggests patients with TTP respond promptly to Rituximab, with a significant reduction in the requirement for PEX. Our results suggest patients with TTP respond promptly to Rituximab and require significantly fewer PEX. In addition, Rituximab causes a reduction in IgG antibodies to ADAMTS 13 and appear to be strongly associated with disease remission. Rituximab therefore appears to be a safe, effective, targeted therapy for TTP.
those likely to have IAA but who had isolated clinical positive findings which could also be present in FA [n=16, group-3]. Patients with a known or obvious FA diagnosis were not included in the study. Chromosome breakage test and FANC D2 immunoblot were performed in PBL in all patients [n=65]. Also, skin primary fibroblasts were analysed [n=40] to detect potential haematopoietic FA reversion. Because chromosome breakage tests are barely efficient in fibroblasts, we performed FANC D2 immunoblot and developed a new flow cytometry test based on MMC-sensitivity in fibroblasts to identify FA/BRC A downstream groups. Results. In total, 4 patients with FA were identified. The only positive clinical findings for those patients were: Patient-1, group-3 (precocious menopause, vocal cord neoplasia at age 38yo), Patient-2, group-3 (BMF at age 10yo following a period of isolated thrombocytopenia, 1 café-au-lait spot/ hypoplasias), Patient-3, group-2 (low birth weight/short stature, ‘pécule’ facies, onset of pancytopenia only at age 25yo) and Patient-4, group-1 (10yo, no positive clinical findings; did resemble IAA). The two patients from groups 1 and 2 were diagnosed with chromosomal breakage test, and further classified with FANC D2 immunoblot in PBL. For the two patients from group-3, based on persistent clinical suspicion of FA after negative breakage test in PBL, somatic mosaicism with complete haematopoietic reversion was diagnosed using FANC D2 immunoblot fibroblast analysis. Importantly, FA diagnosis was definitely excluded in all other patients. Conclusions. In situ ations where the suspicion of FA persists after a negative breakage test in PBL (e.g. congenital physical abnormalities and possible mosaicism), then diagnostic tools should be performed on fibroblasts. As a rule, we found that underdiagnosing FA is very rare if careful history and physical exam are done together with standard chromosome breakage tests in PBL. Because no cases of FA were found in our cohort of patients with IAA presentation and negative breakage test, we suggest that screening can be limited to this technique. The strategy here presented allowed us to identify a few unexpected FA cases in a cohort of BMF patients, and importantly, to definitely rule out FA in others.

0517
THE EFFECT OF COMBINED THERAPY WITH DEFEROXAMINE AND DEFERIPRONE ON MYOCARDIAL IRON AND ENDOTHelial FUNCTION IN THALASSEMAIA MAJOR: A RANDOMIZED CONTROLLED TRIAL USING CARDIOVASCULAR MAGNETIC RESONANCE
M.A. Tanner,¹ R. Galanello,² C. Dessi,² G.C. Smith,¹ M.A. Westwood,¹ A. Agus,¹ R. Assomull,¹ S.V. Nair,¹ J.M. Walker,¹ D.J. Pennell²
¹Royal Brompton Hospital, LONDON, United Kingdom; ²Ospedale Regionale per le Microcitemie, CAGLIARI, Italy; ³University College Hospital, LONDON, United Kingdom

Background. In β-thalassemia major (TM) cardiac failure secondary to myocardial iron loading remains the leading cause of death. Approximately two-thirds of patients maintained on deferoxamine continue to exhibit myocardial iron loading. The oral iron chelator, deferiprone has been demonstrated to remove myocardial iron and it has been proposed that in combination with deferoxamine it may have an additive effect. Myocardial iron can be rapidly and reproducibly quantified using cardiovascular magnetic resonance (CMR) T2* (a measure of interstitial myocardial iron) and endothelial function (as quantified by flow mediated dilatation of the brachial artery (FMD)) can be impaired in TM which may further contribute to cardiovascular pathology. FMD is also reliably measured by CMR. CMR is therefore well suited to assess the efficacy of new therapies for the treatment of iron overload in TM. Aims. To report the changes in myocardial iron loading (changes in T2*) and endothelial function (as assessed by FMD) from a randomized placebo controlled trial comparing the combined therapy of deferiprone and deferoxamine with the standard therapy of deferoxamine alone. Methods. A mobile CMR scanner (1.5T, Siemens Sonata) was transported to Cagliari, Italy. The myocardial T2* was assessed in 167 patients with TM. 65 patients (male 27, female 38, age 29±4.8years) with mild-moderate myocardial iron loading (T2* 8-20ms) were randomized to receive either deferoxamine and placebo, or deferoxamine and deferiprone. Myocardial and hepatic T2* were assessed at baseline, 6 and 12 months. Endothelial function was assessed at 0 and 12 months. Results. Analysis of covariance showed a significant difference between the two groups, with the combined group showing superior effects in reducing both myocardial iron (p<0.017) and hepatic iron (p<0.001). See figure 1. Over the 12 months endothelial function improved significantly in the combined treatment group (from 10.5% to 12.8%, p<0.001) but not in the placebo group (9.9% to 13.4%, p=0.10). Conclusion. In patients with mild-moderate cardiac iron loading the combined therapy of deferiprone and deferoxamine is superior to deferoxamine alone in the removal of myocardial iron and improving endothelial function.

Figure 1. Both heart and liver iron loading significantly improve with combined chelation therapy (continuous line). There is no significant improvement in the placebo group (dashed line).

0518
PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA: LONG-TERM EPIDEMIOLOGICAL STUDY
P. Peffault de Latour,¹ J.Y. Mary,¹ C. Salanoubat,¹ L. Terriou,¹ G. Etienne,¹ M. Mohty,² R. Roth,² S. De Guibert,¹ J.Y. Cahn,² G. Socié²
¹Hopital Saint Louis, PARIS, France; ²Inserm U897, PARIS, France; ³Hotel Dieu, PARIS, France; ⁴Services des Maladies du Sang, LILLE, France; ⁵Service de Médecine Interne, PARIS, France; ⁶Institut Pauli Calmettes, MARSEILLE, France; ⁷Service d’Hematologie Clinique, RENNES, France; ⁸Service d’Onco-Hematologie, GRENOBLE, France

Background. Paroxysmal nocturnal haemoglobinuria (PNH) is a rare acquired disorder of haematopoietic stem cells. Although knowledge about the pathophysiology of the disease is increasing, few studies have been published on the long-term follow up mainly because of the rarity of the disease. Aims. Analyzing a large cohort of patients with PNH on the long term to better determine prognostic factors. Assessing the role, if any, of the introduction of flow cytometry for diagnosis in the presentation and following of the disease. Methods. We have already reported such an analysis on 220 patients in 1996 (Socié et al., Lancet). Data were updated and collected on an additional 258 patients with PNH. Haematological centres were contacted by the way of the French Society of Haematology and/or the French Association of Young Haematologist. The date of diagnosis was based on blood cytometric analysis if there was no prior positive Ham’s test. Data validation is still in progress. Results. Provisionary results are the following. We report here the natural history of PNH among 478 patients (258 female, 220 male). 58 French haematological centers participated in the study. Patients were diagnosed over a 55-year period (1950-2005). The age at diagnosis was 34 (inter-quartile range: 24-47). The median follow up (+ standard deviation) is 5.6 years (+0.4). 50 patients underwent allogeneic bone marrow transplantation. During the evolution, 113 patients presented a thrombosis, 9 a myelodysplasia, and 6 an acute leukemia, respectively. Ninety-six patients died. The Kaplan-Meier survival (+ standard deviation) was 85% at 5 years (+2), 76% at 10 years (+3), and 66% at 15 years (+3). The analysis of prognostic factors are on going at time of abstract submission. Conclusion. This is the largest cohort of patients with PNH reported until now. Definitive results after complete validation of the data base will be presented at the meeting.
0519
HIGH PREVALENCE OF PULMONARY HYPERTENSION AND HEMOLYSIS ASSOCIATED WITH NITRIC OXIDE DEPLETION IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

A. Hill,1 X. Wang,2 R.J. Sapsford,1 G.M. McGawley,1 D.L. Oxborough,1 S.J. Richards,1 H. Kroon,1 R.P. Rother,1 M.T. Gladwin,1 F. Hillmen1
1Leeds General Infirmary, LEEDS, United Kingdom; 2National Institutes of Health, BETHESDA, USA; 3Alexion Pharmaceuticals, Inc., CHESHIRE, USA;

Background. Pulmonary hypertension (PHT) is an emerging common complication of hereditary hemolytic anemias. It has been mechanistically and epidemiologically linked to intravascular hemolysis and decreased nitric oxide (NO) bioavailability. The release of excessive red cell hemoglobin during intravascular hemolysis can exceed the capacity of the hemoglobin scavenging molecule, haptoglobin, leading to the consumption of endogenous NO. While this complication has been described in approximately 30% of adult patients with sickle cell disease and thalassemia, the prevalence of PHT in patients with paroxysmal nocturnal hemoglobinuria (PNH), an acquired disease with the highest levels of intravascular hemolysis observed, has never been determined. PNH patients frequently have symptoms consistent with both hemolysis and PHT including severe fatigue and dyspnea on exertion. Aims. We, therefore, examined for the presence of PHT in PNH and explored potential mechanisms associated with its development by measuring the ability of plasma to instantaneously consume NO. Methods. Doppler echocardiography was performed in 28 hemolytic PNH patients to estimate pulmonary artery pressures. Transmitral flow, Doppler determinations of the severity of valvular regurgitation, and left ventricular stroke volume were assessed and graded. Systolic PHT was prospectively defined by a tricuspid regurgitant jet velocity (TRV) of at least 2.5 m/s at rest. Nitric oxide consumption was assessed using ozone-based chemiluminescence, and red cell hemolysis was determined by plasma levels of lactate dehydrogenase (LDH). Blood was collected using methodologies to limit artefactual hemolysis. Results. Tricuspid regurgitation was observed in 20 out of 28 patients with PNH. Fourteen of these 20 evaluable patients (70%) demonstrated elevated pulmonary artery systolic pressures. Twelve (60%) had mild to moderate PHT (mean TRV 2.6 m/s±0.1) while two (10%) had moderate to severe pressures (mean TRV 3.7 m/s±0.2). Plasma from PNH patients (n=32) consumed 54.6±8.3 micromolar NO while normal subjects (n=9) consumed 2.2±0.6 micromolar NO (p<0.0001). LDH levels correlated with NO consumption (r=0.6342, p=0.0002). Eculizumab is a humanized monoclonal antibody that binds to C5 inhibiting terminal complement activation. In a separate cohort of 7 patients treated with eculizumab for a median of 3 years to reduce hemolysis, the ability to consume NO appeared lower in lower (13.2±4.8 micromolar NO). Conclusions. 1) PHT has a much higher prevalence in PNH than in other hemolytic disorders. 2) Patients with PNH demonstrate high levels of NO consumption that are highly correlated to intravascular hemolysis (LDH) in these patients. 3) Eculizumab therapy is associated with reduced levels of NO consumption. Additional studies are required to determine the contributions of intravascular hemolysis and reduced NO bioavailability to the pathogenesis of pulmonary hypertension in PNH and the possible role of PHT in the morbidity and mortality characteristic of the disease.

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0520
ONTGENY, FUNCTION AND PERIPHERAL HOMEOSTASIS OF Regulatory T CELLS IN THE ABSENCE OF INTERLEUKIN-7

R. Peffault de Latour,1 H. Dujardin,1 P. Hillmen1
1Hopital Saint Louis, PARIS, France; 3Institut Pasteur, PARIS, France; 3Centre Jean Perrin, CLERMONT FERRAND, France; 4National Institute for Medical Research, LONDON, United Kingdom

Background. Mice lacking interleukin 7 (IL-7/-/- mice) are lymphopenic, but show no signs of autoimmunity, contrary to what is observed in other lymphopenic models. Aims. We investigated whether the absence of disease was due to the fact that IL-7 is dispensable for the ontogeny, function and homeostasis of regulatory Foxp3-expressing CD4+ T cells. Methods. Frequencies of CD4+CD25+ T cells in the thymus and spleen of IL-7/-/- mice were assessed by Flow Cytometric analysis. We studied the expression of Foxp3 and eculizumab (anti-CD25) which regulates intravascular hemolysis and reduces NO bioavailability to the pathogenesis of pulmonary hypertension in PNH than in other hemolytic disorders. We, therefore, examined for the presence of PHT in PNH and explored potential mechanisms associated with its development by measuring the ability of plasma to instantaneously consume NO. Methods. Doppler echocardiography was performed in 28 hemolytic PNH patients to estimate pulmonary artery pressures. Transmitral flow, Doppler determinations of the severity of valvular regurgitation, and left ventricular stroke volume were assessed and graded. Systolic PHT was prospectively defined by a tricuspid regurgitant jet velocity (TRV) of at least 2.5 m/s at rest. Nitric oxide consumption was assessed using ozone-based chemiluminescence, and red cell hemolysis was determined by plasma levels of lactate dehydrogenase (LDH). Blood was collected using methodologies to limit artefactual hemolysis. Results. Tricuspid regurgitation was observed in 20 out of 28 patients with PNH. Fourteen of these 20 evaluable patients (70%) demonstrated elevated pulmonary artery systolic pressures. Twelve (60%) had mild to moderate PHT (mean TRV 2.6 m/s±0.1) while two (10%) had moderate to severe pressures (mean TRV 3.7 m/s±0.2). Plasma from PNH patients (n=32) consumed 54.6±8.3 micromolar NO while normal subjects (n=9) consumed 2.2±0.6 micromolar NO (p<0.0001). LDH levels correlated with NO consumption (r=0.6342, p=0.0002). Eculizumab is a humanized monoclonal antibody that binds to C5 inhibiting terminal complement activation. In a separate cohort of 7 patients treated with eculizumab for a median of 3 years to reduce hemolysis, the ability to consume NO appeared lower in lower (13.2±4.8 micromolar NO). Conclusions. 1) PHT has a much higher prevalence in PNH than in other hemolytic disorders. 2) Patients with PNH demonstrate high levels of NO consumption that are highly correlated to intravascular hemolysis (LDH) in these patients. 3) Eculizumab therapy is associated with reduced levels of NO consumption. Additional studies are required to determine the contributions of intravascular hemolysis and reduced NO bioavailability to the pathogenesis of pulmonary hypertension in PNH and the possible role of PHT in the morbidity and mortality characteristic of the disease.

Cell signaling, transcriptional control and apoptosis • I

0521
LYSOSOMAL ROUTING OF G-CSF RECEPTORS DEPENDS ON A SINGLE MEMBRANE-PROXIMAL lysine RESIDUE, IS CONTROLLED BY Socs3 AND PLAYS A CRITICAL ROLE IN G-CSF-INDUCED GRANULOPOIESIS

M. Irandoust, J. Gits, O. Roovers, M. Valkhof, I. Touw, L. Aarts
ErasmusMC, ROTTERDAM, Netherlands

Background. The G-CSF receptor (G-CSF-R) tightly controls proliferation, survival and differentiation of myeloid progenitor cells. Mutations truncating the C-terminus of G-CSF-R are found in severe congenital neutropenia (SCN) patients at risk to develop AML. Myeloid progenitor cells expressing truncated G-CSF-R hyperproliferate in response to G-CSF and show defective differentiation. These truncated G-CSF-R have lost their recruitment site for SOCS3 and are hampered in receptor internalization. Aims. To study the role of receptor internalization and postendocytic routing in the control of G-CSF signaling and to determine the underlying mechanisms of these processes. Methods. We studied internalization rate, post-endocytic trafficking, signal transduction and proliferation/differentiation characteristics of various mutant G-CSF-R. Results. Routing was monitored by confocal microscopy following double labeling of internalized anti-G-CSF-R antibodies and endosomal marker proteins. TNFα (early endosomes), Hrs (presynaptic endosomes), Rab7 (late endosomes/lysosomes) and Lamp1 (lysosomes). Single amino acid mutants of G-CSF-R were generated to study involvement of individual lysines and the role of SOCS3 in receptor routing. Signaling activity was assayed in primary cell cultures and SKD2 cells and in STAT5 reporter and band shift assays. Results. We show that internalized G-CSF-R follow a classical endosomal-lysosomal degradation route. A lysine-null (K5R) G-CSF-R was retained in an Hrs-positive prelysosomal compartment and not targeted for degradation, indicating that lysosomal routing
Erythroid progenitors can be expanded cooperatively with Smad proteins, which interfere with TGF-β signaling. A. Biondi, Congress of the European Hematology Association

We identified genes, upregulated transcriptional activity by sequestering Smads in cells and marked represses the response of hematopoietic cells. Using single K to R G-CSR mutation and add-back mutants, we subsequently established that lysosomal regulation, downregulation of STAT5 activity and induction of myeloid differentiation all depend on the integrity of a single, membrane-proximal lysine residue of G-CSR, i.e., K632. Only the add-back of K632 but not of any of the other lysines to the lysine-null G-CSR-R fully restored G-CSF stimulation as well as proliferation/differentiation signaling characteristics of G-CSR-R. The suppressor of cytokine signaling-3 (SOCS-3) is a prominent negative regulator of G-CSR-induced STAT5 activity and recruits E3-ubiquitin ligase activity via its SOCS-box. We found that SOCS-3-mediated inhibition of G-CSR-induced STAT5 activation strongly depended on the presence of the SOCS3 SOCS-box as well as on G-CSR-R K632. In addition, lysosomal routing of mutant G-CSR-R/Y729F, lacking the SOCS-3 binding site, was severely diminished.

Summary/conclusions. Receptor internalization and routing are important mechanisms by which proliferation signals from G-CSR-R are attenuated and neutrophil differentiation is induced. The presence of a single lysine residue (K632R) and the SOCS-3 recruitment site (Y729) in G-CSR-R are both critical for the control of lysosomal routing and attenuation of G-CSR-induced proliferation. We thus present evidence that SOCS-3 is a major regulator of G-CSR-R routing, most likely via recruitment of E3-ubiquitin ligase for G-CSR-R/K632, thereby playing a critical role in maintaining the proliferation/differentiation balance during G-CSR-activated neutrophil production.

0522

TRANSLATION OF IGBP1 mRNA CONTRIBUTES TO THE REGULATION OF EXPANSION AND DIFFERENTIATION OF ERYTHROID PROGENITORS

G. Grech, 1 M. Blázquez-Domingo, 2 H. Beug, 3 B. Löwenberg, 4 M. Von Lindern 3
1 Erasmus MC, ROTTERDAM, Netherlands; 2 Federation of Eur Microbiology Society, DELFT, Netherlands; 3 Inst. Molecular Pathology, VIENNA, Austria

Background. Erythroid progenitors can be expanded in vitro in the presence of erythropoietin (Epo) and stem cell factor (SCF), while they differentiate to enucleated erythrocytes in presence of Epo only. Previously we showed that SCF-induced delay of differentiation depends on the activation of phosphoinositide-3-kinase (PI3K). An important PI3K-dependent process in cell proliferation is regulation of mRNA at a site. PI3K controls the activity of mTOR (mammalian target of rapamycin), whose activation results in phosphorylation of eIF4E-binding protein (eIF4E-BP). Fully phosphorylated 4E-BP releases eIF4E (eukaryote Initiation Factor 4E), which can subsequently bind eIF4G, the scaffolding protein of the eIF4F cap-binding complex. In particular mRNAs with a structured RNA element in the 5'-untranslated region (mammalian translationally controlled gene) require PI3K activation for efficient translation. SCF but not Epo can induce full phosphorylation of 4E-BP and efficient formation of the eIF4F cap-binding complex. Overexpression of eIF4E inhibited erythroid differentiation, indicating that SCF-induced formation of the eIF4F complex contributes to progenitor expansion. A major step in mRNA translation controlled by eIF4F is polysome recruitment. Aims. Our objective is to identify genes that are translationally controlled upon SCF signalling and to investigate their contribution in the attenuation of erythroid differentiation. Methods. To identify genes whose expression is regulated by signaling-induced polysome recruitment, we compared total and polysome-bound mRNA from factor deprivation (oocytes) and SCF (oocytes plus SCF) restimulated progenitors on gene-expression micro-arrays. Profiling was performed with 4 biological replicates and candidate genes were selected using ANOVA. In subsequent cluster analysis we combined these data with (polysomal) expression profiles of differentiating erythroid cells. Real time PCR was used to investigate if polysome recruitment of candidate transcripts is dependent on PI3K activation and eIF4E expression. Retrospective transcription was used to constitutively express these genes in the erythroid cell model and cell number, cell size and hemoglobinisation was measured to assess the effect on erythroid differentiation. Western blot analysis was used to investigate the resistant constitutive active IGBP1 on the phosphorylation state of mTOR targets. Results. We identified genes, upregulated specifically at the level of mRNA polysome recruitment and downregulated during erythroid differentiation. We further characterised 13 genes whose polysome recruitment is dependent on the PI3K/mTOR pathway. Constitutive expression of these targets in erythroid progenitors revealed that IGBP1 (Immunoglobulin binding protein 1) was able to inhibit erythroid differentiation. We elucidated a mechanism by which the IGBP1/Pp2a complex prolongs the phosphorylation of mTOR targets, possibly the mechanism inhibiting erythroid progenitor differentiation.

Summary/Conclusions. We identified in this study a novel and unique set of genes that are minimally regulated at the level of transcription and are specifically upregulated in response to SCF signalling. We support the importance of translation control to regulate the balance between expansion and differentiation of erythroid progenitors, by showing that IGBP1, a translationally controlled gene, blocks erythroid differentiation and this can be explained by maintenance of mTOR target phosphorylation under differentiation conditions. This suggests that, like SCF signalling and eIF4E overexpression, IGBP1 enhance translation initiation efficiency.

0523

EXPRESSION OF THE TEL-AML1 LEUKEMIA-ASSOCIATED FUSION PROTEIN INHIBITS TGF-BETA SIGNALING

C. Palmi, 1 P. Cardus, 2 A. Novosel, 1 A. Biondi, 3 M. Greaves, 4 A. Ford 5
1 Institute of Cancer Research, LONDON, United Kingdom; 2 Centro Ricerca Tettamanzi, MONZA (MI), Italy

Background. The TEL-AML gene fusion is the most frequent chromosome translocation generated abnormality in childhood leukemia, ~10% of acute lymphoblastic leukemia (ALL). The translocation usually arises before birth probably as an initiating event, and its impact is to generate a clone of overt, clinically silent pre-leukemic B cell progenitors. ALL disease arises following a second, post-natal hit (genetic event occurring up to 14 years later, usually involving deletion of the normal TEL allele. The mechanism by which TEL-AML1 protein transforms and sustains early progenitor B cells is currently unknown. The chimeric gene encodes a protein in which TEL dimerization domains are fused N-terminally to all the DNA binding and activating domains of AML1 and there is experimental data to endorse the probability that the activity of the TEL domain converts AML1 to a transcriptional repressor, probably via the recruitment of co-repressor molecules to the transcriptional complex.

Aims. To investigate how TEL-AML1 expression provides the pre-leukemic clone with selective advantage. In particular, considering the critical role of TGF-β in the growth and differentiation of hematopoietic cells, to test whether TEL-AML1 interferes with TGF-β signalling.

Methods. We performed this study using a TEL-AML1 inducible expression system. We have established cell lines of murine Ba/F 3 pro-B cells that stably express a regulatory, truncated human progesterone receptor ligand-binding domain (pSwitch, Invitrogen) that undergoes a conformational change in the presence of the steroid agonist, mifepristone and conversion to an active form. The active binding domain is then able to bind and activate transcription from an otherwise silent TEL-AML1 gene that we have also stably introduced into these cells. Results. Ba/F 3 cells expressing TEL-AML1 grow more slowly than controls but are more resistant to the anti-proliferative effects of TGF-β (at ~30 ng/mL). Using transient transcription reporter assays with a construct that contains the mouse germine promoter Ig-α, positively regulated by TGF-β, we have shown that expression of TEL-AML1 markedly represses the response of the promoter to TGF-β. The transcriptional response to TGF-β is Smad 1/5/8 dependent and the Runx domain of AML1 is known to bind Smad 1/5/8. We confirmed that TEL-AML1 (which includes the Runx domain) associates with Smad 1/5/8 immunoprecipitation. Conclusions. On the basis of these observations, we propose that while AML1 cooperates with Smad proteins to induce transcription in response to TGF-β, the TEL-AML1 fusion encoded protein interferes negatively with the TGF-β signalling. We speculate that the fusion protein, in virtue of its association with Smads, may decrease the TGF-β transcriptional activity by sequestering Smads away from their co-operators and/or by recruiting co-repressor molecules to the transcriptional complex at the TEL-AML1 consensus DNA domains. Since TGF-β is a critical regulator of cell growth, cellular senescence, differentiation and apoptosis, the alteration of its signaling activity may play an important role in the establishment of the persistent pre-leukemic clones by TEL-AML1 and/or in their progression to overt leukemia.
CBL is a E3-ubiquitin ligase, that negatively regulates M. Michallet, F. Garban, J.H. Bourhis, M. Kuentz, J.L. Harousseau, J.Y. Cahn, MM less than 55 years were Patients M. Attal, Congress of the European Hematology Association Smoldering multiple myeloma (SMM) is a monoclonal absence of FL. Analysis of the three important downstream signaling rate in FLT3-WTCBL-70Z and FLT3-WTv-CBL expressing cells in the lead to aberrant FLT3 signalling in acute leukemias. could play a role in regulating FLT3 RTK activity and, if mutated, could downstream signaling pathways were investigated by western blotting. Results. Stable coexpression of FLT3-WT and CBL-70Z or v-CBL, but not FLT3-WT with CBL-WT or one construct alone, conferred IL-3 inde- pendent growth to Ba/F3 cells. Stimulation with FLT3 ligand (FL) lead to hyperproliferation of FLT3-WTCBL-70Z and FLT3-WTv-CBL expressing cells, but not of FLT3-WTCBL-CBL expressing cells. To determine whether the proliferative advantage of FLT3-WTCBL-70Z and FLT3-WTv-CBL cells is mediated by FLT3 we cultivated the cells in presence of selective FLT3 inhibitors, SU5614 and PKC412. Both inhibitors were able to abrogate the IL-3 independent and FL-stimulated growth of FLT3-WTCBL-70Z and FLT3-WTv-CBL cells. The FLT3-WT receptors were constitutively activated and showed a higher spontaneous dimerization rate in FLT3-WTCBL-70Z and FLT3-WTv-CBL expressing cells in the absence of FL. Analysis of the three important downstream signaling pathways of FLT3 (STAT5-, PI3K/AKT- and MAPK-pathway) could show activation of STAT5 and AKT in FLT3-WTCBL-70Z and FLT3-WTv-CBL expressing cells. Summary. The CBL deletion mutants, CBL- 70Z and v-CBL, but not CBL-WT, confer a transforming potential to hematopoietic cells expressing FLT3. The pro-proliferative phenotype of FLT3-WTCBL-70Z and FLT3-WTv-CBL cells is mediated by an increase in FLT3 tyrosine kinase activity compared to FLT3-WTCBL-CBL cells and can be inhibited by selective FLT3 inhibitors. Here, we provide a new mechanism of transformation mediated by FLT3: mutations of a regulatory protein, that is implicated in negative regulation of RTK kinase activity.

Multiple Myeloma - Clinical / Experimental


University Hospital, NANTES, France; University hospital, CRTEIL, France; University Hospital Hecker, PARIS, France; Institut Gustave Roussy, PARIS, France; Institut Pauol Calmettes, MARSEILLE, France; University hospital pit, PARIS, France

Background. The role of myeloablative allogeneic stem cell transplantation (alloSCT) in the treatment of multiple myeloma (MM) is still an area of controversy. Few series have described the results of such a therapy as part of first-line treatment in younger patients. Methods. From 1985 to 2003, 116 patients with de novo MM less than 55 years were treated by alloSCT within 12 months following diagnosis either in first CR (n = 13), first PR (n = 70), stable disease (n = 14) or primary refractory (n = 19), and registered in the SFGM-TG database. Results. Patients were 67% males, 49 females, median age 41 years (18-55). The donor was a geno-identical one in 105 cases, and pheno-identical in 11 cases. The conditioning regimen consisted of 12 Gy total body irradiation (TBI) + 120 mg/kg Cyclophosphamide in 50% of the cases, TBI + Cy + melphalan in 20% of the cases, and TBI + melphalan in 17% of the cases. I-cell depletion was used in only 8 cases, and GVHD prophylaxis consisted of the combination of cyclosporine A + short course methylxatrace in 85% of the cases. Grade 3-4 acute GVHD was documented in 18.7% and chronic GVHD in 30.5% of the cases, respectively. 100 days after alloSCT, the mortality rate was 28%, and 40% 1 year after alloSCT. The overall survival was 41% at 4 years, and 34% at 12 years, and disease-free survival was 26% at 12 years with a true plateau observed 5 years after alloSCT. Chemosensitive disease at the time of alloSCT and occurrence of chronic GVHD were associated with a better survival (12-year survival 38.2 vs 18.2% p=0.002, and 42 vs 20.1% p=0.002, respectively). Conclusion. With a prolonged follow-up, data from the SFGM-TG show that alloSCT when performed as part of first-line therapy, despite a high initial mortality, may cure one quarter of the patients with de novo MM less than 55 years.

0526 NEW CRITERIA TO IDENTIFY RISK OF PROGRESSION IN SMOLDERING MULTIPLE MYELOMA: MULTIPARAMETRIC FLOW CYTOMETRY ANALYSIS OF BONE MARROW PLASMA CELLS


Hospital Universitario de Salamanca, SALAMANCA, Spain; Hospital Clínico de Valladolid, VALLADOLID, Spain; Hospital Comarcal del Bierzo, LEON, Spain; Hospital Virgen del Puerto, Placentia, Spain; Hospital General Rio Carrin, PALENCIA, Spain; Complejo Universitario de León, LEON, Spain; Hospital General de Segovia, SEGOVIA, Spain; Hospital Virgen de la Concha, ZAMORA, Spain

Background. Smoldering multiple myeloma (SMM) is a monoclonal disorder defined by the presence of a serum monoclonal protein ≥ 3g/dL or bone marrow plasma cells ≥10% and absence of end-organ damage. These patients require close follow-up because the high risk of progression to symptomatic multiple myeloma. Therefore, the definition of new parameters that could be used for the identification of patients at the highest risk of progression could be of great importance in the clinical setting. Aims. To evaluate the impact of immunophenotypic analysis of bone marrow (BM) plasma cell (PC) for the prediction of risk of progression of SMM. Methods. From January 1996 to September 2004, bone marrow aspirate samples from 78 patients, who fulfil the criteria of SMM according to the International Myeloma Working Group criteria, were analysed by multiparametric flow cytometry. Plasma cells were immunophenotypically classified as normal or abnormal according to the expression of CD38, CD45, CD19 and CD56 antigens. Other parameters included were: percentage of plasma cell infiltration by morphology and cytometry, MC, immunoparesis, presence of Bence-Jones pro-
teinuria, haemoglobin, platelets, calcium, and albumin. The median age of the series was 69 years (range 45-88). The monoclonal paraprotein was IgG in 55 (65%), and IgA in 29 (34%), with a median paraprotein level of 2.5 mg/dL. The median follow-up was 50 months. Results. Thirty-six patients (42%) progressed to MM, with a median time to progression (TTP) of 26 months (range 2 to 94 months). Interestingly, flow cytometry showed that the number of aberrant PC (aPC) within the total PC (TPC) population in BM, clearly predict the risk of progression. Thus, in patients with ≥ 95% aPC from TPC the median TTP was 40 months vs not reached in the rest (p=0.0000). Other parameters with a significant influence on progression in the univariate analysis were: the paraprotein level (higher vs lower 3 mg/dL, p=0.0017), the presence of immunoparesis (no paresis vs. one or two Ig, p=0.0001), the presence of Bence-Jones proteinuria (p=0.0017), the total BM infiltration assessed both by morphology and flow cytometry (p=0.0032; and p=0.0001, respectively). Moreover, this cut off level of 95% aPC within TPC, also allow us to discriminate two risk categories upon considering only patients at low risk of progression, based on a low paraprotein level or absence of immunoparesis (p=0.0072 and p= 0.0056, respectively). By multivariate analysis this new parameter (95% aPC from TPC), together with immunoparesis (no vs one or two Ig), BM infiltration by cytometry, and the amount of MC, had independent significant impact on risk of progression. Conclusion. bone marrow immunophenotypical analysis of plasma cells, as multiparametric flow cytometry at diagnosis is useful for predicting progression of SMM into active MM.

0527 A MULTICENTER, RANDOMIZED TRIAL OF ZOLEDRONATE VS OBSERVATION IN EARLY-STAGE, ASYMPTOMATIC MYELOMA


CROB, RIONERO, Italy; IRCCS Casa Sollievo della Sofferenza, S. GIOVANNI RONDO (FC) Italy; La Sapienza University, ROME, Italy; S. Giovanni Addolorata Hospital, ROMA, Italy; S. Luigi Gonzaga Hospital, ORBASSANO (TO), Italy; University, TORIN, Italy; S. Eugenio Hospital, ROME, Italy; S. Giacomo Hospital, ROME, Italy; INT Fondazione Pascale, NAPLES, Italy; DIM, University, GENOVA, Italy

Background. The use of bisphosphonates in patients with asymptomatic, otherwise untreated early-stage myeloma is still debated and currently not recommended by evidence-based guidelines due to a substantial lack of appropriate studies. Zoledronate is a third generation bisphosphonate, which significantly decreases skeletal events in active myeloma and has been demonstrated to have a possible anti-myeloma in vitro and in vivo effect. Aims. The aim of this study was to evaluate whether the prophylactic use of zoledronate is able to reduce the rate of and the time to evolution in overt, symptomatic myeloma requiring chemotherapy in this population of patients. Methods. On June, 2001, ten Italian centers started a randomized clinical trial comparing administration of zoledronate vs simple observation in patients with monoclonal gammopathy fulfilling the diagnostic criteria of asymptomatic, stage Ia, IIA or smouldering myeloma, not requiring chemotherapy. Patients strictly diagnosed as having taut MGUS were excluded. One-hundred sixty patients were enrolled and randomized (1:1) to receive (n. 80) or not (n. 80) zoledronate (Zometa, Novartis Pharmaceuticals, Origgio, Italy) for one year, on an out-patient basis, at the dose of 4 mg as i.v. single monthly infusion. Results. No severe adverse events were recorded throughout the study, with the exception of a single patient treated with zoledronate who developed a reversible picture of osteonecrosis of the jaw. In the observational arm six patients were lost at follow-up after 6-20 months. Asymptomatic hypocalcemia, without need of interrupting the treatment and promptly corrected by substitution therapy, occurred in fifteen of zoledronate-treated patients. Fever developed in seven patients receiving zoledronate, one of whom stopped the treatment after 3 infusions. Overall, no significant reduction of M-component (> 25%) was observed. On intention-to-treat analysis, after a median follow-up of 42 months, there were 19 (25.7%) progressions requiring treatment in the zoledronate group and 24 (30%) within the controls (p=n.s.). Median time-to-progression was 19 and 16 months, respectively (p=n.s.). Among the 36 patients who required chemo-radiotherapy in both arms, bone lesions and/or hypercalcemia at the time of progression were observed in 16/20 (80%) of controls, and in 7/16 (43.7%) of zoledronate-treated patients (p=0.001). Conclusions. Our data indicate that the use of zoledronate in patients with early-stage, asymptomatic myeloma reduce the possibility of developing skeletal events at progression. Although a weak trend in favour of zoledronate arm was observed, in this study there was no statistical evidence about the possibility that zoledronate may also decrease the number of and/or the time to progression of the disease.

0528 ARRAY-CGH DETECTS FREQUENT RECURRENT IMBALANCES IN PLASMA CELL DISORDERS


University of Torino, TORINO, Italy; Evangelismos Hospital, ATHENS, Greece

Background and Aims. Genomic imbalances such as losses and gains occur frequently in hematological cancers. Their characterization in plasma cell disorders (PCD) is largely incomplete and few lesions (mostly identified by conventional cytogenetics and FISH) have been extensively studied for their pathogenetic and prognostic role. There is thus a clear need for a genome-wide screening of cytogenetically-cryptic lesions, through sensitive, robust and reproducible approaches. Array-CGH allows obtaining a comprehensive view of genomic imbalances, with a precise mapping of these aberrations to the genomic sequence. It has proven extremely successful in several diseases including chronic lymphocytic leukemia (CLL) which closely resemble multiple myeloma (MM) in terms of clinical and molecular heterogeneity. We have screened a panel of patients with PCD with the following Aims. 1) to identify the most common, yet undescribed genetic lesions; 2) to confirm these lesions by FISH; 3) to link genomic imbalances and clinical outcome. Methods. CD-138 purified plasma cells were employed. Genomic DNAs, from both the tumor and normal reference cells, labeled with different fluorescent dyes were cohybridized to 1 Mb resolution arrays (Spectral Genomics Inc. USA) containing 2600 Bacteria Artificial Chromosome (BAC) clones, according to manufacturers recommendations. Variations in DNA sequence copy number for each BAC clone were assessed by relative fluorescence signal intensities, providing a locus-by-locus measure of DNA copy-number changes. FISH experiments have been performed to confirm clonal abnormalities (gains/losses) identified by array-CGH. BAC clones were labeled for FISH experiments and interphase nuclei were made, according to standard cytogenetic protocols. Results. 20 patients were studied including 16 MM and 4 PCL. The median number of lesions/patient observed in our panel was 17 (range 4-135). The amount of the genome affected by chromosomal imbalances was highly variable among different patients (median 3.9% range: 0.14%-27%). This number is superior to that reported in CLL (Drandi, ASH 2005) and to a lesser extent to diffuse large B cell lymphoma (DLBCL) (Chaganti, Blood 2005). Of 2600 BACs 954 were always spared from genetic damage, 864 were targeted only in one patient, 401 in two patients, 296 in 3-5 patients and 105 were targeted in six patients or more. By restricting the analysis to the most common lesions we have identified five previously undescribed recurrent lesions occurring in at least 30% of patients, involving the following regions: 19p13.2, 14q12, 16q21.1 11q24, 9q23. We have confirmed the first two lesions by FISH, while for the others experiments are ongoing. Conclusions. 1) In PCD the genome undergoes a high degree of genetic disruption compared to other lymphoid tumors, particularly CLL; 2) a number of highly recurrent lesions have never been identified, and some have already been confirmed by FISH. All these lesions will require further investigation to identify candidate target genes and to verify if they might be prognostically relevant.
M. Zandecki,1 M. Brousseau,1 X. Leleu,1 J. Gerard,1 T. Gastinne,2 A. Godon,1 F. Genevieve,1 M. Dib,1 J. Andrieux,1
1CHU Angers, ANGERS, France; 2Hôpital Hurnez, LILLE, France; 3Hôtel Dieu, NANTES, France; 4Hôpital Jeanne de Flandre, LILLE, France

Background. Cytogenetic performed in multiple myeloma (MM) allow the definition of two pathways for malignant progression, one hyperdiploid and the other hypodiploid. Monoclonal gammopathy of undetermined significance (MGUS) is probably a preliminary step to MM for some patients at least. Cytogenetic status in MGUS is limited to interphase FISH techniques, due to the small amount of abnormal plasma cells and to their low proliferation rate. Aims. To define incidence of hyperdiploidy and hypodiploidy in MM and MGUS, and the distribution of monosomy 13 within each group. Methods: We ascertained DNA content of plasma cells (ploidy) using Feulgen staining and image analysis in 96 MGUS and 169 MM patients. Interphase FISH was performed using centromeric probes to look for trisomies 3, 7, 9, 11 and 15 in 42 MGUS and using rb-1 gene probe for monosomy 13 in 57 MGUS and 150 MM patients. Results. Hyperdiploidy and hypodiploidy were found in 54% and 11.5%, and in 59.5% and 25% of MGUS and MM patients respectively. Mean and median ploidies, for hyperdiploid as for hypodiploid patients, were similar in MGUS and MM. Interphase FISH confirmed the association between trisomies for several odd chromosomes and hyperdiploidy. Monosomy 13 was found in 24.6% in MGUS and in 45.3% in MM; incidence was similar in hyperdiploid MM and hypodiploid MM (38% and 31.9% respectively), whereas it was found in 11.1% of hyperdiploid MGUS contrasting with 76.3% found in hypodiploid MM. Only two patients, both hyperdiploid, evolved to MM after a mean follow-up of 77 months. Conclusions. Our results show that the number of hyperdiploid patients and the amount of chromosomes gained are similar in MGUS and in MM; monosomy 13 was found in equal numbers in both disorders, hypothesizing that events unrelated to monosomy 13 need to occur for evolution of MGUS to MM. In contrast, hypodiploidy is rare in MGUS, and is unrelated to monosomy 13, hypothesizing that hyperdiploid MM might occur either after a MGUS step with deletion 13 as a secondary event, or using another pathway without prior MGUS.
absence of MSCs) as in major MHC mismatched recipients (50% engraftment versus 17% in the absence of MSCs). Furthermore, the MSC-facilitated engraftment was still evident at 4 months after transplantation and the donor chimerism included both lymphoid (CD3+, B220+) and myeloid (GR-1+) lineages. In contrast, infusion of donor-derived MSCs was associated with a significantly increased rejection rate of allogeneic donor BM cells in both multiple minor antigen mismatched transplants (44% versus 0% in the presence of donor MSCs) and major MHC mismatched transplants (80% versus 22% in the presence of donor MSCs). Finally, the addition of third party MSCs derived from C3H mice did not affect the engraftment rate of MHC mismatched transplants. in vivo cytotoxicity data, employing differentially CFSE-labeled splenocytes, showed that the infusion of merely allogeneic donor or third party MSCs in naive mice was sufficient to induce a memory T cell response. Summary/Conclusions. Taken together, these results show that MSCs are capable of modulating immune responses in vivo and that these responses are affected by MHC antigen matching between donors and recipients. In addition, MSCs are not intrinsically immunoprivileged and are capable of inducing a memory T cell response following injection in vivo in immunocompetent hosts. Following cotransplantation in immunocompromised hosts that have received sublethal irradiation, allogeneic MSCs still induce an allogeneic response resulting in graft rejection. Although it is still unclear whether the immunogenicity of allogeneic MSCs is preserved after a full myeloablative conditioning regimen, the possibility that allogeneic or third party MSCs are immunogenic may be taken into account in designing clinical studies in the setting of allogeneic stem cell transplantation.

SUSTAINED RECONSTITUTION OF NADPH-OXIDASE ACTIVITY IN X-LINKED CHRONIC GRANULOMATOUS DISEASE FOLLOWING RETROVIRAL GENE THERAPY AND NONMYELOABLATIVE CONDITIONING

M. G. Ott, 1 M. Schmidt, 1 S. Stein, 1 U. Siler, 1 K. Schwarzwaelder, 1 U. Kochl, 1 K. Kuehlecke, 1 A. Schulz, 1 A. Trasher, 1 D. Hoezler, 1 C. von Kalle, 1 R. Seger, 2 M. Grez 3
1 University of Frankfurt, FRANKFURT, Germany; 1 National Center for Tumor Diseases (NCT), HEIDELBERG, Germany; 2 Georg-Speyer Haus, FRANKFURT, Germany; 3 University’s Children’s Hospital, ZURICH, Switzerland
2 Department of Pediatric Hematology, FRANKFURT, Germany; 3 Eufics, IDAR-OBERSTEIN, Germany; 4 Molecular Immunology Unit, LONDON, United Kingdom; 5 Department of Hematology, FRANKFURT, Germany

Gene transfer into hematopoietic stem cells has been successfully used to correct immunodeficiencies affecting the lymphoid compartment. However, similar results have not been reported for diseases affecting myeloid cells, mainly due to low engraftment levels of gene-modified cells observed in unconditioned patients. Here we report on two adult patients who received gene-transduced hematopoietic stem cell doses in combination with nonmyeloablative bone marrow conditioning for the treatment of X-linked Chronic Granulomatous Disease (X-CGD), a primary immunodeficiency caused by a defect in the oxidative antimicrobial activity of phagocytes. Therapeutically significant gene marking was detected in neutrophils of both patients leading to large numbers (up to 60%) of functionally corrected phagocytes 16 months after gene therapy. This high correction resulted from an unexpected but temporarily restricted expansion of gene transduced myeloid cells in vivo. Gene marking levels in B-cells has remained constant at a level of 20%, while gene marking in T-cells is below 5%. Gene marking in bone marrow was detected at levels between 30% and 40% one year after transplantation. Killing assays in vitro have demonstrated antibacterial and antifungal activity in gene transduced phagocytes and both patients recovered of Staph. aureus and A. fumigatus infections after gene therapy. Both patients have been free of severe bacterial and fungal infections since transplantation. Large-scale mapping of retroviral integration site distribution revealed that activating insertions in the zinc finger transcription factor homologs MDS1/EVI1, PRDM16, or in SETBP1 have expanded gene-corrected long term myelopoiesis 3- to 4-fold in both patients, providing direct evidence in humans that these genes influence regulation of normal long-term hematopoiesis. Although it is likely that insertion effects have reinforced the therapeutic success observed in this trial, our results suggest that gene therapy in combination with bone marrow conditioning is a therapeutic option for inherited diseases affecting the myeloid compartment and can be successfully used to treat CGD.
**0534**

Pten CONTROLS HEMATOPOIETIC STEM CELLS AND PREVENTS LEUKEMOGENESIS

G.M. Oser,1 A. Wilson,2 H. Wu,1 A. Trumpp1

1ISREC, EPALINGES, Switzerland; 2Ludwig Institute for Cancer Research, LAUSANNE, Switzerland; 1Howard Hughes Medical Institute, UCLA, LOS ANGELES, USA; 1ISREC, EPALINGES, Switzerland

Pten (phosphatase and tensin homologue deleted on chromosome ten) encodes a tumor suppressor gene involved in hereditary (Cowden disease) and sporadic human cancers, such as gliomas, endometrial and breast cancers. The Pten phosphatase inhibits the PI3-kinase pathway and plays a key role in apoptosis, cell growth, proliferation and migration. Deficiency of Pten in mice causes early embryonic lethality preventing the analysis of Pten function during development or in the mature organism. Recently it was shown that conditional deletion of the pten gene causes an increase in neuronal stem/progenitor cells due to decreased apoptosis and increased self-renewal (Groszer et al., 2001). Our aim is to study the role of Pten in other self-renewing tissues such as the hematopoietic stem cell compartment. We generated a mouse line in which the pten gene can be deleted in hematopoietic cells including hematopoietic stem cells (HSCs). This was achieved by crossing the conditional pten (ptenflox) allele with the IFN-α inducible MxCre transgene. Cre activity was induced in 4 week old MxCre:ptenflox/lox mice, converting the ptenflox alleles into pten (delta) null alleles in HSCs and other hematopoietic cell types present in the bone marrow. Pten mutant mice show severely enlarged spleens, due to a dramatic expansion of granulocytes, erythrocytes and megakaryocytes. In addition, mutant mice accumulate immature cancer-like cell types and develop transplantable leukemias within 4-6 weeks. Furthermore, normal numbers of HSCs are present in the bone marrow, however the total number of phenotypic HSCs is 6-fold increased in the spleen. Label retaining assays indicate that the quiescent HSC pool in the bone marrow is lost in Pten mutants suggesting that the Pi3-kinase pathway controls the transition of HSCs from a long-term quiescent to a self-renewing mode. In summary these results suggest that Pten activity restricts HSC self-renewal, and that it functions as a tumor suppressor in the hematopoietic system.

**0535**

SAFETY AND EFFICACY OF THE TERMINAL COMPLEMENT INHIBITOR ECULIZUMAB IN A PHASE III TRIAL IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

P. Hillmen,1 N.S. Young,1 J. Schubert,1 R.A. Brodsky,2 G. Socié3 P. Muus,1 A. Röth,1 J. Szer,1 M.O. Elebute,2 R. Nakamura,4 P. Browne,11 A.M. Ristano,1 S.A. Rollins1 C.F. Mojčík1 R.P. Rother1 L. Luzzatto1

1Leeds Teaching Hospitals NHS Trust, LEEDS, United Kingdom; 2National Institutes of Health, NHLBI, BETHESDA, MD, USA; 3Saatlard University Medical School, HOMBURG/Saar, Germany; 4Johns Hopkins University Medical Center, BALTIMORE, MD, USA; 5Hospital Saint Louis, PARIS, France; 6UMC St. Radboud, NIJMEGEN, Netherlands; 7University Hospital of Essen, ESSEN, Germany; 8Royal Melbourne Hospital, MELBOURNE, Australia; 9St George Hospital, LONDON, United Kingdom; 10City of Hope National Medical Center, DUARTE, CA, USA; 11St James’ Hospital, DUBLIN, Ireland; 12Mediche Federico II University, NAPLES, Italy; 13Alexion Pharmaceuticals, CHESHIRE, CT, USA; 14Nat Inst for Cancer Research (IST), GENOVA, Italy

Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired genetic disorder resulting in the clonal expansion of somatically mutated hematopoietic stem cells with a deficiency of glycosylphosphatidylinositol (GPI)-anchored proteins from the surface of all blood cells. Absence of the GPI-anchored terminal complement inhibitor CD59 from the surface of erythrocytes and platelets results in hemolysis and platelet activation, respectively. Excessive or persistent intravascular hemolysis can result in anemia, fatigue, thrombosis, pain, pulmonary hypertension, poor quality of life (Qol), and frequently a dependency on transfusions to maintain hemoglobin levels. Currently there are no approved or effective therapies that reduce intravascular hemolysis and improve the associated clinical morbidities in PNH. Aims. The pivotal phase III clinical study, TRIUMPH (Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria), evaluated the efficacy and safety of eculizumab, a humanized monoclonal antibody against C5 that inhibits terminal complement activation compared to placebo in a cohort of PNH patients. Methods. Patients were randomized to receive either placebo or eculizumab administered intravenously at 600 mg weekly for 4 weeks and then 900 mg every 2 weeks commencing the fifth week for a total of 6 months of therapy. The co-primary endpoints were stabilization of hemoglobin levels and reduction in transfused blood units. Prespecified measures of hemolysis and Qol were assessed. Results. Eighty-seven PNH patients (39 sites, 11 countries) were randomized 1:1 to receive either eculizumab or placebo. Eculizumab therapy was safe and well tolerated in the study. Both primary endpoints were met with statistical significance: 1) stabilization of hemoglobin levels was achieved by 48.8% of eculizumab-treated patients and by 0% of placebo-treated patients (p=0.000000014); 2) median transfused packed red blood cells (PRBCs) were 0 units in the eculizumab-treated group compared with 10 units in placebo (p=0.000000006). Fifty-one percent of eculizumab-treated patients were completely transfusion-independent through week 26 (study end), while every placebo-treated patient received at least one transfusion by week 14 (p=0.000000005). Eculizumab treatment dramatically reduced intravascular hemolysis, as evidenced by an 85.8% decrease in the lactate dehydrogenase area under the curve relative to placebo (p<0.0000000001). Concomitantly, eculizumab treatment resulted in an increase in the proportion of PNH type III RBCs from 28.1% at baseline to 56.9% by week 26 while the proportion in the placebo group remained constant (p=0.00005). Fatigue, as measured by the FACIT-Fatigue Qol instrument was significantly improved (p=0.00006), and significant improvements were also demonstrated in Qol subscales of the EORTC QLQ-30 instrument including fatigue (p=0.0000006), global health status (p=0.00002), physical functioning (p=0.0000005), emotional functioning (p=0.005), cognitive functioning (p=0.002), role functioning (p=0.0001), social functioning (p=0.005), pain (p=0.002), dyspnea (p=0.0008), appetite loss (p=0.00004) and insomnia (p=0.014). The 2 most common adverse events in the trial were headache and nasopharyngitis. Summary/Conclusions. Eculizumab stabilized hemoglobin levels, decreased the need for transfusions and provided clinically meaningful improvements in fatigue and other Qol parameters in patients with PNH through reduction of intravascular hemolysis. Long-term eculizumab treatment in PNH is effective and well tolerated.
**POSTER SESSION II**

**Transfusion medicine**

**0536**

**BLOOD-SHOT EYES: AUTOLOGOUS SERUM EYE DROPS FOR GRAFT-VERSUS-HOST DISEASE AND OTHER DISORDERS**


1Australian Red Cross Blood Service, MELBOURNE, Australia; 2Royal Victorian Eye and Ear Hospital, MELBOURNE, Australia

Background. Autologous serum eye drops have been used for treatment of ocular surface disease including chronic corneal ulceration and severe dry eye caused by graft-versus-host disease (GVHD). Stevens Johnson syndrome and Sjogren’s syndrome. Autologous serum eye drops are hypothesized to be superior tear replacement to commercially available lubricants because (1) serum contains similar elements to natural tears, such as epidermal growth factor & vitamin A, facilitating corneal healing and a practical interval between use, (2) immunoglobulins may provide antimicrobial benefit and (3) the autologous source limits antibody formation & allergic reactions, allowing longer term therapy. Aims. To provide safe, high quality autologous serum eye drops for treatment of patients with refractory corneal ulceration and dry eye syndromes. Methods. Collection, processing and testing of units occurs at the Australian Red Cross Blood Service (ARCBS) GMP-licensed facility. From 2000-2005, eye drops were prepared using aseptic technique from autologous whole blood collected into blood bags or serum separator tubes. Following centrifugation, serum was extracted & separated into 50 mL aliquots for storage in sterile vials at -30°C. When required, aliquots were thawed, diluted with 120 mL sterile 0.9% saline and transferred into sterilised 5mL eye drop bottles. All manipulations were performed in a class II biohazard cabinet using sterile consumables. Since September 2005 we use the following fully closed system: Whole blood donations (up to 450 mL ± 10%) are collected from patients meeting ARCBS autologous donor eligibility criteria. Collection bags without anticoagulant (Baxter trio dry packs) facilitate clotting and serum removal following centrifugation. Serum is diluted to 20% by adding 200 mL sterile 0.9% saline to each 50mL serum vial via sterile connection. A sample is taken for microbial testing, and the remaining volume transferred to 20 metres tubing of a Macopharma blood bag. Tubing is segmented at ~7 cm intervals, creating approximately 200 segments (individual doses) from a single donation. These are frozen at < -18°C. Labelled storage containers are stored in local hospital blood bank freezers and/or by patients at home. Patients use the drops according to clinical need in consultation with their doctor. One donation can provide supplies for approximately 6 months. Information sheets are provided to patients and hospital laboratory staff reinforce appropriate product storage and handling. Results. We have collected blood from 52 patients. Clinical indications include GVHD, Sjogren’s syndrome/scleroderma, Stevens Johnson syndrome, and epithelial defects following corneal grafts. One patient was excluded (hepatitis B positive). The diagnosis of B19 associated with PRCA was made according to the presence of B19-specific IgM antibody and viral DNA in sera. To assess whether the B19 was transmitted via the blood product, we performed PCR direct sequencing analysis of B19 in the patient and his blood donor’s sera. The DNA sequence of 2 distinct regions of the genome in B19 virus (N1 and Vp1) were amplified and then directly sequenced. Sequencing of N1 and Vp1 regions from the blood donor serum were identical to those from the patient was completely corresponded to those of blood donor serum. The results suggest that B19 virus is horizontally transmitted from the blood products, and it may be the cause of erythroid crisis in the patient. The patient was treated with intravenous immunoglobulin (5 g/body for 3 days) without any response. Erythropoiesis of the patient began to recover spontaneously around 50 days after the treatment, and B19 virus DNA became negative by PCR analysis. Conclusion. This is the first case report demonstrating the transmission of B19 via RBC transfusion did cause erythroid crisis in the recipient, by using genomic PCR direct sequencing method. Blood products containing B19 DNA may possess a potential risk, especially for immunocompromised patients. Therefore, more sensitive screening for detecting B19 virus should be applied especially for transfusion in these patients.

**0538**

**USE OF TaGVHD WARNINGS IN PATIENTS RECEIVING PURINE ANALOGUE CHEMOTHERAPY**

F.J. Cutler,* C. Niciu,* J.A. Murphy,* R.L. Soura

1Scottish National Blood Transfusion Serv, GLASGOW, United Kingdom; 2Western Infirmary, GLASGOW, United Kingdom; 3Monklands Hospital, AIRDRIE, United Kingdom

Background. Transfusion associated graft versus host disease (TaGVHD) is a rare but serious complication of blood transfusion with mortality rates >90%. It occurs following the transfusion of immunocompetent donor lymphocytes. These engraft and proliferate in a susceptible host causing widespread tissue damage. TaGVHD can be prevented by y irradiation of cellular blood components for patients in at risk groups. A major risk group is patients who have received purine analogue chemotherapy, principally Fludarabine. The annual SHOT report demonstrates that incorrect component transfused remains the commonest transfusion error and many of these are accounted for by not selecting irradiated blood. Aims. To find if patients who had received Fludarabine had 1) a BC/NS/B TaGVHD sticker in their notes 2) a flag on the hospital transfusion laboratory (HTL) IT system indicating the requirement for irradiated blood 3) a warning in the nursing profile regarding the requirement for irradiated blood. An additional aim was 4) to confirm correct component transfused. Of results at one site. Methods. Patient case records who had received Fludarabine chemotherapy were identified via pharmacy records. 2 Patient case records were traced and inspected for a BC/NS/B TaGVHD sticker or other warning indicating the need for irradiated blood. 3) The HTL IT system was examined for a flag indicating the requirement for irradiated blood and checked to determine if blood subsequently transfused had been irradiated. 4) Nursing profiles for the patients were traced and examined for comments on the requirement for irradiated blood. Results. 1) Case records were obtained for 28/34 patients at site 1 and 14/14 patients at site 2. 2) At site 1 only 7/28 (25%) had any warning for the requirement for irradiated blood and only 2 (7%) had the BC/NS/B TaGVHD sticker. At site 2 all 14 (100%) case records contained a warning regarding the requirement for irradiated blood, however, 0/14 had the BC/NS/B sticker. 3) Only 1/34 patients (site 1) and 1/14 patients (site 2) did not have a flag on the HTL IT system regarding the requirement for irradiated blood and neither of
these patients had been transfused. All cellular products transfused sub-sequentially to Fludarabine therapy had been irradiated. 4) 21/34 nursing profiles were inspected (site 1) and 14/21 had some indication of the requirement for irradiated blood. Site 2 has a unified nursing and medical case record. Conclusions. 1) The high rate of warning flags in The HTL IT system indicates that medical and/or biomedical staff are aware of the need for irradiated blood in these patients. 2) The BCSH/NBS stickers are not being used and it would seem likely that patients are unaware of this potential risk from transfusion. 4) Although transfusion within the patient’s institution may be safe, results indicate that transfusion at another site is likely to be potentially hazardous. We suspect that these findings are not unique to these 2 centres. The findings of this study have led us to alter our practice with pharmacy staff distributing the BCSH/NBS patient information leaflet and sticker at the time of first Fludarabine prescription.

**0539**

FACTORS DETERMINING THE RISK OF SEVERE (WHO GRADE 3 AND 4) HEMORRHAGE IN HEMATOLOGIC PATIENTS

A. Carvalhalis,1 E. Drozd2

1Instituto Portugus de Oncologia, PORTO, Portugal; 2Medical University of Warsaw, WARSAW, Poland

Current indications for platelet transfusion in the management of thrombocytopenia hinge on studies, that used minor bleeding as the end point. Minor bleeding may not be a good correlate for life-threatening hemorrhage, that poses real risk of death from that cause. Therefore, we have attempted to verify these indications through evaluation of severe hemorrhages. In this study, we have analyzed retrospectively circumstances of 146 severe hemorrhages (20 grade 3 hemorrhages, and 126 grade 4, among them 109 fatal) that have occurred among 1590 patients hospitalized because of various hematologic disorders with a goal to identify factors that might have contributed to the occurrence of hemorrhage. It was found that unintentional violation of platelet transfusion policy (for transfusion for patients with platelet count below 10×10^9/L) might have been responsible for 8 such hemorrhages, at the most. Similarly, 8 hemorrhages have occurred in patients with normal or increased platelet count. Frequency of remaining 130 hemorrhages was inversely correlated with platelet count. Tendency for increased number of hemorrhages started with platelet count below 50×10^9/L, became significant below 40×10^9/L, and further increased until below 20×10^9/L with plateau afterwards. The highest frequency of hemorrhages was in patients with various forms of acute leukemia, either primary or secondary and aplastic anemia (18-60% of all patients with given form of disease had severe hemorrhage) and was much lower in various lymphomas (1% and 6%). Moreover, almost half of hemorrhages in acute leukemia has occurred in patients with early disease (within 50 days of diagnosis). The lowest frequency of hemorrhages was for IT, when only one hemorrhage among 72 patients has occurred. For patients with platelet count between 20 and 50×10^9/L concomitant presence of transfusing factor abnormalities was an important factor contributing to the occurrence of hemorrhage. Unexpectedly, the presence of severe infection had no effect on hemorrhage occurrence in that group. These data may suggest that in order to effectively prevent life-threatening hemorrhages in patients with early acute leukemia it may be necessary to increase transfusion threshold to at least 20×10^9/L, in this group of patients only. Moreover, the lower number of grade 3 than grade 4 hemorrhages in that cohort may suggest that there is a disproportion between benign and severe hemorrhages in thrombocytopenic patients possibly related to the undefined differences in the resistance of blood vessels in particular patients to the injury.

**0540**

LONG-TERM ERYTHRO-EXCHANGE IN THE TREATMENT AND PREVENTION OF SEVERE SICKLE CELL DISEASE RELATED COMPLICATIONS IN SICILY

T. Lombardo,1 P. Fazzino,1 S. Pennisi1

1Osp. S. Bambino, CATANIA, Italy; 2Servizio Talassemia, CATANIA, Italy; 3Serv. Trasfusionalte. Osp. Vitt. Em Il, CATANIA, Italy

Background. Sickle Cell Disease (SCD) is a severe health problem in Sicily. Among the Sicilian population some individuals have frequent vaso-occlusive complications, whereas others have sporadic/mild episodic complications. Although the number of SCD patients in Sicily and in the other Italian regions is considerable, the natural history of the disease is far from being completely understood. The main reasons for that are the limited number of published works and the lack of national registries. On the other hand, patients affected by SCD, 6 males and 3 females with a mean age of 38.2 years, developed 1 episode of SCD crisis. Although the complications associated with SCD are numerous, both direct and indirect, essentially all are associated with recurrent vascular occlusions. If the goal of therapy is to increase the capacity for oxygen transport and drastically reduce the levels of HBs, the therapy should be erythro-exchange and not simple transfusion therapy. Erythro-exchange is indicated for the treatment of various severe complications of SCD, such as acute pulmonary syndrome, stroke, and acute syndromes involving multiple organs. In this study, from November 1995 through December 2005, we investigated a selected group of patients affected by SCD who developed one or more severe SCD-related episodes. The aim was to evaluate if a long-term exchange therapy is effective or reduces hospitalization rates of SCD complications, and to compare this management program with exchange therapy disadvantages. Materials and Methods. We studied 9 patients affected by SCD, 6 males and 3 females with a mean age of 38.2 years (range 24-62 years); informed consent was obtained from all subjects. All patients refused Hydroxyurea treatment for personal reasons. Five patients were classified as high risk SCD complications patients. 4 patients did not have any risk factor for SCD complications, such as HbSS. Two Out Of The 9 Patients Developed One Stroke Episode, Five Acute Chest Syndrome, And Two Recurrent And Severe Vaso Oclusive Episodes. Acute Episode Treatment: analgesic regimen, hydration, oxygen therapy, antibiotics associated with early erythro-exchange. A second exchange was performed after 5-4 days to stabilize HBs levels between 20% and 35%. Long-Term Care And Follow-Up: exchange transfusion was applied therapeutically as a prophylactic regimen and the goal of the protocol was to maintain an HBs of ≤55% and ≥35% pre and post pheresis. Transfusion guidelines include the use of units matched with an extended phenotype. Patients were monitored for allo-immunization or recurrence of SCD complications. Transfusion therapy, allo-immunization or recurrence of SCD complications. Discussion. in conclusion we can say that this procedure provided a dramatic resolution of SCD episodes and long-term therapy in our patients. Although the risk of hemorrhage recurrence, this program has proved to be very useful in treating SCD related complications. Finally, in the light of our results, we believe that this therapeutic approach could improve both the length and quality of life for SCD related patients.

**0541**

HAEMOVIGILANCE FOR THE TRANSFUSION THERAPY OF PATIENTS WITH THALASSAEMIA


1G.H. Athens G. Gennimatas, ATHENS, Greece; 2Regional Haemovigilance Network (PEDA), THESSALONIKI, Greece; 3Coordinating Haemovigilance Centre (SKEA, ATHENS, Greece

Background. Multi-transfused patients are exposed to an increased risk of developing alloimmunization and other immune or non-immune mediated transfusion-associated adverse reactions as well as contracting transfusion-transmitted infections. Aims: Against this background, haemovigilance systems offer useful information about current practices both in the laboratory and in the clinical setting of transfusion, as well as a tool for evaluating quality management procedures and providing blood safety indices. In this way, quality improvement may be monitored at national and local level, to the benefit of all patients in need of transfusion as well as those whose lifestyle depends on regular transfusion therapy. Methods. In the frame of this haemovigilance system initiated in November 1995, we analyzed immune, non-immune and infectious adverse reactions and adverse events associated with the transfusion of red cell concentrates (RCCs) in patients with thalassaemia. We examined numerator data, such as the absolute number of adverse events/adverse reactions reported in 1997-2004 during or after transfusion. These data were analyzed by the type of reaction, severity, imputability and morbidity. Apart from iron overload - identified as the predominant complication of multi-transfusion with RCCs - we studied the incidence of non-haemolytic febrile reaction (NHFR) in relation to leukoreduction (pre-storage, bedside and laboratory post-storage), the transfusion reaction rate (TRR) and the patients' reaction rate (PRR). Acute and delayed haemolytic reactions and the incidence of alloimmunization and autoimmunization were also investigated and analyzed in relation to red cell antigen-matching policy. Transfusion of incorrect RCCs, TRALI, TA-GvHD, allergic and anaphylactic reactions, infectious
and other adverse reactions were also examined. The data were then analyzed in relation to the number of transfusions (units of RBCs). Results: NHFTR incidence was 0.87% of blood units. TRR was 0.87% and PRR 7.2%. Further analysis showed that the TRR was 0.2% and PRR 1.8% in patients transfused with pre-storage leukodepleted RBCs, compared to 0.6% and 5.2% respectively with bedside filtered RBCs. Delayed haemolytic reaction was diagnosed in 47 patients (64%) had one antibody, 25% two antibodies and 13% multiple antibodies. Alloimmunization was diagnosed in 3.5% of the patients. The most common antibodies detected were of the Rh system, Jka, Kpa, Leet-B, FyH-B and S. Autoimmunization of the IgG type was diagnosed in 1.5%.

E. Economou-Petersen, K. Stamoulis, I. Papassideri

RESULTS.

K. Stamoulis, I. Papassideri

Immunoblotting analysis of the Triton insoluble cytoskeletons, revealed oxidized/denatured hemoglobin or hemichromes. The same storage conditions/oxidative stress. The specific carbonylation of a set of RBC membrane and cytoskeleton proteins with prolonged storage in CPDA is shown for the first time and supports the concept of protein oxidation as a part of storage lesion. The importance of RBC oxidative damage in the storage lesion is not well documented. Aims. To determine the possible storage-induced membrane and cytoskeleton protein oxidation in CPDA-preserved non-leukodepleted RBCs bag in the course of transfusion period storage. Methods. RBC concentrates from six eligible blood donors were prepared according to the standard operating procedures. Each RBC unit was followed up during the storage period of 35 days and shortly afterwards. Membrane skeletons were prepared by Triton X-100 extraction of ghosts. The membrane ghosts and skeletons of days 0-2 of these units, in addition to fresh preparations from ten healthy subjects, were used as controls. Total ghosts and membrane skeletons were analyzed by SDS-PAGE densitometry and immuno-blotted against a variety of erythroid-specific antibodies. Carbonylated protein content was determined following 2,4-dinitrophenylhydrazine derivatization and SDS-PAGE coupled with Western blotting. Results. Immunoblotting with dinitrophenol-specific antibody revealed increased RBC membrane and cytoskeleton protein carbonyls with prolonged storage in CPDA blood bags. A quantitative and statistical important difference in carbonylation was detected in membrane and cytoskeleton proteins isolated from RBCs stored for different time periods. In comparison to control membranes, there was an evident increase in the number and the intensity of the carbonylated protein bands appearing in the immunostained gel, ranging from MW 240 kDa to 15 kDa. The membrane skeletons stored even for long times in CPDA did not exhibit signs of severe proteinolysis, as confirmed by immunoblotting analysis of spectrin, actin and 4.1 proteins. Summary/Conclusions. We conclude that the protein carbonylation of RBC membrane and cytoskeleton during banking in CPDA is increased probably in association with the diminution in total antioxidant capacity of RBCs. Since the stored RBCs convey less glucose to the pentose phosphate pathway, due to the subsequent decrease in NADPH and ATP levels, there are expected to be less protected against oxidative stress. The specific carbonylation of a set of RBC membrane and cytoskeleton proteins with prolonged storage in CPDA is shown for the first time and supports the concept of protein oxidation as a part of storage lesion. These data could give additional, useful information in evaluating improved conditions for storage of RBCs intended for transfusion.

0543

MEMBRANE AND CYTOSKELETAL PROTEIN CARBONYLATION IN NON-LEUKODEPLETED CPDA-PRESERVED RED BLOOD CELLS

A. Kriebardis, M. Antonelou, K. Stamoulis, E. Economou-Petersen, L. Margaritis, I. Papassideri

‘University of Athens, ATHENS, Greece; ‘National Blood Center, ATHENS, Greece

Background. Despite the arrest of the normal aging process, ex vivo storage causes a number of reversible and irreversible biochemical and mechanical changes to the red blood cells (RBCs) and accumulation of bioactive substances in storage medium, collectively referred to as storage lesion. Some of the negative effects of RBC transfusion are associated with the storage lesion. The importance of RBC oxidative damage in the storage lesion is not well documented. Aims. To determine the possible storage-induced membrane and cytoskeleton protein oxidation in CPDA-preserved non-leukodepleted RBCs bags in the course of transfusion period storage. Methods. RBC concentrates from six eligible blood donors were prepared according to the standard operating procedures. Each RBC unit was followed up during the storage period of 35 days and shortly afterwards. Membrane skeletons were prepared by Triton X-100 extraction of ghosts. The membrane ghosts and skeletons of days 0-2 of these units, in addition to fresh preparations from ten healthy subjects, were used as controls. Total ghosts and membrane skeletons were analyzed by SDS-PAGE densitometry and immuno-blotted against a variety of erythroid-specific antibodies. Carbonylated protein content was determined following 2,4-dinitrophenylhydrazine derivatization and SDS-PAGE coupled with Western blotting. Results. Immunoblotting with dinitrophenol-specific antibody revealed increased RBC membrane and cytoskeleton protein carbonyls with prolonged storage in CPDA blood bags. A quantitative and statistical important difference in carbonylation was detected in membrane and cytoskeleton proteins isolated from RBCs stored for different time periods. In comparison to control membranes, there was an evident increase in the number and the intensity of the carbonylated protein bands appearing in the immunostained gel, ranging from MW 240 kDa to 15 kDa. The membrane skeletons stored even for long times in CPDA did not exhibit signs of severe proteinolysis, as confirmed by immunoblotting analysis of spectrin, actin and 4.1 proteins. Summary/Conclusions. We conclude that the protein carbonylation of RBC membrane and cytoskeleton during banking in CPDA is increased probably in association with the diminution in total antioxidant capacity of RBCs. Since the stored RBCs convey less glucose to the pentose phosphate pathway, due to the subsequent decrease in NADPH and ATP levels, there are expected to be less protected against oxidative stress. The specific carbonylation of a set of RBC membrane and cytoskeleton proteins with prolonged storage in CPDA is shown for the first time and supports the concept of protein oxidation as a part of storage lesion. These data could give additional, useful information in evaluating improved conditions for storage of RBCs intended for transfusion.

0544

PLATELETS RECOVERY AND TRANSFUSION NEEDS AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION


Institut Paoli Calmettes, MARSEILLE, France

Few data are currently available regarding platelets transfusion needs and the kinetics and predictive factors for platelets recovery after RIC allo-SCT. In this study, we analyzed the profile of platelets recovery and transfusion needs in the first 100 days after sibling PBSC RIC in a single institution series of 166 consecutive transplantations. Patients and graft characteristics were: age 49 y. (range: 18-70), diagnoses: 66 myeloid malignancies (40%), 64 lymphoid malignancies (39%), and 36 metastatic solid tumors (21%), 112 pts (67%) received an ATG-based RIC, while 54 pts (33%) received a low dose irradiation-based RIC. 75 pts (45%) developed grade 2-4 acute GVHD. Platelets recovery (>20 G/L) was...
observed at a median of 9 days (range: 0–99). The kinetics profile of platelets recovery is shown in the figure below. In the whole study population, the nadir was observed around day +7 after allo-SCT, and a plateau was reached about day +35. Filtered and irradiated donor apheresis platelets were used and patients needed a median of 1 unit (range: 0–55). In this series, 83 pts (50%) did not require any platelets transfusion during the follow-up period (median follow-up: 442 days) and 85 patients (50%) received at least one transfusion of platelets (54 were not transfused beyond day +100 after allo-SCT). Platelets count prior to RIC allo-SCT (median count 144 G/L; HR 0.44 (0.28–0.7) p=0.002), conditioning regimen (use of ATG; HR 1.86 (1.08–2.5) p=0.025) and the occurrence of acute (HR 1.71 (1.08–2.7); p=0.001) and severe GVHD (HR 2.36 (1.35–3.8) p=0.0006; 52% of patients with grade 3-4 acute GVHD were transfused) were the parameters significantly associated with platelets transfusion needs in Multivariate analysis. In this cohort, 145 pts could be assessed for platelets recovery at day +100: among them, 99 (68%) had a platelet count >99 G/L. Univariate analysis found the incidence of severe cardiovascular complications. Extracorporal elimination is used for selective removal of LDL-cholesterol in severe hypercholesterolaemia are genetic disorders, which are associated with high incidence of severe cardiovascular complications. Extracorporal elimination is used for selective removal of LDL-cholesterol in severe hypercholesterolaemia. We hypothesized that LDL-apheresis reduces total plasma cholesterol and partially improves impaired haemostasis too. The aim of this work was to verify this hypothesis. Methods. Repeated LDL-apheresis procedure (treatment interval 17.5±1.6 days) based on immunoadsorption has been used to collect 6×10^11 plts (2 units) and one unit of PRBC. Sample collections and analyses were done on day (d) 0 (donation day), d2 and d7. We determined blood cell counts (Sysmex SE-9500, Müller), metabolic markers are shown in the Table. Results of thrombelastography showed a statistically significant difference (p<0.05) only on d2: MCF 72.6±2.3 (T-PC) and 69.8±2.6 (A-PC), MCE 243.2±26.4 (T-PC) and 209.8±26.4 (A-PC); during storage at 4°C the results of thrombelastography did not change significantly. Results of HSR showed a significant difference (p<0.05) between the two

**0545**  
CHANGES OF HAEMOSTASIS INDUCED BY LDL-APHERESIS  
M.N. Blazek, M. Blaha, J. Maly, V. Blaha, M. Cermanova, M. Pecka, L. Slovacek  
Faculty Hospital, Charles University, HRADEC KRALOVE, Czech Republic  
Background. Familial hypercholesterolaemia and familial combined hyperlipidaemia are genetic disorders, which are associated with high incidence of severe cardiovascular complications. Extracorporal elimination is used for selective removal of LDL-cholesterol in severe hypercholesterolaemia as combined strength of conservative and invasive lipid-lowering therapy may reduce progression of atherosclerosis in these high-risk patients. Activity of haemostasis plays an important role in the development of atherosclerotic complications. Aims. We hypothesized that LDL-apheresis reduces total plasma cholesterol and partially improves impaired haemostasis too. The aim of this work was to verify this hypothesis. Results of thrombelastography showed a statistically significant difference (p<0.05) only on d2: MCF 72.64±2.34 (T-PC) and 69.88±2.6 (A-PC), MCE 243.21±26.4 (T-PC) and 209.88±26.4 (A-PC); during storage the results of thrombelastography did not change significantly. Results of HSR showed a significant difference (p<0.05) between the two
groups only on d0: 59.58±12.31% (T-PC) and 36.66±17.13% (A-PC). HSR increased significantly from d0 to d2 for the group of A-PC (d2: 53.55±15.47) and decreased significantly from d2 to d7 in the T-PC (d2: 62.86±9.05%, d7: 51.96±12.40%). Swirling effect was observed over the entire time period for all products although 2 A-PC showed aggregates on d0. Conclusion. The mean plt yield on d0 of the T-PC and the A-PC

O. Dogan, B. Tait, M. Haeusler, M. Agan C. Hogan

Crossmatches were performed by N. Chung, B. Cho, P. Jang, LuminexÒ), recipients commenced mycophenolate mofetil a week before transplantation, PP five days before surgery and subsequently maintained in both groups, although pH, glucose and bicarbonate were partially significantly lower, lactate, potassium and LDH partially significantly higher in the A-PC than in the T-PC. Thrombelastography of the T-PC showed significantly better in vitro function parameters only on d2, so did the HSR results of T-PC on d0. If the partially improved in vitro metabolic and functional parameters of the T-PC are of in vivo relevance has to be evaluated in clinical trials.

0547

IVIG AND RITUXIMAB ALLOWS SUCCESSFUL SOLID ORGAN TRANSPLANTATION IN PATIENTS WITH A POSITIVE CROSSMATCH AND DONOR SPECIFIC ANTI-HLA ANTIBODIES


1Australian Red Cross Blood Service, MELBOURNE, Australia; 2Royal Melbourne HOSPITAL, MELBOURNE, Australia; 3Vic Transplantion&Immungene
tics Service, MELBOURNE, Australia

Background. Intravenous immunoglobulin (IVIG) in high doses is used increasingly for immunomodulation of various disorders. An illustrative case is end-stage renal failure (ESRF) where until recently, transplantation of dialysis-dependent patients with a positive cytotoxic crossmatch (XM) associated with donor specific anti-HLA antibodies (DSAb) was not possible due to high incidence of severe acute humoral rejection. Up to 20% of ESRF patients have DSAb, potentially excluding them from transplantation. However, with the use of low dose IVIG with plasmapheresis (PP), or high dose IVIG alone, successful transplantation of such patients has been reported recently (Jordan et al., 2003). Rituximab has also been postulated as appropriate treatment of antibody-mediated rejection (AMR) in this context. Aims. To demonstrate that HLA-mismatched kidneys can be successfully transplanted using immunomodulation with IVIG and PP, and to evaluate the role of rituximab in AMR of such cases. Methods. Crossmatches were performed by complement dependent cytotoxicity (CDC) on potential donor and recipient pairs. If positive with T or B cells with demonstrable DSAb as defined by a combination of CDC and solid phase assay (ELISA, LumineXÒ), recipients commenced mycophenolate mofetil a week before transplantation, PP five days before surgery and subsequently tacrolimus. IVIG 2 g/kg was given 48 hours pre-transplant and one month after transplantation. Any transplant that demonstrated subsequent AMR was treated with PP, IVIG and intravenous rituximab 375 mg/m². Results. We describe 6 successful renal transplants in which a positive XM with DSAb was overcome by pre-transplant PP, and high dose IVIG (0.1 g/kg) and one dose of rituximab 375 mg/m². The renal function of the transplanted organs is excellent; creatinine and eGFR equivalent or better than donor (70 mL/min/1.73m²). There have been no episodes of cellular rejection, opportunistic infections, or post-transplant diabetes mellitus. Summary/Conclusions. XM positive transplants can be performed successfully and safely with a regimen of PP, high dose IVIG and lower doses of conventional immunosuppression. Rituximab, together with IVIG and PP is successful treatment in episodes of AMR in these patients. This reinforces the important role of IVIG and rituximab in modern renal transplantation programs, and may be extended to other solid organ transplant programs in which the haematologist may be involved.

0548

TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE IN FOUR IMMUNOCOMPETENT PATIENTS


1Istanbul University, CAPA-ISTANBUL, Turkey; 2H. Istanbul Medical School, ISTANBUL, Turkey

We observed transfusion associated graft versus host disease (TA-GVHD) in four male patients (ages: 61, 52, 56 and 57 years). All of the four patients showed the typical clinical and laboratory findings of TA-GVHD, as follows: high fever, diarrhea, erythodermia, hepatitis and pancytopenia. Oral mucositis was also observed in these patients. Skin biopsies performed in all the patients and were compatible with GVHD microscopic findings. Bone marrow aspiration and biopsy were performed in three of the patients and revealed hypocellularity. There are some other clinical similarities between our patients. All of the patients showed signs of infection and were treated with intravenous immunoglobulin (IVIG) and packed red cell transfusions. There were no episodes of acute renal failure, although two patients died (ages: 52, 56 years). Transfusion indications of the patients were coronary artery bypass grafting (CABG) surgery in three patients and upper gastrointestinal bleeding in one patient. The symptoms had begun in median 11 days, and the patients died in median 21 days with multiorgan failure. Broad spectrum antibiotics, corticosteroids and packed red cell transfusions were used for all patients. Cyclosporine A and chloroquine treatment were also used in two of the patients. HLA typing and sex chromatin analysis using FISH method were done in peripheral blood lymphocytes of the first patient and two of his daughter. The results demonstrated the presence of a donor homozgyous for HLA-B65, HLA/DR1, 01 haplotypes and of a chimeraism for sex chromatin in this patient’s blood. These findings confirmed that the engraftment was supplied by one of the daughters of the patient. In the second patient we extracted and stored DNA by using patient’s hair and donor’s peripheral lymphocytes. The other two patients could not be studied for HLA antigens because of sudden death events. Summary. TA-GVHD is rare, but nearly always fatal complication of blood transfusion. Although immunocompromised patients have an important risk factor for the TA-GVHD, it can also be developed in immunocompetent individuals. Cardiovascular surgery, transfusions from in-family blood donors, genetic homozygity that can be increased by consanguinity are all the risk factors for occurrence TA-GVHD. There is some common extended major histocompatibility haplotypes in white populations, and the chance of transfusion from a donor homozgyous at a particular HLA locus to a patient heterozygous at the same locus, reported as 0.21 percent for one transfusion. This probability will be raised with respect to transfusion number and in the presence of the paternal consanguinity. Conclusions. TA-GVHD can easily be misdiagnosed as drug reactions or viral infections that may have similar clinical and laboratory findings. Because of the ineffectiveness of treatment opportunities, prevention of TA-GVHD is of paramount importance. Patients at risk should be identified and transfused with irradiated cellular blood products.

0549

THE CELLULAR COMPONENTS INDUCED IMMUNOSUPPRESSION VIA REGULATORY T CELLS IN ALLLOGENEIC TRANSFUSION

P. Jeong, P. Jang, B. Cho, N. Chung, H. Kim, C. Han

The Catholic University of Korea. INCHON, South-Korea; 2Uijeongbu St. Mary's Hospital, UIJJUNGBU, South-Korea; 3St. Mary's Hospital, SEOUL, South-Korea

Background. Transfusion save a lot of patients by supply of blood components, although there are some tolerable adverse effects. However, some clinical data showed that transfusion might be induced high risk of post-operative infection and higher relapse or mortality rate in cancer patients. There is controversial for relation between transfusion and immune dysfunction. Aims. We investigated whether immune dysfunction might be induced after transfusion of cellular components. Methods. We used 5 weeks old male BALB/c (H-2d, recipient), female C3H/He (H-2k, donor), and C57/BL (H-2b, third party). We obtained irradiated spleen cells (SP) from BALB/c or C57/BL, and injected to C3H/He mouse via tail vein with intraperitoneal IL-2 administration. Some mice received con
current injection with same condition for 2 days. After 24 hours, we harvested the immune cells (BM), thymus, and spleen. For more parameters, we used mitogen (MLR) and tested with mixture ratio. There was high level of IL-2, shown profound decrease of cell proliferation and, in some ratio, specific for H-2 complex. Two day transfusion did not show inhibition of cells but proliferation. For inhibitory effects of transfusion, we performed MLR with mixture of control and B-CH SP. B-CH SP were induced inhibitory effects according to mixture ratio. The ratios were 1:10 to 1:10 in supernatants from mixture with control and B-CH SP. Also, there were markedly increased CD4+CD25+ cells in BM, SP, and thymus with no change of other immune cells after 24 hours. However, in 24 hours treated cases, there were increased some adhesion molecules and co-stimula
tory markers. In cytotoxicity, SP after transfusion did not have cyto-

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Aplastic anemia

**0551**

**A STUDY OF HLA AND KIR GENES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA PATIENTS**


1’University of Naples Federico II, NAPOLI, Italy; 2’Istituto Nazionale Tumori di Napoli, NAPOLI, Italy; 3’Dip. Biol. e Pat. Cellulare e Molecolare, NAPOLI, ITALY; 4’University of Naples Federico II, NAPOLI, ITALY

**Background.** Paroxysmal Nocturnal Haemoglobinuria (PNH) is characterised by the occurrence of haemolytic anaemia, thrombophlebitis and cytopenia. The expansion of a stem cell bearing a somatic mutation in the phosphatidyl-moiolostol glycan-α (PIG-α) gene, which is involved in the biosynthesis of the glycosyl-phosphatidyl-inositol (GPI) anchor, characterises this very rare haematopoietic disorder.

**Aims and Methods.** In order to investigate the involvement of immune-dependent mechanisms in the pathogenesis of PNH we addressed the analysis of HLA and KIR gene distribution in 12 PNH patients and in 217 controls of the same ethnic origin by PCR-SSP typing. In addition, 15 patients affected by Aplastic Anaemia (AA), whose immune-mediated pathogenesis has been already demonstrated, were enrolled in the study. The statistical evaluation of data was performed by using Student’s t test and Fisher two tailed exact test. Results. Our preliminary results demonstrate a significant increase of the HLA haplotype B*14, CW*08 in PNH patients compared to healthy controls (36.3% vs 3.3%; p<0.0005) while a not significant increase of this haplotype was observed in our group of AA patients (13.3% vs 3.3%; p=0.087). In addition, an increase of DRB1*15 was found in PNH (45.4% vs 20.1%; p=0.053) but not in AA patients. KIR analysis showed a decreased expression of KIR-2DS3 (10% vs 52.7%; p=0.110) and an increased expression of 2DS2 (80% vs 52.1%; p=0.077) genes in PNH patients respect to controls. Conclusions. The critical involvement of HLA molecules in the regulation of the adaptive immune response and the relevance of KIR-repertoire for the functional effectiveness of NK and cytotoxic effectors have been largely recognised. In this context, our data support the hypothesis that complex immune-mediated mechanisms could underlie the dominance of the GPI-defective clones in PNH. The occurrence of ethnical differences as well as the number of patients enrolled in this study are expected to account for the apparent divergence with the increased frequency of DR2 observed in 21 Japanese and 16 American PNH patients.

**0552**

**EXPRESSION PATTERNS OF GLYCOSYLPHOSPHATIDYLYNOSITOL-ANCHORED PROTEINS (GPI-AP) THROUGHOUT THE DIFFERENT NORMAL BONE MARROW CELL MATURATION PATHWAYS: A FRAME OF REFERENCE FOR UNDERSTANDING PNH**


University of Salamanca, SALAMANCA, Spain

**Introduction.** Glycosylphosphatidylinositol-anchored proteins (GPI-AP) are a heterogeneous group of proteins deficiently expressed in patients with paroxysmal nocturnal hemoglobinuria (PNH). Despite the physiological and pathogenetic relevance of different GPI-AP in PNH patients, no study has been reported in which the exact patterns of expression of a large number of GPI-AP are quantitatively evaluated in normal bone marrow (BM) cells, classified according to their lineage and maturation stage. **Aim.** In the present study, we have quantitatively analyzed the expression of eleven different GPI-AP (CD14, CD16, CD24, CD48, CD52, CD55, CD59, CD66b, CD87, CD110 and CD157) during maturation of the neutrophil, monocytic, erythroid, lymphoid, basophil and plasmacytoid dendritic cell (pDC) lineages in normal BM as a frame of reference for the understanding of the abnormal patterns of expression of GPI-AP observed in the BM of PNH patients. **Material and Methods.** Ten normal BM samples from an iden-
tactical number of healthy donors were analyzed by flow cytometry, using different 6-color stainings - depending on the specific cell lineage under study-, to analyze the expression of the above referred GPI-AP. **Results.** Our results show that expression of most GPI-AP varies during normal BM maturation, and different profiles were frequently observed depending on the cell lineage or the GPI-AP analyzed. Accordingly, the expression of CD55, CD59, CD110, CD14, CD87, CD157 and CD48 were observed during monocytic maturation. Different levels of expression of CD55, CD59 and CD58 were detected during the erythroid maturation. Maturation into basophils was associated with a higher expression of CD55 and a lower reactivity for CD59, both on this lineage and pDC. Finally, changes in the expression of CD55, CD59, CD24 and CD48 were observed along the B-cell maturation, whereas CD59 remains stable. **Conclusion.** Our study shows that the expression patterns of most GPI-AP vary along the different normal BM maturation pathways, and provides a detailed map GPI-AP expression during normal hematopoietic differentiation, which could serve as a basis for the identification and characterization of changes occurring in PNH.

**0553**

**PREGNANCY-INDUCED PURE RED-CELL APLASIA, A DISTINCT SYNDROME**

A. Laber, K. Moffett, A. Choudry
University of Louisville, LOUISVILLE, KY, USA

**Background.** Pure red-cell aplasia (PRCA) is a rare hematologic disorder. Several conditions have been associated with the development of PRCA including malignancy, infections, thymoma, autoimmune disorders, and rarely pregnancy. Previously, we have described a patient who developed PRCA on three occasions, 2 triggered by pregnancy and 1 secondary to medroxyprogesterone. Here, we systematically review the information on all published cases of pregnancy-induced (P)-PRCA. **Aim.** To characterize the syndrome of P-PRCA. **Patient and Methods.** Published cases of PRCA induced by pregnancy were identified through MEDLINE (1966-July 2005; search terms: pregnancy and red-cell aplasia, pure) and references from journal articles, books and abstracts. We excluded patients who developed PRCA prior to pregnancy, or had other etiologies. This analysis focused on the patient characteristics; clinical aspects of PRCA; pregnancy features, infant characteristics, treatment and outcomes. **Results.** Ten patients with 15 P-PRCA episodes have been reported. **Patient characteristics.** Age ranged from 15 to 40 years. Gestational age at presentation varied from the first to the third trimester. No patient had other causes of PRCA. **P-PRCA.** Hemoglobin level at presentation ranged between 2.5 to 9 g/dL. Bone marrow evaluation showed pure erythroid hypoplasia with no abnormalities in the other cell lines. All patients received red blood cell transfusions, and 6 of them were treated with corticosteroids. Time to recovery of hemoglobin to a normal level ranged from 2 to 12 weeks post-partum, but was not described in three reports. **Pregnancies.** Four pregnancies ended with delivery via a Caesarean section, 1 at 30 weeks and 3 between 36-40 weeks of gestation. Three via vaginal delivery and 4 authors did not list the mode of delivery, all at full term. Two women underwent artificial aboritions as treatment for PRCA. **Infants.** Fetal outcome included healthy infants in 8 cases and demise in 5. The cause of infant death was prematurity in 4, including 2 secondary to artificial aborition and premature birth in 2. Infant blood values were normal in the 9 reported cases. Following- up. Five subjects had subsequent pregnancies, 3 complicated by PRCA; 1 normal and 1 spontaneous abortion without PRCA. One woman developed PRCA secondary to the contraceptive medroxyprogesterone acetate 3 years after her first episode and 4 years before her second occurrence of P-PRCA. **Conclusions.** P-PRCA is a self limited syndrome with a high risk for relapse during subsequent pregnancies. It can be managed by blood transfusions. Progesterone might cause PRCA in these women. Physicians should closely monitor women with a history of P-PRCA if they become pregnant again or receive hormones for contraception or other reasons.

References


**0554**

**IMMUNOSUPPRESSIVE THERAPY WITH ANTI-LYMPHOCYTE GLOBULIN, CYCLOSPORINE AND PREDNISOLONE IN THE TREATMENT OF APLASTIC ANEMIA A SINGLE CENTRE EXPERIENCE**

B. George, V. Mathews, A. Viswabandya, A. Srivastava, M. Chandy
Christian Medical College, Vellore, VELLORE, TAMILNADU, India

Immunosuppressive therapy offers a reasonable chance of cure in patients with aplastic anemia who do not have a HLA identical donor for allogeneic BMT. Between 1986-2005, 208 adult patients (age> 15 years) received immunosuppressive therapy with either Anti-lymphocYTE globulin (ALG) or Anti thymocyte globulin (ATG). Equine ALG (Lymphoglobulin, Pasteur Mérieux) was given at the dose of 15 mg/kg/day for 5 days while equine ATG (ATGAM, Pharmacia Upjohn) was given at 40 mg/kg/day for 4 days. Following administration of ALG/ATG, oral prednisolone was started at the dose of 1 mg/kg/day for 10 days and in the absence of serum sickness, tapered over the next 7 days. From 1997, once steroids were tapered, Cyclosporine was started at 5 mg/kg/day in 2 divided doses for a period of 3-6 months and then tapered over 6-12 months depending upon the response. There were 145 males and 63 females with a median age of 36 years (range: 16-69). The median time from diagnosis to ALG was 3 months (range: 1-120). Ninetytwo patients (44%) fulfilled the criteria for Non-severe aplastic anemia (NSAA) while 86 (41%) had severe aplastic anemia (SAA) and 30 (15%) had very severe aplastic anemia (VSAA). ALG was given to 185 patients (88%) while ATG was given for 25 (12%). Cyclosporine was given to 101 patients (54.8%). Twenty four patients expired within one month of ALG administration related either to infection or bleeding while response was seen in 119 patients (57.2%). Among patients who showed a response, 51 had a complete response while 68 had a partial response. If the patients who expired within 1 month were excluded, the response rates were 64.6%. There was no difference in response between VSA, SAA and NSAA (50%, 57%, 59.8%) but there was a significant difference between patients who received Cyclosporine versus those who did not (67.3% vs 41.1%; p<0.001). On follow up, 8 patients evolved to paroxysmal nocturnal hemoglobinuria (PNH) while 1 patient transformed to a myelodysplastic syndrome (MDS). Four patients subsequently underwent allogeneic bone marrow transplantation and are alive and free of disease. At a median follow up of 26 months, the overall survival (OS) is 66% and the disease free survival (DFS) is 50.4%. In patients who were alive more than 1 month post ALG, the DFS was 57% with an overall survival of 69%. Immunosuppressive therapy with ALG/ATG offers a reasonable chance of response in patients with aplastic anemia with a sustained response in a number of these patients. Clonal evolution to PNH or MDS still remains a problem in patients receiving immunosuppressive therapy.

**0555**

**SEVEN TURKISH PATIENTS WITH NIJMEGEN BREAKAGE SYNDROME: CLINICAL CHARACTERISTICS AND MUTATION ANALYSIS**

C. Ucar,1 U. Caliskan,1 H. Gulen,1 M. Tekin,1 A. Zamani,1 A. Erbay,1 E. Kazanci,2 C. Vergin1

1Selcuk University Meram Medical Faculty, KONYA, Turkey; 2Seluk University, KONYA, Turkey;
Celal Bayar University, MANISA, Turkey; Ankara University, ANKARA, Turkey; Selcuk University, KONYA, Turkey;
Behcet Uz Children Hospital, IZMIR, Turkey

**Background.** Nijmegen breakage syndrome (NBS) (OMIM 251260) is a rare autosomal recessive disorder characterized by growth retardation, microcephaly, developmental delay, distinctive facial appearance, immunodeficiency and predisposition to malignancies. NBS is caused by mutations in the NBS1 gene which maps to chromosome 8q21. Mutations in the NBS1 gene were first found to be associated with this syndrome in 1998. The gene product, nibrin, is a novel protein which is the member of the Mre11/Rad50 protein complex suggesting that the gene is involved in DNA double strand break repair. Most of the previously identified patients have belonged to Slavic populations, such as Poland, Czech Republic, and Ukraine. Almost all of patients from Slavic origin
were found to carry a homozygous five base pair deletion (657del5) in the 6th exon of this gene. Recently a Turkish patient with NBS has been reported and who was found to be homozygote for 657del5. Aim: We reported seven patients with NBS from three different families in Turkey. Families of the three homozygotes, whom we identified, originated from a Central Anatolian city of Konya (2 families) and an Aegean city, Izmir. These families denied any relationship with Slavic populations. Methods: All probands in these families were phenotypically diagnosed as having NBS based on growth retardation, microcephaly, developmental delay and facial features in addition to lymphoepithelial malignancies. Cyto genetic and immunological investigations also supported the diagnosis. Results: We identified three Turkish families in which probands were diagnosed as having NBS and found to be homozygote for 657del5. Evaluation of haplotypes created with help of three flanking microsatellite markers revealed that the 657del5 allele in three Turkish families had a single origin, which was identical with that found in the Slavic populations. Conclusion: This study demonstrated that NBS has not been very rarely diagnosed in Turkish population. Our detection of homozygotes in these unrelated families presenting with malignancy implies that NBS is still underdiagnosed in Turkey. 657del5 mutation in Turks shows the same origin described in Slavs. This result suggests the presence of population admixture in modern Turkey.

**0556**

**BONE MARROW EXPRESSION PROFILE OF RAS, P53, AND MDM2 GENES IN CYTOPENIC DISORDERS**

E. Schulz,1 I. Lorand-Metze,2 F.F. Costa,3 S.C. Costa,1 S.T.O. Saad1
1State University of Campinas Brazil, DRESDEN, Germany; 2State University of Campinas, CAMPINAS, Brazil

Ras, P53 and Mdm2 genes are active regulators of cell growth, division, and death. These 3 genes form a cascade in which each gene is able to modify the transcription and expression of the other gene. In order to understand the mechanisms of cytopenias in different hematologic disorders, we investigated the expression of these genes in 16 patients with mono, bi or pancytopenia, excluding myelodysplastic syndromes (complementary data already published in Blood 2004 104: Abstract 3433). The expression of P21ras, P53, and Mdm2 proteins was detected in bone marrow cytopenons stained by APAAP (alkaline phosphatase anti-alkaline phosphatase procedure) using monoclonal antibodies Y15-219, PAb 1801 and 2A10, respectively. N-, K-, H-ras and p53 gene mutations were assessed by PCR-SSCP (polymerase chain reaction/single strand conformation polymorphism) to exclude the presence of mutations in these genes. The quantitative wild-type expression of p21ras, p53 and mdm2 proteins in the cytopenons (combination of number of cells and staining intensity) was compared to the values of expression of these proteins in 7 normal bone marrows. We found that patients with severe aplastic anemia (n=4), bone marrow hypoplasia (n=3), and toxic leucopenia (n=2) as diagnosis exhibited total loss of cytoplasmic expression or notorious hypoexpression of these proteins. Two cases of connective tissue disease (CTD) (1 undifferentiated connective tissue disease with positive PANA; 1 systemic lupus erythematosus) strongly overexpressed both P21ras and wild-type P53, while both cases of megaloblastic anemia (MA) aberrantly overexpressed wt-P53 protein. The other 3 patients with familiar leucopenia, hepatitis B thrombocytopenia and hypersplenism due to schistosomiasis presented normal values of P21ras, P53, and Mdm2 proteins. We observed that P21ras, P53, and Mdm2 proteins are downregulated in benign hypoplastic disorders of the bone marrow. Conversely, P53 and P21Ras proteins are upregulated in bone marrow disorders in which the cytopenia and the activation of these proteins are likely triggered by DNA lesions as well as subsequent apoptosis as part of the pathophysiology of the disease in CTD and MA. Hence, we suggest that P21Ras, P53, and Mdm2 genes are tightly and actively modulated in benign cytopenic disorders of the bone marrow and play a pivotal role in the molecular mechanism of these diseases.

**0557**

**REDUCED INSULIN SECRETION IN NORMOGLYCEMIC PATIENTS WITH β-TALASSEMAIA MAJOR**

N. Angelopoulos1, A. Zervas,2 S. Livadas,1 I. Adamopoulos,3 D. Giannopoulos,4 A. Goula,4 G. Tolis1
1‘Hippocratic Hospital, ATHENS, Greece; 2Endocrine Unit, Ag.Sophia Hospita,
ATHENS, Greece; 3General Hospital of Kalamata, KALAMATA, Greece

Background. Diabetes mellitus in patients with thalassaemia major is caused by hemosiderosis due to transfusional iron overload. However the exact mechanisms responsible for the progression from normoglycaemia to overt diabetes in these patients are still poorly understood. Aims. To assess insulin sensitivity and secretion in the fasting state in regularly transfused patients with β-thalassaemia major with normal glucose response during oral glucose tolerance test and estimate its possible relation to iron overload. Methods. We assessed fasting glucose, insulin and C-peptide levels from 24 patients with β-thalassaemia major and 18 healthy age- and body mass index- matched controls. Insulin sensitivity and insulin release index were calculated according to the HOMA model. The correlation to age, body mass index and serum ferritin was further analyzed. Results. Fasting glucose levels of patients were increased compared to controls (5.50±0.12 mmol/L vs. 4.67±0.13, mean±SEM, p<0.001). A decrease in b-cell secretion in the fasting state (estimated by SCHOMA) was observed in thalassaemic patients (SCHOMA 88.47±11.11 vs. 184.29±23.72 in controls, p<0.001). Further intragroup analysis of patients to impaired (IFG) and normal (NGF) fasting glycaemia group, revealed an increased SCHOMA in NGF compared to IFG patients (110.63±17.68 vs. 66.31±10.88 respectively, p<0.05) but no difference was found regarding estimated insulin sensitivity (ISI-HOMA) between the two groups. Plasma values of C-peptide correlated positively with ferritin (r=0.42, p=0.04) and SCHOMA (r=0.45, p=0.02) and negatively with ISIHOMA (r = 0.43, p=0.05). Conclusions. These results support the concept that an impairment of b-cell function, as reflected by a decreased insulin secretion index, already exists in β-thalassaemic patients with normoglycaemia before any changes in glucose tolerance can be detected in oral glucose tolerance tests.

**0558**

**RECOMBINANT ERYTHROPOIETIN AS TREATMENT FOR THE HYPOREGENERATIVE ANEMIA OF HEMOLYTIC DISEASE OF THE NEWBORN**

H. Donato1, G. Schwartzman2, C. Garcia3, V. Nain4
1‘Bio Sidus, BUENOS AIRES, Argentina; 2Polichimico Bancario, BUENOS AIRES, Argentina; 3Sanatorio de la Trinidad, BUENOS AIRES, Argentina

Background. Intrauterine transfusions (IUTs), red cell transfusions (RCTs) and exchange transfusions (EXTs) are usually administered, in utero or during the first days after delivery, for the treatment of hemolytic disease of the newborn (HDN) due to Rh or ABO incompatibility. These transfusional practices induce a severe suppression of the erythropoiesis evidenced by reticulocytopenia, bone marrow erythroid hypoplasia, and inadequately low levels of serum erythropoietin (EPO). Spontaneous reactivation of erythropoiesis only occurs after 2-4 months of age. Consequently, a late hypopregenerative anemia gradually develops between 2nd and 6th weeks of life, and affected infants frequently
require RCTs. A few authors reported that recombiant EPO (rhEPO) is useful for its treatment and prevention in neonates with RH HDN who received IUTs or intratranche EXTs. Furthermore, since the neonatal bone marrow is not able to sustain an adequate erythropoietic response throughout several weeks, this hypoprog Bogenerative anemia frequently develops in infants with severe ABO or RH HDN who received no transfusion. No trial involving this population has been published. Aims. To evaluate the efficacy of rhEPO for the treatment of HDN due to RH, ABO or other antigens incompatibility, regardless of whether patients received or not IUTs, RCTs, or EXTs. Methods. After the first week of age, infants started treatment with epoetin α (Hemotec), 250 U/kg, subcutaneously, 3 times a week, when their hematocrit (Htc) dropped to levels requiring RCT, with a clear inadequate reticulocyte response. Patients were closely monitored throughout the following days and RCTs were administered, according to the criteria of the treating physician, if a further decrease of Htc occurred or if clinical symptoms and signs of anemia developed. The treatment was discontinued when the Htc reached normal levels. All patients were given iron (6 mg/kg/day) and folic acid (1.2 mg/day). Results. Twenty six infants were included (Rh HDN=19; ABO HDN=6; KpA HDN=1); 9 patients (ABO=6, Rh=3) had not been administered any IUTs, EXTs or RCTs, and 8/19 RH HDN had received IUTs. Mean age at starting the treatment was 27±5.8 days (range: 5-65). Htc and reticulocyte count (Rtc) showed significant increases after 7 and 14 days of treatment (Table).

Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hematocrit (%)</th>
<th>Reticulocyte count (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>All Patients</td>
<td>24.6 27.8*</td>
<td>30.0*</td>
</tr>
<tr>
<td>Rh-kpA Patients</td>
<td>20.0 27.5*</td>
<td>29.5*</td>
</tr>
<tr>
<td>ABO Patients</td>
<td>26.5 26.8*</td>
<td>31.0*</td>
</tr>
</tbody>
</table>

*p<0.001; *p<0.01; **p<0.05; *NS

No difference was observed between infants with Rh or ABO HDN. Comparison between patients with RH HDN receiving or not IUTs showed no significant difference for: a) the Htc increase from day 0 to 7 (7.4±5.8% vs. 3.0±2.8%, respectively), or from day 0 to 14 of treatment (6.6±7.9% vs. 4.8±5.5%, respectively); b) the Rtc increase from day 0 to 7 (7.2±5.8% vs. 5.2±3.3%, respectively), or from day 0 to 14 of treatment (7.9±7.4% vs. 5.3±4.7%, respectively). Five neonates (19.2%) required one RCT at days 2, 3, 7, 16 and 24 of treatment (ABO=1, Rh with kPa=5); b) the Rtc increase from day 0 to 7 (4.6±5.8% vs. 3.0±2.8%, respectively), or from day 0 to 14 of treatment (4.6±5.8% vs. 3.0±2.8%, respectively). Five neonates (19.2%) required one RCT at days 2, 3, 7, 16 and 24 of treatment (ABO=1, Rh with kPa=5). Twenty patients (71%) were diagnosed with myelopathy with the combination of tibial SEP and neurological findings. Two patients whose dorsal sural SNAP were not recorded had normal tibial SEP responses; therefore, these patients were considered to have isolated peripheral neuropathy. Summary/Conclusions. As a result, we conclude that dorsal sural nerve conduction studies and tibial SEP were performed. Results. Although dorsal sural sensory nerve action potentials (SNAPs) were not recorded in 15 (54%) of 28 patients, only 9 (32%) of them had polyneuropathy with conventional conduction studies. Furthermore, patients with dorsal sural SNAP had mean lower amplitude, mean longer latency and slower velocity response when compared to controls. Twenty patients (71%) were diagnosed with myelopathy with the combination of tibial SEP and neurological findings. Two patients whose dorsal sural SNAP were not recorded had normal tibial SEP responses; therefore, these patients were considered to have isolated peripheral neuropathy. Summary/Conclusions. As a result, we conclude that dorsal sural nerve conduction studies is a reliable method for detection of early peripheral neuropathy in B12 deficiency. On the other hand, in concordance with previous studies, dorsal tract involvement is more common in patients with B12 deficiency.

0560 DORSAL SURAL NERVE CONDUCTION STUDY IN VITAMIN B12 DEFICIENCY WITH MELOGASTIC ANEMIA

Trakya University School of Medicine, EDIRNE, Turkey; Trakya University School of Medicine, EDIRNE, Turkey

Backgrounds. Peripheral neuropathy is frequently observed in B12 deficiency. In spite of this, knowledge about peripheral neuropathy in B12 deficiency is little because the severity of clinical involvement of the central nervous system clearly outweighs signs and symptoms detectable due to peripheral nervous system involvement. Aims. We primarily investigated peripheral neuropathy with dorsal sural nerve conduction study, which is a new method for detection of early peripheral neuropathy, in B12 deficiency with megaloblastic anemia. Also, as posterior column involvement is the most frequent reported and complicated neuropathy in B12 deficiency, the sensory evoked potentials (SEPs) were studied in all patients. Methods. Twenty-eight B12 deficiency patients (15 male, 13 female, mean age 65.8 years) with megaloblastic anemia and 18 age- and sex-matched controls were included. Dorsal sural nerve conduction studies, conventional motor/sensory nerve conduction studies and tibial SEP were performed. Results. Although dorsal sural sensory nerve action potentials (SNAPs) were not recorded in 15 (54%) of 28 patients, only 9 (32%) of them had polyneuropathy with conventional conduction studies. Furthermore, patients with dorsal sural SNAP had mean lower amplitude, mean longer latency and slower velocity response when compared to controls. Twenty patients (71%) were diagnosed with myelopathy with the combination of tibial SEP and neurological findings. Two patients whose dorsal sural SNAP were not recorded had normal tibial SEP responses; therefore, these patients were considered to have isolated peripheral neuropathy. Summary/Conclusions. As a result, we conclude that dorsal sural nerve conduction study is a reliable method for detection of early peripheral neuropathy in B12 deficiency. On the other hand, in concordance with previous studies, dorsal tract involvement is more common in patients with B12 deficiency.
and IgM (4.2% vs. 6.1%, p=0.623; OR = 2.69; CI = 0.27-26.56) were comparable between SCA patients than in controls. Summary/Conclu-
sion. ACA IgG and IgM and APS IgG are strongly associated with SCA among Bahraini patients, and their presence is potentially linked to hematological-coagulation abnormalities frequently seen in SCA.

0562

ERYTHROPOIETIN PRODUCED BY A HUMAN CELL LINE HAS ONLY TRACE LEVELS OF POTENTIALLY IMMUNOGENIC N-GLYCOLYNEURAMINIC ACID RESIDUES

Z. Shahrokhi¹, S. Flatman², M. Davies², A. Baycroft², M. Heartlein³
¹Shire Human Genetic Therapies Inc, CAMBRIDGE, USA; ²Lonzza Biologics plc, SLOUGH, United Kingdom

Background. Recombinant erythropoietins are used extensively in the management of anaemia associated with chronic kidney disease or can-
cer. At present, all of these agents are produced in Chinese Hamster Ovary (CHO) cell lines. This leads to glycosylation patterns that differ greatly from that of endogenous human erythropoietin. This may be important as, in some bioactive substances (including growth factors), glycosylation patterns are thought to affect bioavailability, pharmacoki-
etics and functionality. Of particular interest is the presence of N-gly-
colyneuraminic acid (Neu5Gc) residues, as this substance is not pro-
duced naturally in humans. As a result, it has immunogenic potential and tests show that individuals have circulating antibodies to Neu5Gc. Aims. To produce an erythropoietin in a human cell line and characterize the structure, with a particular focus on Neu5Gc residues. Methods. An overview of the gene-activation technology (Shire Human Genetic Ther-
apies, Inc.) used to produce erythropoietin (epoetin delta) is shown in Figure 1. The erythropoietin-producing cell line was created by transfec-
tion of a human cell line with DNA containing the appropriate target-
ing and gene-activating sequences. A variety of techniques have been used to characterize the resultant erythropoietin including: amino acid sequencing, peptide mapping with reverse-phase, high-performance li-
quid chromatography (HPLC)/mass spectrometry, oligosaccharyl pro-
filing and MALDI-TOF of released glycans. Sialic acid and Neu5Gc were quantified following labelling of the released glycans by reverse-phase HPLC analysis with fluorescence detection (limit of detection for Neu5Gc: 0.06 nmol/nmol epoetin delta). Neu5Gc content of recombi-
nant erythropoietins was also assessed for comparison using the same

Figure 1. Technique used to produce epoetin delta.

0563

STUDY OF THE INNOVATIVE PARAMETERS RET-Y & RBC-Y IN PATIENTS WITH HYPOCHROMIC, MICROCYTIC ANEMIA

A. Agorasti, D. Konstantinidou
General Hospital of Xanthi, XANTHI, Greece

Background. The RET-Y and RBC-Y (generated by Sysmex analyzer) are the mean value of the forward scatter light histogram within the reticulocyte and mature red cell population respectively, expressed in arbitrary units (AU). The RET-Y and RBC-Y are related to cell size and to cell content (mainly). Aim. The aim of this study was the assessment of the RET-Y and RBC-Y in patients with hypochromic, microcytic ane-
mia and their correlation with soluble transferrin receptor (sTfR), stTfR/log ferritin ratio (sTfR-F index) and red blood cell indices (MCV, MCH, MCHC). Methods. We enrolled 116 patients with hypochromic, microcytic anemia (group A: iron deficiency n=39, group B: β thal-
lossemia trait n=53, group C: O-Arab trait n=24) and 42 healthy individ-
uals (group D). Blood counts were performed with Sysmex XT-2000i

0564

LEBANESE G6PD DEFICIENCY: EVALUATION OF THE NEONATAL SCREENING AND PHENOTYPE DESCRIPTION

C. Khayat Djambas¹, M.T. Abi Warde², I. Khneisser², J. Loiselet², A. Megarbane²
¹Hotel Dieu de France Hospital, BEIRUT, Lebanon; ²Genetic Department Saint Joseph University, BEIRUT, Lebanon

Lebanese neonatal screening for G6PD deficiency is started in 1996 after a study showing an incidence of 12/1000 and hemolytic anemia in 77.8% of those, mainly precipitated by beans. Molecular study in 56 of the Lebanese Mediterranean Form in 50%, the Lebanese Mediterranean Form in 50% of the G6PD carriers were screened till January 2005 (20% of the Lebanese neonate), 503 have def-
icieny in G6PD: 1% of boys and 0.04% of girls. We conducted a study evaluating the screening and describing the phenotype of the Lebanese G6PD deficiency population. All the 503 patient family were contacted by phone. Only 125 could be reached. One refuses to cooperate. The 122 constituted the A population. From the 122, fifty were taken randomly. They constituted the B population with the 7 presenting hemolytic anemia in the A population. A questionnaire was done by phone with the A population and a more developed one with the B population. The same investigator has done all the questionnaires. There was 11 girls and 111 boys in the A population with a mean age of 4 years 3 months. 3/122 (2.46%) did not know about the result of the screening. 62/119 (52.12%) were informed of the deficiency orally by phone, 57/119 (47.82%) received the written list of ‘substance to avoid’ with the diag-

nosis. 7/122 (2 girls and 5 boys) present hemolytic anemia with 2 of
them needing transfusion. 3 of the 7 were the ones not knowing their deficiency. Mean age of hemolysis was 2 years 6 months. Beans ingestion precipitated hemolysis in 6 and viral infection in one. Beans ingestion was intentional in 3 cases. 20/122 (16.4%) lives near beans plantation and did not develop hemolysis. After announce of the deficiency 89/122 (73%) had consulted a physician. No one had consulted a hematologist. 30/122 (16.4%) did not know that G6PD deficiency could precipitate hemolysis. 80/122 (65.5%) ignores the transmission’s modality of the disease. Age, sex, socio demography of the B population was similar to the A population. In the B population 31/57 (54.54%) have family story of G6PD deficiency. Twelve have done the screening because of the family story. 15/57 (31.5%) consider the disease as a serious one and only 31/57 (54.54%) consider this deficiency for life. 8/57 (14.5%) have presented neonatal jaundice needing hospitalization, five of them (62.5%) received phototherapy. 42/57 (83%) knows that oxidative agent could precipitate hemolysis. From known oxidative agent only avoidance of beans showed significant reduces of hemolysis between exposed populations and none exposed. After hemolytic episode parents do not protect their children from other oxidative agent. Neonatal screening of G6PD deficiency reduces the risk of hemolytic anemia from 77.8% to 3.36%. 2.46% of the detected where not informed. Information is still lacking and need more reflection and follows up. Long list of substance to avoid do not necessarily guaranty avoidance of substance and compliance. Beans are the main substance to avoid.

0565
M EASURE OF RETICULOCYTE HAEMOglobinisation BY WHole BLOOD HaEMatology AnalYSERS: CorRELATION OF CHr (BAyer A DVIA 120) with Ret-he (S YSMEX XE-2100)
1. Papassotiriou, M. Nicolau, C. Tsitsikas, S. Polychronopoulou, A. Stamoulakatou
Aghia Sophia Children’s Hospital, ATHENS, Greece

The haemoglobin content of the reticulocytes (CHr) has been used as a diagnostic tool in the diagnosis of anemias and in monitoring erythropoiesis (1). The reticulocyte channel of Sysmex XE-2100 whole blood analyser provides a parameter defined as Ret-Y which corresponds to the mean value of the forward-scattered-light histogram within the reticulocyte population. By applying the regression plot Ret-He=5.569exp 0.001Ret-Y, Ret-Y can be mathematically transformed into Ret-He an haemoglobin equivalent for reticulocytes, expressed in picograms (2). High correlation between Ret-He and the CHr parameter has been demonstrated, especially when monitoring iron deficiency anemia (5). The objective of this study was to establish the relationship between Ret-He and CHr parameters in children. A total of 300 random peripheral blood samples were analyzed. Blood samples comprised 200 healthy individuals, 30 patients with sphaerocytosis and 70 patients with haemoglobinopathies (mainly $\beta$-thalassemia and Sickle C.D Disease /$\beta$ thalassaeemia). Blood counts were performed using two systems: Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan) and Bayer Advia 120 (Bayer, Tarrytown, NY, USA). The within-run imprecision of Sysmex XE-2100, concerning erythrocyte parameters, HB, Hct, MCV and reticulocyte count was 0.8-1.4% and that of Bayer Advia 120 0.5-1%. The between-run imprecision of Sysmex XE-2100 was 1.5-4% and of Bayer Advia 120 1.2-1.5%. The reference range for Ret-He was 28.8±5.4 pg (17.6-36.7 pg) and for CHr 27.9±4.3 pg (18.8-36.0 pg). Both values were normally distributed and showed a linear fit of the regression line. The regression equation calculated in this study was: Ret-He=1.16xCHr - 4.36, at 99% confidence level (r=0.94, p<0.00001). Conclusively, the reticulocyte channel of Sysmex XE-2100 offers a new reticulocyte index Ret-He which shows excellent correlation with the equivalent parameter of Bayer Advia 120 Chr. Both parameters are equivalently functional in diagnosing different anemias and in therapeutic monitoring of iron-restricted erythropoiesis.

References:

0566
EVALUATION OF MYOCARDIAL IRON DEPOSITION ASSESSED WITH M.R.I. IN YOUNG THALASSEMIC PATIENTS RECEIVING ONE YEAR OF DEFERASIROX VERSUS DEFEROXAMINE
A. Christoforidis,1 I. Tsatra,2 K. Karasmanis,3 E. Zevgarioudou,2 A. Koussi,1 I. Tsiourides,1 M. Athanassiou-Metaxa3
1Aristotle University of Thessaloniki, THESSALONIKI, Greece; 2Hippokration General Hospital, THESSALONIKI, Greece; 3Papageorgiou Hospital, Radiology Dept., THESSALONIKI, Greece

Deferasirox (Exjade®) is a new once-daily, oral iron chelator, recently approved by FDA, while is awaiting EU regulatory approval. A multicenter clinical trial, recently published, indicated that daily administration of 10 mg/kg of deferasirox at a dose of 10 mg/kg maintained iron concentration (LIC), whereas doses of 30 mg/kg achieve significant LIC reduction. Aim of this study was to evaluate the effectiveness of deferasirox in removing iron from the heart in comparison to deferoxamine, with the use of Magnetic Resonance Imaging (M.R.I.). In our center 11 young patients with $\beta$-thalassaemia major, aged 10 to 16.5 years with a mean age of 14.2±2.5 years, participated in a large multicenter, Phase III, comparative trial of deferasirox versus deferoxamine. Seven patients were randomized in the deferasirox group and 4 in the deferoxamine group. Doses were assigned according to baseline LIC assessed with percutaneous liver biopsy. In line with previous clinical management at our center, these patients were studied with myocardial MRI, as part of their routine monitoring, at the begging of the trial and one year after. MR images of heart were acquired during systolic phase, using electrocardiogram-triggered, flash 2D sequences, with 5 mm thickness and FOV 360x240mm. Region of interest (ROI) measurements were performed in the air and in the left ventricular myocardium. The natural logarithm of the signal intensity of the studied tissue to air ratio [ln (mean signal intensity of tissue / SD of air)], was calculated, with estimating normal values above 3.2. All patients completed one year of the study with no major adverse event. None of them was presented with any symptoms of cardiopathy, and heart echo, routinely performed according to the protocol design, was normal in every patient. All MRI’s values were within normal range, something which can be attributed to the young of age. Mean heart MRI values at the begging of the study were 4.29 for the deferasirox and 4.3 for the deferoxamine group.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>n</th>
<th>MRI start</th>
<th>MRI end</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deferasirox</td>
<td>10 mg/kg</td>
<td>1</td>
<td>4.21</td>
<td>3.6</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>5</td>
<td>4.37</td>
<td>4.25</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>1</td>
<td>3.98</td>
<td>4.64</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7</td>
<td>4.29</td>
<td>4.21</td>
<td>0.32</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>35-50 mg/kg</td>
<td>4</td>
<td>4.30</td>
<td>4.42</td>
<td>0.28</td>
</tr>
</tbody>
</table>

One year after, heart MRI values were 4.21 and 4.41 respectively with no statistically significant difference. Of particular interest is the fact that one patient receiving deferasirox at a low dose of 10 mg/kg showed a significant reduction in MRI values (4.21 versus 5.60), whereas patient receiving high dose of 50 mg/kg managed to reduce myocardial iron deposit as indicated by the significant increase of MRI values (5.96 versus 4.64). Results are shown on the following Table. In conclusion, deferasirox at a daily dose of 20 mg/kg seems to be equivalent to defer oxamine doses of 40-50 mg/kg in maintaining myocardial iron concentrations. Similarly to liver, effect of deferasirox in removing myocardial iron is dose-dependent Low dose of deferasirox (10 mg/kg) seems to be ineffective, whereas one patient receiving high dose showed an encouraging improvement in myocardial MRI values. Randomized, controlled studies are needed for safer conclusions.

0567
DIVERSE MOLECULAR DEFECTS ASSOCIATED WITH IDIOPATHIC ERYTHROCYTOSIS REFLECT THE HETEROGENEITY OF THIS DISORDER
M.J. Percy,1 F.G.C. Jones,1 T.R.J. Lappin,3 M.F. McMullin1
1Belfast City Hospital, BELFAST, United Kingdom; 2Queen’s University, BELFAST, United Kingdom

Background. Idiopathic erythrocytoses are a heterogeneous group of disorders characterised by an absolute increase in the red cell mass and associated with variable erythropoietin (Epo) levels. The diagnosis of idiopathic erythrocytosis (IE) is one of exclusion in patients who do not
fulfil the criteria for the myeloproliferative disorder of polycythaemia vera (PV) and have no identified secondary causes such as a high affinity haemoglobin or Epo producing tumour. The recent discovery of the universal Janus Kinase (JAK2) mutation, V617F, associated primarily with MPD, now makes it possible to identify a clonal stem cell defect in those individuals who previously would have not fulfilled the criteria for PV and thus were included in the IE group. In the majority of IE cases the molecular defect is undefined. Aims. To identify the underlying genetic defects in IE individuals and establish if any of this group of patients would be positive for the PV associated V617F JAK2 mutation. Methods. DNA samples were prepared from more than 120 British and Irish erythrocytosis patients and PCR-direct sequencing of the following genes was performed: the cytoplasmic region of the Epo receptor (EpoR), all three exons of the von Hippel Lindau (VHL) protein and the catalytic domain of the prolyl hydroxylase PHD2. Results. Sequencing the EpoR identified a teenage boy with a truncation mutation, G6002A, which removed the terminal 70 amino acids from the receptor. This same mutation was first described in a Finnish skier but microsatellite analysis indicated that both mutations had arisen independently. Screening for the Chuvash VHL mutation, Arg200Trp, in the IE group detected 8 families from the Indian sub-continent who had members homozygous for this mutation. In addition, two Caucasian individuals both with erythrocytosis, D1 and E1, were heterozygous for the same mutation. Although E1 also possessed the G144R VHL mutation, D1 did not exhibit a second defect in the VHL gene but expressed the wild type allele. Most recently, a novel mutation, C950G, in PHD2 has been identified to cause erythrocytosis in 3 members of one family due to an aberration in the Epo negative feedback pathway. Finally, 64 IE individuals were screened by ARMS-PCR to indicate the prevalence of the V617F JAK2 mutation and only one individual was found to possess this mutation. Summary. Although mutations in the oxygen sensing pathway represent the major identified cause of IE so far, they account for only a minor proportion of the total number of patients with IE. Thus the molecular basis of a significant cohort of patients remains elusive but these individuals are a valuable resource that may provide insights into the mechanisms regulating red cell haemostasis and oxygen sensing.

0568
CHARACTERISATION OF BONE MARROW POSITIVE MITOGEN-STIMULATED DIRECT ANTIGLOBULIN TEST IN PATIENTS WITH REFRACTORY ANAEMIA
F.G. Imperiali, A. Zaninoni, M. Colombi, A. Iurlo, A. Zanella, W. Barcellini
Fondazione IRCCS Policlinico, MILANO, Italy

Background. Autoimmune phenomena, particularly directed against RBC, are described in Myelodisplastic syndromes (MDS). We already reported positive BM cultures in patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS) by a new method named mitogen-stimulated-direct antiglobulin test (MS-DAT). Aims. We characterised the target BM cell of the MS-DAT positivity in MDS patients.

Methods. MS-DAT was performed by stimulating BM cells with PMA and PHA and antibodies were detected in supernatants by competitive solid phase ELISA. BM cells were separated by magnetic beads in CD45+ (myeloid cells) and CD45- cells (erythroblasts) and supernatants of positive and negative cultures tested on both BM populations. Results. Eleven out of 23 patients showed positive MS-DAT in BM (cut off value 150 ng/mL±3SD), and positive patients had increased erythroblast counts and signs of hemolysis (i.e. higher reticulocytes, indirect bilirubin, LDH, and lower haptoglobin) compared with MS-DAT negative ones. MS-DAT positive BM supernatants had negligible reactivity with CD45- cells both from BM MS-DAT positive and negative patients. Conclusion. Our results show an autoimmune reactivity against erythroblasts in RA and RARS patients with peripheral signs of hemolysis.

0569
SERUM PRO-HEPCIDIN AND IRON STATUS IN THALASSAEMIA
A. Meco, L. Duca, P. Delbini, L. Nava, L. Zanghi, M.A. La Rosa, A. Crifò, M.D. Cappellini
Pollicino G. Martino Medical School, MESSINA, Italy; Dep.Int.Med.Maggiore Policlino, IRCCS, MILAN, Italy; Dep.Paediatrics Pollicino G.Marti- no, MESSINA, Italy

Background. Hepcidin is a antimicrobial-like hormone peptide synthesized in the liver. It seems to be a key regulator of iron homeostasis inhibiting intestinal iron absorption, recycling iron in the macrophages and mobilizing iron from hepatic stores. Hepcidin expression is induced by iron overload and inflammation and is suppressed by anaemia and hypoxia. Prohepcidin is a small plasma peptide believed to be a hepcidin precursor. Thalassaemia syndromes are a heterogeneous group of inherited anaemias resulting from reduced or absent synthesis of α- or β-globin chains of haemoglobin, where hepcidin is regulated by opposing factors such as ineffective erythropoiesis, anaemia and iron overload. In these conditions iron overload is mainly due to blood transfusions as well as increased iron absorption. Aim. To investigate serum pro-hepcidin in a cohort of Thalassaemia Major (TM) and Thalassaemia Intermedia (TI) patients and to evaluate a possible relationship with iron status. Patients and Methods. Thirty-three TM regularly transfused patients, twelve TI patients and twelve normal subjects were studied. TI patients had no or very few transfusions during their life, the last one being at least 5 years ago. Blood from TM was taken at least 48 hours after chelation therapy and just before blood transfusion. Iron status was evaluated by serum ferritin, percentage of transferrin saturation and non-transferrin bound iron (NTBI). Serum ferritin was determined by standard procedures; NTBI was assayed in serum by HPLC after nitrilotriacetic acid (NTA) chelation. Serum pro-hepcidin was measured by ELISA competitive binding assay (DRG,Germany). Results. Positive correlations were found between pro-hepcidin and ferritin (r=0.423, p<0.01), and between pro-hepcidin/ferritin ratio and NTBI (r=0.356, p=0.05) in TM patients. We report the results in Table 1.

<table>
<thead>
<tr>
<th>SF (ng/mL)</th>
<th>NTBI (nM)</th>
<th>Pre-hepcidin (ng/mL)</th>
<th>Hb (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM (n=43)</td>
<td>875±560°</td>
<td>0.86±1.23°</td>
<td>453±36°</td>
</tr>
<tr>
<td>TI (n=12)</td>
<td>1183±777°</td>
<td>4.06±1.59°</td>
<td>548±35°</td>
</tr>
<tr>
<td>Normal Subjects</td>
<td>170±80</td>
<td>-0.72±0.53</td>
<td>154±44</td>
</tr>
</tbody>
</table>

*p<0.0002 vs normal subjects; °p=0.0008 vs normal subjects; *p=0.0001 vs TM normal subjects

Conclusions. In thalassaemia pro-hepcidin levels were increased for the degree of iron load and for the possible effect of concomitant minor infections. In thalassaemic syndromes where iron overload and anaemia have opposing effect, the increased erythropoietic stress may influence hepcidin production. Understanding the mechanisms of iron homeosta- sis in patients with thalassaemia is of great significance in understanding the pathogenesis of iron load and planning novel treatments.
0570

THE ABSOLUTE RETICULOCYTE COUNTS AND IRON OVERLOAD CORRELATE WITH PULMONARY HYPERTENSION OBSERVED IN PATIENTS WITH SICKLE CELL/β-THALASSEMIA

E. Voskandeli,1 G. Tsetsos,1 A. Tsoutsias,1 E. Spyropoulou,1 C. Terpos2
1Thalassaemia Center, Laikon Gen. Hospital, ATHENS, Greece; 2Bioatriki Medical Center, ATHENS, Greece; 1,2 General Airforce Hospital, ATHENS, Greece

Background. Echocardiographic studies have reported that approximately 30% of screened adult patients with sickle cell anemia have pulmonary hypertension (PH) defined as systolic pulmonary artery pressures of above or equal to 35 mm Hg or regurgitant jet velocity (TRV) of above or equal to 2.5 m/sec. PH is increasingly observed in hemolytic anemias, including sickle cell disease and thalassemia in particular thalassemia intermedia. Brain natriuretic peptide (BNP) is released from the ventricles during pressure strain and its levels would correlate with severity of PH. Aims. The aim of this study was to evaluate the prevalence of PH in correlation with hemolytic findings and BNP levels in a cohort of patients with double heterozygous sickle cell trait and β-thalassemia (HbS/β-thal). Methods. We studied 52 patients (19 males and 33 females) with HbS/β-thal (thal 0: 35 pts and thal ±: 17 pts) who were followed up regularly in the Thalassemia Center of Laikon Hospital. Their median age was 53 years (range: 21–62 years). All pts were evaluated for the presence of PH using Doppler echocardiography and then applying the modified Bernoulli equation (Pulmonary artery systolic pressure=4V² + right atrial pressure). Exclusion criteria of this study include: 1) evidence of left ventricular failure; 2) vaso-occlusive crisis during the last 15 days; 3) atrial fibrillation or ventricular tachycardia; 4) mitral value regurgitation (MVR) >2/4+ or mitral value stenosis; and 5) severe pericardial perfusion. In all patients we measured Hb, leucocyte and platelet counts, reticulocyte counts, serum lactate dehydrogenase (LDH), bilirubin, ferritin, creatinine, Hb F and BNP levels. Twenty-four (46%) patients were on hydroxyurea administration for a median time of 9+5.5 years. Results. Thirteen (25%) patients had PH, according to established criteria. All patients had mild symptoms, such as fatigue or dyspnea on slight exertion. The administration of hydroxyurea did not affect the presence of PH. Patients with PH had elevated values of reticulocyte counts and serum ferritin and a borderline increase of HbF compared with non PH patients. No other parameter was different between the two groups of patients (Table).

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient with PH (n=13)</th>
<th>Patients without PH (n=39)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median; range)</td>
<td>41±9.4</td>
<td>36±13.9</td>
<td></td>
</tr>
<tr>
<td>Gender (n)</td>
<td>7M/6F</td>
<td>12M/27F</td>
<td></td>
</tr>
<tr>
<td>O2 on hydroxyurea (n)</td>
<td>(46.1%)</td>
<td>(46.1%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Hb (g/dl, mean±SD)</td>
<td>9.1±1.4</td>
<td>8.8±1.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Retics (x1000/mm³, mean±SD)</td>
<td>230±66</td>
<td>175±65</td>
<td>0.01</td>
</tr>
<tr>
<td>LDH (U/L, mean±SD)</td>
<td>772.5±359.7</td>
<td>782.7±348.7</td>
<td>0.46</td>
</tr>
<tr>
<td>Bilirubin (mg/dl, mean±SD)</td>
<td>2.3±1.7</td>
<td>2.4±1.2</td>
<td>0.45</td>
</tr>
<tr>
<td>Creatinine (mg/dl, mean±SD)</td>
<td>0.7±0.1</td>
<td>0.8±0.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Ferritin (µg/L, mean±SD)</td>
<td>1192.6±1124.2</td>
<td>449.1±694.8</td>
<td>0.02</td>
</tr>
<tr>
<td>BNP (ng/ml, mean±SD)</td>
<td>202.0±226.2</td>
<td>310.6±656.6</td>
<td>0.18</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>16.9±3.8</td>
<td>13.1±9.8</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Conclusions. The results of this ongoing study have shown that the frequency of PH in our cohort of HbS/β-thal patients is similar with that observed in patients with sickle cell disease. The correlation between PH with reticulocyte counts and ferritin suggests that the degree of hemolysis and iron overload may be implicated in the pathogenesis of PH in HbS/β-thal. There was no correlation between serum BNP or LDH and the presence of PH; however, this may reflect the number of patients available in the present study.

0571

CHARACTERISATION OF A DOUBLE MUTATION, THE NOVEL PRO92HIS AND PREVIOUSLY DESCRIBED GLU255-, OF NADH-CYTOCHROME BS REDUCTASE ASSOCIATED WITH APPARENT TYPE I RCM

M.J. Percy,1 L.J. Crowley,1 D.M. Layton,1 T.R.J. Lappin,1 M.J. Barber2
1Belfast City Hospital, BELFAST, United Kingdom; 2University of Florida College of Med., TAMPA, FLORIDA, USA; 1Imperial College, LONDON, United Kingdom; 1Queen’s University, BELFAST, United Kingdom

Background. The clinical disorder of recessive congenital methaemoglobinemia (RCM) is characterised by a deficiency of NADH-cytochrome b5 reductase (cb5r). There are two phenotypic forms of this disorder, type I and type II and although both types exhibit cyanosis from birth type II disease is accompanied by severe neurological defects. The cb5r enzyme consists of two distant sub-domains that comprise the FAD- and NADH-binding lobes, linked together by a hinge region. It participates in several pathways including the reduction of methaemoglobin, cholesterol biosynthesis and fatty acid metabolism. More than 40 different mutations have been described for cb5r that result in both forms of RCM and with the development of a heterologous expression system it is possible to characterise novel mutations in terms of enzymatic function and protein stability. Aims. To identify the molecular defect causing apparent type I RCM in a young girl and to characterise the identified cb5r variants using a bacterial expression system. Patient and Methods. A DNA sample was prepared from an apparent type I RCM patient and PCR-direct sequencing of the DIA1 gene, which encodes cb5r, was performed. The resultant variants were generated using a bacterial expression system. For comparison the wild-type cb5r and the previously described RCM variant, Pro95His, were also prepared. All proteins were purified to homogeneity and characterised for enzyme activity and thermostability. Results. Sequencing detected a heterozygous deletion of GAG, bases 27,100 to 27,102 (NCBI accession number NT_011520) in exon 9 resulting in loss of Glu255. In addition, a homozygous C to A change at base 16,076 (C16,076A) in exon 4 predicting an amino acid change to proline at codon 92. Uniquely, in this case one allele carries a double mutation. Previously, a change of proline to histidine at codon 95 was described for an individual with type II RCM. Investigation of both the Pro92His and Pro95His mutations indicated they impacted moderately on the enzymatic activity of cb5r without dramatic changes towards the NADH substrate or affecting the redox potential of the FAD prosthetic group. The thermostability of both Pro92His and Pro95His variants was greatly decreased, indicated by a reduction of TS0 by 11o and 9oC respectively compared to wild type enzyme. In contrast, the Pro92His and Glu255- double mutant exhibited substantially decreased enzyme activity, lower affinity towards NADH and reduced thermostability. Summary. Characterising the Pro92His and Glu255 variants, described in a type I RCM patient, indicated that the Pro95His mutation did not dramatically affect the function of cb5r but greatly decreased the thermostability of the protein. In contrast, the double mutation affected both the catalytic activity and the stability of the protein. The previously described Pro95His mutation also exhibited decreased protein stability but when present in combination with the Tyr42Ter mutation, resulted in type II RCM. Consequently, the pathophysiology of RCM appears to be influenced by the residual activity of the individual cb5r variants and the heterologous expression system provides a valuable tool in delineating between both types of RCM.

0572

COST ASSESSMENT OF B THALASSEMAIA MAJOR: THE ITACA STUDY

L.G. Mantovanini,1 S. Ravera,1 M.D. Cappellini2
1Center of Pharmacoeconomics Univers Milan, MILAN, Italy; 2Congenital Anemia Center Maggiore Hosp, MILAN, Italy

Background. People with severe anemia like β-thalassemia major require blood transfusions as life-long therapy. Regular blood transfusions can never cause iron overload that may damage vital organs, particularly the liver, the heart and the endocrine glands. Iron chelation therapy is essential to prevent end-organ damage and improve survival. Currently, the most available drug is Desferoxamine (DFO), parenterally administered by continuous infusion. Deferiprone (L1) is an oral iron chelator that is indicated for patients with the previously described DFO therapy, or for patients in whom DFO was proven to be ineffective. So far, only little is known about the cost of care with subjects with thalassemia undergoing iron chelation treatment and about their satisfaction and quality of life. Aims to investigate the costs, compliance,
A FAMILY WITH A MILD PHENOTYPE DESPITE MULTIPLE MUTATIONS IN THE α- AND β- GLOBIN GENE CLUSTER

University Medical Center Utrecht, Utrecht, Netherlands

Background. Hemoglobinopathies refer to a diverse group of disorders caused by an abnormal structure of the hemoglobin molecule. Thalassemias are hereditary disorders characterized by defective production of one of the beta- or alpha-globin chains. The aim of this study was to classify the aberrant hemoglobin and genetic variant results of the proposita and her family by means of molecular analysis. Methods. DNA isolation was performed using the QIAamp DNA Blood Kit (Qiagen). Hemoglobin variant analysis was performed by cation exchange HPLC (Biorad). DNA sequence analysis was performed using the ABI 310 genetic Analyzer (Applied Biosystems). Results. The proposita (female, age 20) was referred to our laboratory upon suspicion of thalassemia (Hb 9 mmol/L, MCV 72 fl, MCH 1.62 fmol, erythrocytes 5.56 x 10^12/L). She complained of fatigue but had no other clinical symptoms. Quantification of hemoglobin variants showed 11.3% HbA1, 4.4% HbA2, 59.9% HbC, and 23.0% HbF relative to total hemoglobin. On one allele of the patient we detected an HbC mutation (codon 6 GAG to AAG) in cis with the nonfunctional ψα and YG 10C>G promoter sequence variant (unpublished results). At the second allele three molecular alterations were found in the β-globin gene cluster: the 90C>T β-globin promoter mutation that is known to cause β-thalassemia and two novel mutations in the promoter of the YG gene (271C>T) and YG gene (403TGC→CTTAA). Further analysis of the patient’s α-globin gene cluster revealed also the presence of a heterozygous α-thalassemia (5.7 deletion). DNA analysis of four family members revealed, in addition, several other sequence deviations in the γ-globin genes. Three common mutations were detected in the γ-globin gene promoter: 222,226del (AGCC,A), 309A>G, and 369G>C, whereas one novel mutation was detected in the γ-globin gene promoter: 499T>A. The first of these mutations is known to lower γ-globin expression, the second is associated with increased HbF levels in normal healthy adults, the third is not associated with increased HbF levels in normal adults, and the functionality of the latter is unknown at present. Conclusions. In the here presented family a total of ten different mutations were found in the globin genes: one in the β-globin gene cluster and nine in the α, γ, and δ-globin genes. In spite of this, the proposita and all family members displayed a mild clinical phenotype. It is possible that, ultimately, this beneficial mild phenotype results from the interplay between the various identified genetic variants which function as phenotypic modifiers. This case shows that molecular analysis subsequent to biochemical analysis can be beneficial, but that the need for such analysis should always be considered in relation to the clinical practice.
es confounds diagnosis based on hematology alone, and definitive diagnosis is only achievable through DNA analysis. To date Hb Heraklion has been observed in a single Greek case and Hb Taybee in sporadic Israeli-Arab cases. There is minimal experience for the management of such atypical cases, and our previous experience indicates that it is probably insufficient to monitor clinical status in patients with hemoglobinopathies based on hemoglobin levels alone.1

Reference

0576
EPOETIN DELTA, ERYTHROPOIETIN PRODUCED BY A HUMAN CELL LINE, IS EFFECTIVE IN THE TREATMENT OF RENAL ANAEMIA

R. Pratt
Shire Pharmaceuticals, WAYNE, USA

Background. Several recombinant erythropoietins are currently available for the treatment of anaemia associated with chronic renal failure and cancer. All of these agents are produced in Chinese Hamster Ovary cell lines and as a result have glycosylation patterns that differ from endogenous erythropoietin. Epoetin delta (Dynepo®, Shire) is an erythropoietin produced in a human cell line through gene activation. Aims. To assess the efficacy and safety of different doses of epoetin delta in patients with anaemia and chronic renal failure requiring haemodialysis. Methods. In this multicentre, double-blind study, haemodialysis patients with anaemia (haemoglobin < 10.0 g/dL) who had not previously received an epoetin were randomized to receive epoetin delta (15, 50, 150 or 300 IU/kg) or epoetin alfa (50 IU/kg) three times a week. In the initial correction phase, patients received the allotted dose until 'correction success' was reached (two consecutive weekly haemoglobin measures ≥ 11.5 g/dL or one measurement ≥ 101 previously untreated patients success) then entered a maintenance phase, during which the dose was titrated to maintain haemoglobin levels at ≥ 10.5 g/dL. The maximum duration of treatment was 12 weeks. Maintenance success was defined as haemoglobin ≥ 10.5 g/dL at week 12. Total success was defined as achievement of both correction success and maintenance success. Data from the groups assigned the two highest doses of epoetin delta were pooled and compared with results from the lowest dose group. Results. In total 78 patients were randomized and 75 received treatment (epoetin delta 15, 50, 150 and 300 IU/kg: 21, 14, 13 and 13 patients, respectively; epoetin alfa 50 IU/kg: 14 patients). Baseline haemoglobin levels were similar in the pooled epoetin delta and epoetin alfa groups (8.66±0.94 and 8.57±0.82 mg/dL). The proportion of patients achieving total success was higher in the pooled highest dose epoetin delta group (150 and 300 IU/kg) compared with the lowest dose (15 IU/kg) group (55.6% vs. 4.5%; p=0.0002). Analysis of dose trend across the epoetin delta groups showed a significant trend for an increase in total success and correction success with increasing doses (15, 50, 150, 300 IU/kg: total success, 4.5%, 21.4, 50.0, 61.5%, respectively, p=0.0001 for trend; correction success, 9.1, 21.4, 57.1, 61.5%, respectively, p=0.0002 for trend). There were no significant differences in success rates between epoetin delta 50 IU/kg and epoetin alfa 50 IU/kg. The incidence of treatment-emergent adverse events was similar in the epoetin delta and epoetin alfa groups. Adverse events thought to be possibly related to epoetin delta occurred in 11.5% of patients and most were mild or moderate in severity. There was no evidence of dose-related adverse events. Conclusions. Epoetin delta is effective in increasing haemoglobin levels in patients with haemoglobin < 10g/dL as a result of chronic renal failure, and shows at least similar efficacy to epoetin alfa at an equivalent dose. Safety profiles were similar for the two agents. No patient receiving epoetin delta developed anti-erythropoietin antibodies.

Cytogenetics and Molecular Cytogenetics

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ONCOGENIC DEREGULATION OF HOXA GENES BY CHROMOSOMAL RERARRANGEMENTS IN T-CELL LYMPHOPBLASTIC LEUKEMIAS (T-ALL)

Hôpital Saint-Louis, PARIS, France

T-cell acute lymphoblastic leukaemias (T-ALL) are highly malignant tumours which derive from partially differentiated T-cell progenitors. We recently reported the identification of a new recurrent chromosomal rearrangement in human T-ALL, targeting the major homeobox gene cluster HOXA and the T-cell receptor genes locus TCRB (Soulier et al., Blood 2005). This rearrangement was found in four patients out of a series of 92 T-ALL and corresponded to inv(7)(p15;q34) and t(7,7) (p15;q35), in 5 and 1 cases, respectively. The 4 HOXA breakpoints were analysed at the molecular level by Southern blot in the 4 cases, and cloning of the two derivative breakpoints in two cases. The breakpoints clustered within a 2.6 kb region in the HOXA locus. In order to analyse the molecular consequences of this rearrangement, the expression of the 11 HOXA genes was analysed on micro-array data and by specific RT-PCR. We found that the whole HOXA gene cluster expression was deregulated in the rearranged cases, on both side of the breakpoint cluster region, compared to other T-ALL. Mechanisms of this deregulation remain elusive. Two additional groups of T-ALL demonstrated a global HOXA cluster deregulation, namely the CALM-AF10 and the MLL-rearranged T-ALL cases. These results strongly suggested that the deregulation of HOXA genes is oncogenic in T-ALL. Global gene expression analysis and unsupervised hierarchical classification in the 92 cases T-ALL series demonstrated that the TCRB-HOXA associated cases clustered in an homogeneous subgroup, which shared common expression profile with the TLX1/HOX11 and TLX3/HOXL2 associated subgroups. This suggested use of common biological oncogenic pathways in these homeobox genes associated T-ALL. Like other T-ALL, these cases frequently demonstrated NOTCH1 gene activation mutations and CDKN2A/p16/ARF deletions, consistent with multi-events oncogenesis. We then analysed expression of two alternative transcripts, HOX9b and HOX10b, considering the clusterization of the breakpoints between the HOXA9 and the HOXA10 genes in the TCRB-HOXA translocated cases. Interestingly a massive expression of the HOX10b transcript was demonstrated, whereas no significant expression was detected in the CALM-AF10 and MLL-associated T-ALL cases, or in other T-ALL cases. The HOX10b transcript has been detected during early embryogenesis in mice and in leukemic cell lines. It encodes a short HOX10b protein which retains the homeobox domain of the regular HOX10 protein (HOX10A) but lacks the N-terminal regulation domain. We found no expression of HOX10b during T-cell differentiation by analysing normal human thymus samples, showing that its expression in T-cell leukemic cells was ectopic. Considering the specific expression of the HOX10b transcript in the TCRB-HOXA cases, we are currently analysing the phenotypic consequences of the HOX10b gene overexpression in mouse models using retroviral transduction of bone marrow progenitors.

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CORRELATION BETWEEN CHROMOSOMAL ABNORMALITIES AND IMMUNOHISTOCHEMICAL PROFILE IN DIFFUSE LARGE B CELL LYMPHOMAS REVEALS DISTINCT LYMPHOMAGENESIS PATHWAYS WITH CLINICOPATHOLOGIC SIGNIFICANCE AND PROGNOSTIC VALUE

Centre Henri Becquerel, ROUEN, France

Background. DLBCL constitutes a heterogeneous group. The genetic and molecular mechanisms underlying their diverse clinical presentations and outcomes have been partially clarified by the recent application of DNA microarrays and next generation sequencing technologies. Comparative genomic studies of DLBCL also revealed a broad spectrum of clonal genetic abnormalities and complex karyotypes, including, chromosomal translocations, deletions, duplications and other complex alterations. However the potential clinicopathological relevance of these alterations is still poorly defined. Patients and Methods. 101 previously untreated patients...
diagnosed with de novo DLBCL at our hospital between 1987 and 2003 were selected (median age = 59 years, 50 males, IPI 0-1: 32%; 2-5: 46%; 4-5: 22%). The inclusion criteria were the availability of appropriate paraffin-embedded tissues and a karyotypic analysis using R-bandng method. Hierarchical clustering analysis based on immunostaining with a large panel of antibodies (including cell-cycle control, apoptosis, immune response and B-cell differentiation markers) was performed and correlated with recurrent cytogenetic abnormalities and outcome. The germlinal center B-cell-like (GC) and the non-GC phenotypes were defined using CD10, BCL6 and MUM1 immunostaining. Results. Among the 101 studied patients, 10 karyotypes were considered as normal. The most frequent numerical genetic abnormalities were monosomy 15 (19%), trisomy 15 (13%), 7 (8%) 11 (17%) 12 (18%) 16 (17%) and XQ (16%). The most frequent structural abnormalities involved 1p (31%), 1q (38%), 2p (21%), 3p (16%), 3q (45%), 4q (21%), 5q (16%), 6q (36%), 7q (19%), 8q (15%), 9p13 (26%), 11q (15%), 14q32 (49%), 18q (5%). The t(4;14), t(3;4) and t(8;14) were observed in 21%, 20% and 2% of cases respectively. The GCB phenotype was observed in 40% of cases and is significantly related to t(14;16) (36%), trisomy 12 (56%), and 18q21 (45%) or 2p (31%) rearrangements. The non-GC phenotype was observed in 54% of cases and correlated with 3p (23%) and 3q (57%) rearrangements. DLBCL with t(14;18) are preferentially CD10+ (72%), BCL2+ (68%) and MUM1 negative (56%). By contrast, DLBCL with t(3;4) were more often p53+ (41%), MUM1+ (94%), usually expressing the anti-apoptotic protein Bcl-2 (70%) but were BCL2 negative (88%). Using an unsupervised hierarchical clustering approach based on the expression of a large panel of antibodies, 82% of cases could be properly reclassified only by considering the presence of a t(14;18) or of a t(3;4), indicating clearly 2 distinct cells of origin. Finally, p53 protein expression correlated with 1p7q and 3q27 rearrangement. Genetic abnormalities with a significant unfavourable prognosis impact were the 17p, 3p, 8q24 and 9p13 rearrangements. A scoring system, including all unfavourable genetic abnormalities was strongly predictive of the outcome, independently of the GC/ non-GC phenotype and was confirmed in an independent series of 87 DLBCL. In addition to these clonal genetic abnormalities, BCL2, CDS and p53 rearrangements were associated to a poor clinical outcome. Conclusion. This study demonstrates correlation between chromosomal abnormalities and immunohistochemical profile in DLBCL, and reveals distinct lymphomagenesis pathways with clinicopathologic significance and prognostic value. These results contribute to a molecular database which could allow the identification of new relevant genes involved in lymphomagenesis. 0579 PDGFRB FUSIONS TO TPM3 IN THE T(1;5)(Q23;Q33) OF CHRONIC EOSINOPHILIC LEUKEMIA AND TO NDE1 IN THE T(5;16)(Q33;P13) OF CHRONIC MYELOMONOCYTIC LEUKAEMIA R. Rosati,1 L. R. La Starza,1 A. Bardi,2 L. Luciano,2 C. Matteucci,2 V. Pierini,3 S. Romoli,2 B. Crescenzi,2 F. Pan2, A. Cuneo,4 M.F. Martelli,1 J. Cools,5 P. Marynen,5 C. Mecucci1 1Hematology, PERUGIA, Italy; 2Hematology, University of Ferrara, FERRARA, Italy; 3Hematology, Federico II University, NAPLES, ITALY, 4Center for Genetics and VIB, LEUVEN, Belgium Background. Ph-negative chronic myeloid leukemia, namely atypical chronic myeloid leukemia and chronic myelomonocytic leukemia (CMML) with bone marrow and/or peripheral blood eosinophilia are associated with PDGFRB/5q33 translocations. The 3′ region of PDGFRB encoding the kinase domain fuses with the 5′ region of a partner gene encoding an oligomerization domain, which determines the constitutive activation of PDGFRB tyrosine kinase. To date, ten different PDGFRB gene partners have been identified and several PDGFRB translocations with undefined gene partners have been also observed. Aim. Identification of new PDGFRB partners in patients with 5q33 translocations. Methods. Patient 1, a 21-year-old man with chronic eosinophilic leukemia showed a 46,XY,t(1;5)(q23;p33) karyotype in 28/29 metaphases. The patient underwent α-IFN and oncorbicide treatment for ten years and switched to imatinib after identification of a PDGFRB rearrangement. Patient 2, a 36-year-old woman with Noonan syndrome and exon 3 PTPN11 missense germline mutation, was admitted because of CMML. Cytopheric analysis showed the following karyotype: 46,XX,t(5;16)(q53;p15) (11)/46,XX[3]. After assessing PDGFRB involvement in vitro imatinib mesylate therapy was administered and haematological, cytogenetic, and FISH remission was achieved after six months. FISH with two cosmids for PDGFRB/5q33 (9-4 and 4-1) was performed in both patients. DNA clones for the 1q23 band in patient 1, and for 16p13, in patient 2, were applied to narrow breakpoints and to select candidate partners. RT-PCR with gene-specific primers were performed to amplify TPM3/PDGFRB and NDE1/PDGFRB fusions from patients 1 and 2, respectively. Amplions were sequenced for confirmation. Functional assays were performed on the NDE1/PDGFRB fusion by testing transduced Ba/F3 cells for IL-3 independent growth. Sensitivity of the NDE1/PDGFRB fusion protein to imatinib mesylate was assessed. Results. Cosmids for PDGFRB gave a red/green fusion signal on normal 5 and a split signal with cosmid 9-4 retained at der(5) and cosmid 4-1 translocated to der(1) (patient 1) or der(16) (patient 2). In patient 1, the 1q23 breakpoint fell within clone RP11-205M9. In patient 2, DNA clone CTD-2303E13 NDE1/16p13 and cosmids 27/29 for the 3′MYH11/16p13 were present on normal 16 and on der(5) while cosmids 14/18 for the 5′MYH11/16p13 and clone RP11-8H13 MR1/16p13 gave one signal on normal 16. In patient 1, RT-PCR for TPM3/PDGFRB amplified a chimeric transcript joining TPM3 exon 7 to PDGFRB exon 11. In the second case, RT-PCR showed fusion between exon 5 of NDE1 and exon 11 of PDGFRB. Ba/F3 cells transduced with NDE1/PDGFRB fusion were transformed to IL-3 independent growth. The NDE1/PDGFRB fusion protein was shown to be sensitive to imatinib. Conclusion. We identified TPM3/1q23 and NDE1/16p13 as two new PDGFRB/5q33 partners in patients with CEL and CMML, respectively. Interestingly, the TPM3 gene is already known for its involvement in the t(1;2)(q25;p23)/TPM3-ALK of anaplastic large cell lymphomas and for its association with ETV6/RUNXI in papillary thyroid carcinomas, while NDE1 haploinsufficiency has been associated with the onset of a MDS-like syndrome in mice. Acknowledgements. DNA clones were kindly provided by Dr. M. Rocchi, University of Bari, Italy. Supported by: MIUR, FIRB, AULL, and Fondazione Cassa di Risparmio, Perugia, Italy. B.C. is supported by a grant from FIRC.
META-ANALYSIS OF 966 B-CELL NEOPLASMS WITH 8q24 ABERRATIONS IDENTIFIES DISTINCT CYTOGENETIC ABERRATION PATTERNS OF BURKITT AND NON-BURKITT LYMPHOMAS

M. Baudis,¹ R. Siebert³

¹University of Florida, GAINESVILLE, USA; ²Institut für Humangenetik, KIEL, Germany

Background. Burkitt lymphoma (BL) is cytogenetically characterized by a translocation juxtaposing the MYC locus on band 8q24 next to the IGH locus on 14q32 or one of the light chain loci on 2p12 and 22q11. However, translocations affecting the MYC locus are not exclusive for BL but also occur in other B-cell neoplasias. Aims. Based on published karyotypes derived from conventional cytogenetic analyses, we intended to define the typical cytogenetic signature of Burkitt lymphoma and to delimit this signature from other B-cell lymphomas (B-NHL) with 8q24 translocations. A typical cytogenetic signature could be used as adjunct for clinical diagnosis, and may point towards loci targeted by disease-specific genetic events.

Methods. We performed a meta-analysis of karyotypes from 966 B-cell neoplasms from the Mitelman database with 8q24 breakpoint in the main clone using software from the Progenetix project. 461 cases were diagnosed as Burkitt lymphoma or leukemia. The remaining cases consisted (in decreasing number) of ALL (NOS), DLBCL, B-NHL (NOS), myeloma, FCL, and other B-NHL entities. Results. 440 BL cases lacked a translocation involving 8q26-q27 or 18q21. Of those typical BL, 258 had chromosomal imbalances (average 1.3 imbalances, median 1 imbalance per case). The 346 non-BL B-NHL with cytogenetic translocations indicating a MYC/IG fusion showed a greater overall chromosomal instability (average 5.7 imbalances, median 3 imbalances). In BL, recurring gains involved 1q21-q22 (21%), chromosome 7 (7%) and chromosome 12 (5%). Losses were uncommon, with a maximum of 4.5% on 17p. Other regions affected by chromosomal aberrations in B-NHL like 9q, 6q, 13q and 18q were rarely involved in BL. No differences were observed for lymphomatous and leukemic variants of BL. Gains on 1q and 12 were nearly exclusive in BL, with co-occurrence in only one case. In contrast, the 21 BL cases with additional 3q26-7 or 18q21 break exhibited typical B-NHL aberrations, such as 6q24 loss (15%) or 18q gain (10%) but no gain on 1q. The non-BL-B-NHL cases with cytogenetic translocations indicating a MYC/IG fusion displayed a heterogeneous pattern of imbalances. As in BL, the most common gains involved 1q21-22 (24.9%), 7 (19.4%) and 12 (10%). However, chromosomes 19 (10.1%), 3 (9.8%), 9 (9.4%) and others (21, 11q, 5, 15) had frequent gains, too. Recurring losses involved 6q21 (11.9%) and 13q (11.9%). In contrast to the BL subset, limitation to single chromosomal imbalances was much less common (37% for 0 or 1 imbalance in non-BL B-NHL vs. 70% in BL, p<0.001 for distribution). Summary. Burkitt lymphomas with cytogenetic translocations indicating a MYC/IG fusion contain only few, usually single genomic imbalances. The low complexity of BL underscores the etiologic importance of the IG/MYC fusion in this disease. The mutually exclusive pattern of imbalances may point to alternative genomic events co-operating with IG/MYC translocations in BL. BL cases with additional B-NHL abnormalities may be part of a distinct disease group. Non-BL-B-NHL with 8q24 translocations display a heterogeneous pattern and larger number of chromosomal imbalances. Our analysis exemplifies the importance of large data collections for determining relevant cytogenetic aberration patterns.

Figure 1. Imbalances in BL vs. other B-cell malignancies.

MOLECULAR CHARACTERIZATION OF DISTINCT HOT SPOT REGIONS ON CHROMOSOME 7q IN MYELOID LEUKEMIAS

R.T. Obermiller,¹ M. Habdank,¹ F.G. Rücker,¹ S. Miller,² S.W. Scherer,³ H. Döhner,¹ K. Döhner¹

¹University Hospital of Ulm, ULM, Germany; ²Hospital for Sick Children, TORONTO, Canada

Background. Loss of whole chromosome 7 (7-) or deletion of the long arm (7q-) are recurring chromosome abnormalities in myeloid leukemias. In recent years, several groups initiated the molecular characterization of the deletion and translocation breakpoints. Based on these results a common deleted segment (CDS) of approximately 2 Mb in size was identified in chromosomal band 7q22 flanked by the microsatellite markers D7S1503 and D7S1841. Recently, we mapped the translocation breakpoint of a (7q7)(p13q22) within this genomic segment and identified a novel gene (MLL5, mixed lineage leukemia 5) that represents a candidate gene for chromosome 7 associated leukemias. With respect to deletions affecting the distal part of chromosome 7q a to 4 to 5 Mb sized CDS was defined encompassing chromosomal bands 7q35 to q36. However, several other CDS and translocation breakpoints on 7q have been described so far, suggesting the existence of more than one disease-related gene. Aims. To identify and characterize translocation and deletion breakpoints of myeloid leukemias including acute myeloid leukemia (AML). Methods. Using software from the Progenetix project, 461 cases were diagnosed as AML (70% M0 to M7) and myelodysplastic syndromes (MDS) (30%). Results. AIMS. 458 cases were included in this study. The mean interval between the samples was 26 months (range 12-77). Samples without clonal abnormalities were considered as failures. Using conventional cytogenetic banding techniques, 15/36 (36%) initial and 19/39 (49%) follow-up samples had an abnormal karyotype. The origin of respectively 19 and 37 distinct breakpoints was investigated. Conclusions. The breakpoint of an unbalanced translocation from a patient with secondary AML between the markers D7S1925 and D7S1395. This region was defined encompassing chromosomal bands 7q35 to q36. The breakpoint region was identified in 34 cases and was located close to the proximal border of the CDS. Conclusion. Our data further indicate the remarkable heterogeneity of deletion and translocation breakpoints on 7q and revealed several hot spot regions that may serve as important starting points for the identification of pathogenetically relevant genes.

SERIAL ANALYSIS OF CHROMOSOME ABERRATIONS IN MULTIPLE MYELOMA: HIGH PREVALENCE OF STRUCTURAL ABNORMALITY OF CHROMOSOME 1 DURING DISEASE PROGRESSION

K.L. Wu, H.B. Beverloo, P. Sonneveld

Erasmus MC, Rotterdam, The Netherlands

Background. Two major genetic subtypes of multiple myeloma (MM) have been proposed: the hyperdiploid subtype characterized by multiple trisomies and low prevalence of del(13q), and the non-hyperdiploid subtype characterized by IgH translocations and del(13q). Primary IgH translocations have been considered as initiating events in the pathogenesis of MM. The role of del(13q) and other chromosome abnormalities in disease progression has not been clarified. Aims. To investigated the evolution of chromosomal abnormalities during disease progression.

Methods. Analysis of the cytogenetic abnormalities of serial bone marrow samples from MM patients that entered in the clinical cytogenetic database at Erasmus MC. Results. Seventy-five serial samples obtained from 36 patients at diagnosis (26 samples) and during progression of the disease (49 samples) were included in this study. The mean interval between the samples was 26 months (range 6-77). Samples without clonal abnormalities were considered as failures. Using conventional cytogenetic banding techniques, 15/36 (36%) initial and 19/39 (49%) follow-up samples had an abnormal karyotype. The origin of respectively 19 and 37
marker chromosomes could not be identified. Of the remaining 32 samples, 15 were in diploid and 17 in hyperdiploid, respectively. Serial studies showed an increased number of chromosomal abnormalities during disease progression. The mean number of aberrations increased from 11 (range 1-38) to 18 (range 2-36). Trisomies of chromosomes 5, 9, 11, 15 and 19 were the most common numerical abnormalities. Monosomy of chromosome 18 was identified in 5/18 initial and 7/19 follow-up samples with non-translocated karyotype. Amplification of chromosome 1 was the most common structural abnormality (25/162) (15%) and were detected in 2/13 initial and 10/19 follow-up samples. Both the short and long arms of chromosome 1 were involved and no-specific locus was predominantly affected. The rearrangement of chromosome 1 consisted in the majority of unbalanced translocations and resulted in the formation of t(1;19). Aberrations of chromosomes 8 and 9 were second in frequency (6%) and were detected in 1/13 initial and 9/19 follow-up samples. FISH analysis was performed in 56 samples and showed an abnormality in 15/22 (68%) initial and 25/34 (74%) follow-up samples with probes specific for RB-1 (13q14) and D15S19 (13q14.3) loci and for the cen tromere regions of chromosome 9 and 11. Del(13q) was observed in 10/22 (45%) initial and 14/34 (41%) follow-up samples. Summary: Cytogenetic abnormalities in multiple myeloma are not random. Disease progression is correlated with increasing complexity of cytogenetic karyotype, which consist mainly of structural aberrations acquired during later stages of the disease. Aberrations of chromosome 1 are common in multiple myeloma. In particular, unbalanced translocations of 1p/1q have been delineated as genetic event associated with progressive disease and unfavourable prognosis. Del(13q) is not associated with disease progression.

References

OSB5
CHROMOSOMAL ABERRATIONS ARE DETECTED IN 80% OF CLL PATIENTS BY METAPHASE CYTOGENETICS: A STUDY OF 132 CLL CASES WITH CORRELATION TO FISH, IGVH STATUS, AND CD38 EXPRESSION
S. Dicker, S. Schnittger, T. Haferlach, W. Kern, C. Schoch
MIL Munich Leukemia Laboratory GmbH, MUNICH, Germany

Chronic lymphocytic leukemia (CLL) is a heterogenous disease from a clinical as well as from a genetic point of view. Compared to fluorescence in situ hybridization (FISH) conventional metaphase cytogenetics plays only a minor prognostic role in chronic lymphocytic leukemia (CLL) so far due to technical problems resulting from limited proliferation of CLL cells in vitro. Here we present a simple method for in vitro stimulation of CLL cells which overcomes this limitation. CLL cells were induced to metaphase generation by culturing leukaemic cells with media with the addition of the immunostimulatory oligonucleotide DSP30 plus interleukin 2. In our unselected patient population 125/132 cases could be successfully stimulated for metaphase generation. 101/125 cases showed chromosomal aberrations. The aberration rate is comparable to the rate detected by interphase FISH, which was performed in parallel. Conventional cytogenetics detected additional aberrations in 47 patients compared to FISH analysis. A complex aberrant karyotype, defined as ≥3 aberrations, was detected in 30/125 patients compared to only one such case as defined by FISH. Samples with 17p deletions in FISH had a complex aberrant karyotype in 85% of cases. Conventional cytogenetics frequently detected balanced and unbalanced translocations. A significant correlation of the poor prognosis unmutated IgVH status with unbalanced translocations and of the likewise poor prognosis CD38 expression to balanced translocations and complex aberrant karyotype was found. We demonstrate that FISH analysis underestimates the complexity of chromosomal aberrations in CLL. Therefore, conventional cytogenetics may define subgroups of patients with high risk of progression.

OSB6
T(4;11) PATIENTS REVISED: T(4;11) PATIENTS CARRY TWO MLL FUSION ALLELES
C. Meyer,1 E. Kowarz,1 T. Dangermann,2 T. Klingebiel,3 R. Marschalek1
1Johann Wolfgang Goethe-University, FRANKFURT/MAIN, Germany; 2Institute of Pharmaceutical Biology/DCL, FRANKFURT/MAIN, Germany; 3Pediatric Hematology and Oncology, FRANKFURT/MAIN, Germany

The chromosomal translocation t(4;11) is the most frequent chromosomal aberration of the human MLL gene, associated with infant and early childhood acute lymphoblastic leukemia (ALL) or therapy-related acute leukemia. The disease phenotype is correlated with poor Prednisone-response and outcome. Thus, leukemia patients carrying t(4;11) translocations are treated according to high-risk acute leukemia therapy regimen. Compared to other MLL translocations, no cellular or animal model system is currently available which mimics the human t(4;11) translocation phenotype. It has been reported that the conditional expression of an MLL•AF4 fusion protein causes cell cycle arrest instead of proliferation. Therefore, t(4;11) translocations seem to belong to another disease mechanism that cannot be explained by the current disease model. Here we present the first in vivo experiments where the MLL•AF4 fusion protein is feasible for the oncogenic character. The AF4 fusion partner is a potent oncoprotein in mammalian cells. The oncogenic properties were located in the N-terminal portion of AF4, the same portion fused to the MLL in the AF4+ MLL fusion protein. The latter fusion protein was able to growthtransform mammalian cells equally well as H-ras, a well known oncoprotein. However, this finding is in contrast to the fact that only about 80% of all t(4;11) patients seem to encode both reciprocal fusion genes (MLL•AF4+AF4•MLL+ patients), while for 20% of these patients, only the presence of the MLL•AF4 fusion gene (MLL•AF4+/AF4•MLL-...
patients) could be successfully identified either by RT-PCR or by direct genomic PCR. This controversial data prompted us to investigate MLL•AF4+/AF4•MLL- leukemia patients in more detail by using a FISH PCR-based method. This allows to identify and characterize chromosomal aberrations of the human MLL gene in an unbiased fashion. 13 individual MLL•AF4+/AF4•MLL- leukemia patients out of 76 (t(4;11) leukemia patients were identified (65 were MLL•AF4+/AF4•MLL+ leukemia patients). The 13 MLL•AF4+/AF4•MLL- leukemia patients were analyzed for the presence of rearranged genomic MLL sequences. 10 patients displayed a complex rearrangement between chromosome 4 and 11 (and sometimes a third chromosome) that involved at least the MLL, the AF4 and a third partner. Funded by grant 2002.061.1 from the Wilhelm Sander Foundation to R.M., T.K. and T.D.

**0587**

**FISH-MLL ABNORMALITIES IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA AND ASSOCIATION WITH FLT3 AND MLL INTERNAL DUALPLICATION**

E. Giugliano, G. Rege-Cambrin, A. Serra, The MLL gene on chromosome 11q23 is frequently involved in haematological malignancies. It is possible to subdivide the MLL abnormalities in two groups: 1) rearrangements, usually as translocations or insertions, and partial tandem duplication (PTD); 2) amplification of the 11q23 region, leading to the presence of multiple copies of the MLL gene, located either intrachromosomally, as har and iso11q, or extrachromosomally in dmin and numerical aberrations of chromosome 11. MLL/PTD is the in-frame fusion of a duplicated portion of the MLL gene. Internal tandem duplication (ITD) or mutations have been demonstrated as a activating mechanism also in another oncogene involved in AML, FLT3 gene, which encodes for a tyrosine kinase receptor widely expressed in haemopoietic lineage. The FLT3/ITD is observed in approximately 20% of unselected de novo adult AMLs, with a higher frequency around 30-40% reported for patients with normal cytogenetics. It is associated with poor prognosis in most series. It has been reported that FLT3/ITD is more common in patients with MLL/PTD than in cases with MLL translocations. Recently, a role for coduplication of MLL and FLT3 genes has been suggested in AML as possible marker of a common genotoxic stress. Aim. We investigated the incidence of MLL abnormalities in 207 patients with de novo acute myeloid leukemia, diagnosed following FAB criteria and treated according to the GIMEMA protocols. We used quantitative cytogenetics and fluorescent in situ hybridization (FISH) analysis with a MLL probe. The patients were also tested for the presence of an internal duplication of the MLL and FLT3 gene and for the FLT3 D835 mutation. Methods and Results. Cytogenetic analysis on bone marrow was successful in 175 cases and showed aberrations of chromosome 11 in 12 patients (6.9%). FISH analysis performed with MLL Dual-Color probe (Vysis) was available in 194 cases and demonstrated the MLL involvement in 25 cases (12.9%). Ten patients were rearranged (5.2%); 15 cases showed overrepresentation of MLL gene without evidence of rearrangement (7.7%). FLT3/ITD or D835 mutation were observed in 27.4% and MLL/PTD in 5.3% of the patients. FLT3 abnormalities were present in 20% (5/25) whereas MLL/PTD was observed in 18.7% (3/16) of the cases with involvement of MLL at FISH analysis. Conclusions. The FISH investigation of MLL contributes to the identification of multiple copies of the gene in marker chromosomes, rings, double minutes, har. The presence of MLL amplification is not rare in acute AML and the FISH analysis allows to improve the characterization of MLL involvement when compared with conventional cytogenetics. The incidence of FLT3 alterations is similar in MLL abnormal patients (20%) when compared to the whole AML population (27.4%); on the contrary, MLL/PTD is confirmed to be more frequent in patients with aberrations of chromosome 11 (18.7% vs 5.3% of unselected AML). The rate of MLL/PTD was superior in FLT3 positive (7.7%) than in FLT3 negative patients (4.4%). In this study the coduplication of FLT3 and MLL/PTD had a low incidence around 2.3% in all cases and did not correlate with cytogenetic MLL abnormalities.

**0588**

**A NEW CRYPTOGENIC MOLECULAR LESION UNDERLIES 6P CHANGES IN SECONDARY ACUTE MYELOID LEUKEMIA/MYELODYSPLASTIC SYNDROME (AML/MDS)**

R. La Starza, A. Aventin, C. Matteucci, B. Crescenzi, S. Romoli, N. Testoni, V. Pierini, S. Ciollri, C. Sambani, A. Locasciulli, M. Pechot-Culatofo, M. F. Martelli, F. Marynen, C. Mecucci

Hematology, University of Perugia, PERUGIA, Italy; *Hospital Sant Pau, BARCELONA, Spain; †University of Perugia, PERUGIA, Italy; ‡University of Florence, FLORENCE, Italy; †Health Physics and Environmental Hygiene, NCSR DEMOKritos, GRC; †Hematology, S Camillo Hospital, ROME, ITALY; †Lab de Biopathologie, Inserm U114, MARSEILLE, France; †Center for Human Genetics and VIB, LEUVEN, Belgium

Background. Secondary AML/MDS are frequently associated with complex karyotypes involving chromosomes 5, 7, 11, 12, 17, and 21. Specific genetic pathways are related to physical and/or chemical toxicities, such as -5/-5q- to alkylating agents or 11q23 and 11p15 changes to topoisomerase II inhibitors. 6p aberrations are cytogenetically heterogeneous and often belong to complex karyotypes with del(5)(q)/-5 and/or del(7)(q)/-7. Aim. Molecular characterization of 6p rearrangements in secondary AML/MDS. Methods. We selected nine patients with secondary AML/MDS and one Fanconi Anemia patient with MDS with a rearrangement on the short arm of chromosome 6. Karyotypes of G-banded metaphases were described according to ISCN (1995). Metaphase FISH with a panel of 38 DNA clones for 6p12-25 bands was performed in all cases and 4p12-16, 11q23, 11p15, 10p15 metaphase and/or interphase FISH, CGH and/or multi-FISH were performed in selected cases. Results. 6p rearrangements were isolated in 4 patients and included in complex karyotypes in 6. Numerical or structural aberration typically associated with therapy-related AML/MDS, i.e. -5/-5q, -7/-7q, monosomy 18 were respectively found in four, three, and three patients. In three cases full or partial trisomy of the 6p arm was present. 6p(10) in one case and dup(6)(p) in two cases. The remaining 7 patients showed 6p unbalanced translocations with diverse chromosome partners or unidentified material. In 5 patients with unbalanced translocations, FISH detected cryptic duplications of a genomic region contiguous to the translocation breakpoints, at band p21, while in two patients a low copy gain with five copies of DNA clones mapping at band p21, were present on der(6) and/or inserted in other derivative chromosomes. In all cases a common over-represented 6p21 region was narrowed to a 5-6 megabase DNA segment extending from the TNF gene to ETV-7. Two patients did not show 6p21 gain. Conclusion. 6p21 gains, either as duplication/trisomy or low copy gain, emerged as a new recurrent genomic lesion in secondary AML/MDS with 6p abnormalities. Remarkably, they may be cryptic at conventional cytogenetics and underlie different types of chromosome changes. Putative candidate genes, such as the MHC complex, NOTCH-4, BAK, IANCE, ETV-7, HMGIY and FKBP51, map on the common over-represented 6p21 region. Low copy gains occurred in both treatment and environmentally induced AML/MDS as well as in the FA patient, toxic insults and congenital instability appear to share the same genetic pathway. Acknowledgements. The karyotypes were kindly provided by Dr. M. Rocchi, University of Bari, Italy. Supported by: CNR-MUR, FBB. Associazione Sergio Luciani, Fabbrano, and Fondazione Cassa di Risparmio, Perugia, Italy. B.C. is supported by a grant from FIRC (Fondazione Italiana Ricerca sul Cancro).

**0589**

**CYTOTOGENIC AND FISH STUDY IN 203 B-CLL PATIENTS**


University Hospital, OLOMOUC, Czech Republic

Background. The progress in molecular genetic characterization of chronic lymphocytic leukemia (CLL) revealed the prognostic role of IgVH mutational status, of phenotypic changes involving expression of CD38 and ZAP-70, as well as, of chromosomal abnormalities defined by molecular cytogenetic Methods. Interphase fluorescence in situ hybridization (iFISH) is able to detect the most common chromosomal abnormalities of 13q, 17p deletions and trisomy 12. Aim: The purpose of this study was to determine the chromosomal abnormalities in 203 CLL patients using cytogenetic and molecular cytogenetic methods, and to correlate the molecular genetic findings with disease status (stable versus progressive), with immunoglobulin variable heavy chain (IgVH) mutational pattern, and with other clinical parameters. Methods and patients. 123 males and 80 females (median of age 62 years) were examined by con-
ventional cytogenetic examination on TPA stimulated cells from peripheral blood (167), bone marrow (54) and/or lymph node (2), and by FISH on fixed cells. The locus specific and centromeric probes were used (ABBOT-VYSIS) for FISH. CGH and M-FISH were used to detect abnormalities deletions of ATM, RB1 and trisomy 12 were detected in 19 patients (9%) – deletions of RB1 gene together with deletion of ATM gene in 11 of them, and – deletions of RB1 gene together with deletion of p53 in 8 patients in this group. Three abnormalities deletions of ATM, RB1 and trisomy 12 were detected in only one patient. Summary. FISH is rapid and sensitive method for determination of chromosomal aberrations of prognostic relevance in CCL patients. The deletion of 15q14 was the most frequent (43%) chromosomal aberration detected by FISH in our cohort of CLL patients. The correlation of molecular cytogenetic results with IgVH mutational pattern and with clinical data were analyzed and will be presented.

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A A ROYAL CYTGENETIC STUDY OF MANTLE CELL LYMPHOMA AT DIAGNOSIS AND FOLLOW-UP: EVIDENCE FOR A ‘TEMPORALLY ORDERED’ CYTGENETIC EVOLUTION?


Background. Apart from t(11;14)(q13;q32), MCL is also characterized by other nonrandom cytogenetic findings. These additional aberrations are well studied at diagnosis and believed to represent clonal evolution during lymphomagenesis, but little is known about karyotypic changes that occur after diagnosis in terms of clonal evolution.

Methods. The study included 33 patients with MCL. In all cases, an interphase FISH assay was performed at diagnosis to determine potential aberrations deletions of the involved regions. Commercial probes were employed for the detection of different chromosomal abnormalities deletions of ATM, p53, trisomy 3q and 13q, and deletions of p16 and RB1. Results. The most frequent additional findings at diagnosis were deletions in 15q14 (45.5%), 13q- (36.4%), followed by p16 deletion (3 cases; 1 homozygous), p53 deletion (2 cases), and +12, duplication of the CCND1/IGH fusion gene and BCL6 triplication, in one case each. 11 of the 14 cases studied at follow-up showed karyotypic evolution, with acquisition of p16 deletion (6 cases; 4 homozygous), TEL deletion (5 cases; 2 on the basis of monosomy 12), duplication of the CCND1/IGH fusion gene (3 cases), p53 deletion (2 cases), and -MYC amplification (1 case). There was no case with acquisition of ATM deletion, 13q- or +12, but in two cases with 13q- in a minor subclone at diagnosis the aberration was estimated to involve the total of the lymphoma cells at relapse. Interestingly, new BCL6 aberrations were seen in 3 cases (triplication in one and amplification in the other two, including the case with gene triplication at diagnosis) and were detected at the third or the fourth repetition of the screening. The longest survival after detection of these aberrations was 3 months. Conclusions. The data suggest that in most cases of MCL clonal evolution also occurs during the course of the disease, with the acquisition of multiple additional chromosomal lesions. Despite the small number of patients in our series, it seems that some of the aberrations (like ATM deletion or 13q-) are most commonly already present at diagnosis, while others (such as monosomy 12 and TEL deletion) appear more often or even exclusively on follow-up. From the clinical point of view, we found that the most informative finding is the over-representation of the BCL6 gene, apparently associated with aggressive behavior and perhaps the terminal stage of MCL.

PROGNOSTIC SIGNIFICANCE OF COMPLEX CHROMOSOMAL REARRANGEMENTS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

L. Baibica, Z. Zemanova, L. Pavlivstova, J. Brezinova, M. Moravcova, H. Klamova, K. Michalova

‘General Faculty Hospital and First Med Fac, PRAGUE, Czech Republic; Institute of Hematol. and Blood Transfl, PRAGUE, Czech Republic

Background. Ph chromosome i.e. translocation t(9;22)(q34;q11) is a specific chromosomal aberration in bone marrow cells of patients in chronic phase (CP) of CML. During progression of the disease from the chronic to the accelerated phase (AP) and/or blast crisis (BC), clonal evolution with non-random secondary numerical and structural aberrations is frequently observed. Complex chromosomal rearrangements (CCR) are rather rare and the significance and frequency of different anomalies are poorly understood. Aims. The aim of our study was a comprehensive analysis of complex chromosomal rearrangements found in bone marrow cells of 22 patients with CML by molecular cytogenetic methods, determination of chromosomess and chromosomal parts which are involved in CCR during progression of the disease and estimation of frequency of non-random changes if they exist. Methods. For the assessment of BCR/ABL fusion gene at the time of diagnosis RT-PCR and/or interphase FISH with locus-specific probe (Abbott-VysisTM) were used (200 independent interphase nuclei analyzed). CGH and FISH were used to detect abnormalities deletions of 7q, gains of 3q and few incidence of translocations. In some patients further molecular analyses were performed by real-time RT-PCR according to EAC protocol using β-2-microglobuline as a control gene. Multicolor FISH (mFISH) was carried out using the ‘24XYce’ MetaSystems 24 color kit (MetaSystemsTM) to identify precisely complex chromosomal rearrangements in 22 patients. Most of the patients were in the CP at diagnosis. In the course of the disease clonal evolution with complex chromosomal rearrangements appeared in eight patients who remained in CP, two patients progressed to AP and the rest of them to BC. Results. The majority of the structural changes were unbalanced. Variant Ph translocations (involving chromosomes 9, 22 and one or more other chromosomes) were found in ten patients, the rest of the cohort had a classical Ph translocation associated with additional structural aberrations. The most frequent combinations involved in CCR were found to be Nos. 2 (6x), 7 and 17 (5x), 1, 3, 4 and 5 (4x). Chromosomal regions 1p, 2p, 5q, 7p and 17p were often involved in CCR and the bands repeatedly affected were 17p11.2 (4x) and 7p15 (2x). No one of complex translocation was seen more than once. Conclusions. The results of this study demonstrate the very high instability of the genome of malignant cells at the chromosomal level than was expected on the basis of classical cytogenetic analyses. We also proved that CCR are associated with rather poor prognosis and respond poorly to antileukemic treatment. Analysis of CCR by mFISH is important as we believe that such examinations of large cohorts of patients could confirm the significance and non-randomness of this instability and to find out possible recurrent chromosomal aberrations specific for disease progression. Precise determination of breakpoints on chromosomes involved in CCR of bone marrow cells of CML patients can give new dimension to our understanding of genetic mechanisms which can play role in progression of malignant disease.

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tology) and to know the implication of PAX-5 gene in this pathology. Methods. Among a series of 141 SMZL we applied spectral karyotyping (SKY) in 25 cases with complex karyotype. In patients with t(11;14) we studied by FISH the involvement of PAX-5 gene using a split probe (Dako, Denmark). Results. In 3 out of 25 cases with t(11;14) (p13;q32) detected by SKY, rearrangement of PAX-5 was confirmed. Our three patients harbored complex karyotype. The most frequent additional aberrations were gains of chromosome 1 (5 cases) and gains of chromosome 3 (2 cases). They showed morphology and immunophenotypic features typical of SMZL. All three cases presented bone marrow involvement and two showed a splenic diffuse pattern uncommon in this pathology. Summary/Conclusions. In all patients t(11;14) was found after the application of spectral karyotyping (SKY) technique confirming that complex rearrangements could mask this anomaly when are studied by conventional cytogenetic. Our findings confirm the rare but recurrent involvement of t(11;14) in SMZL cases and that this anomaly is not specific for a subtype of NHL. The prognosis of PAX-5 rearrangements in SMZL demands unclar and a further follow-up of patients is necessary to better understand the role of this aberration. Acknowledgements. This work has been partially supported by grants from Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo (PI 051072) and Fundación La Marató de TV3 (Cancer). We want to thank Juan Cruz Cigudosa for his help in the tune-up of SKY technique and Carme Melero for their expert technical assistance.

**0893**

**I-FISH ANALYSIS OF IMMUNOFLOURESCENTLY LABELED PLASMA CELLS IN PATIENTS WITH MULTIPLE MYELOMA**

L. Pavlistova,1 Z. Zemanova,1 J. Skuhrovceva,1 I. Spicka,2 E. Gregora,1 A. Dohnalova,3 K. Michalova1
1General Faculty Hospital and First Med Fac, PRAGUE; Czech Republic; 2Gen Fac Hospital, PRAGUE, Czech Republic; 3FHKV, PRAGUE, Czech Republic; 4Inst. of Physiology, First Medical Faculty, PRAGUE, Czech Republic.

**Background.** Early detection of specific chromosomal aberrations in plasma cells of patients with MM may have diagnostic, prognostic and therapeutic implication. One of the most frequent and prognostically most significant clonal aberrations in MM are rearrangements of IgH gene at 14q23 region (generally poor prognosis), deletions of RB1 gene at 13q14 and/or loss of whole chromosome 13 (moderately adverse or medium prognosis). The translocation t(11;14)(q13;g32) is associated with longer overall survival and, in contrast to other IgH rearrangements, it is considered to be a favorable prognostic factor. However, the detection of genetic aberrations involved in MM by conventional cytogenetic and/or classical I-FISH methods may be limited by low proliferative index of plasma cells. The sensitivity and specificity of I-FISH analysis may significantly increase previous immunofluorescent labeling of malignant myeloma cells. This method allows identification of chromosomal changes even in cases with low bone marrow infiltration. Aims. The aim of the study was to assess the frequency of the most significant chromosomal abnormalities (abnormalities of IgH gene, deletions of RB1 gene) in a series of MM patients with advanced immunofluorescently labeled non-dividing plasma cells of patients with MM by I-FISH and to evaluate their prognostic significance. Methods. I-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). For I-FISH directly marked locus specific DNA probes (Abbott-Vysis) were used. Detection of deletion/monosomy of chromosome 13 was performed by LSI 13q14 (RB1) and LSI 13q34 DNA probes. Aberrations of 14q32 region were proved by LSI IgH rearrangement probe. For detection of specific IgH translocations LSI IgH/CCND1 and/or LSI IgH/FGFR3 probes were used. Cytogenetic findings were correlated with different clinical and laboratory parameters. Results. Altogether 114 newly diagnosed MM patients were examined by I-FISH. Deletion of RB-1 gene was found in 22 (19%) patients and monosomy 13 was identified in other 34 (30%) of them. Combination of both aberrations was proved in 6 (5%) cases. Aberration of IgH gene was found in 60 (57%) from 106 evaluated patients (deletions, partial trisomies and monosomies and numerical changes involving chromosome 14 were also found). Sixteen out of 33 cases (48%) evaluated for t(11;14)(q13;g32) were positive. Another six patients were examined for t(4;14)(p16;q32) and translocation was proved in four of them. Patients with aberration of 13q had significantly shorter event-free survival (EFS). Strong association with advanced clinical stages was also proved. Between IgH positive and IgH negative cases, difference in EFS was not statistically significant due to heterogeneity of IgH positive patients. In most cases t(11;14) is associated with other chromosomal aberrations and prognostic relevance of these findings remains to be cleared. Summary: I-FISH on plasma cells detected by immunofluorescent staining permits to increase yield of number of chromosomal abnormalities in MM patients. This method significantly contributes to the higher sensitivity and specificity of diagnostic procedures and is important for determination of prognosis and treatment of MM patients. Our results confirmed 13q aberrations as a marker of poor prognosis (p=0.008).

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**0954**

**DETECTION OF STRUCTURAL ABERRATIONS OF CHROMOSOME 7 IN MYELOID MALIGNANCIES USING COMBINATION OF MOLECULAR CYTOGENETIC TECHNIQUES**

J. Brezinova,1 Z. Zemanova,1 J. Melichercikova,2 M. Siskova,1 J. Cermak,1 K. Michalova1
1Institute of Hematology and Blood Transf, PRAGUE; Czech Republic; 2Cen-ter of Oncocytogenetics, PRAGUE; Czech Republic; 1sts Med. Dept. Gen. Fac-ulty Hosp., PRAGUE; Czech Republic.

**Background.** Complete or partial loss of chromosome 7 is a frequent chromosomal aberration in myeloid disorders such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Allelotypic studies have delineated at least three distinct loci, that are frequently deleted: 7q22, 7q31 and 7q35. It has been hypothesized that there are localized tumor suppressor genes that contribute to the pathogenesis of these disorders. Aims. Using combinations of conventional and molecular cytogenetic techniques we have focused on the analyses of deletions and translocations involving chromosome 7 in bone marrow cells of patients with MDS and AML. Correlation of clinical characteristics, outcome and survival of patients according to cytogenetic findings were evaluated. Methods. Using conventional cytogenetic and/or classical I-FISH methods may be limited by low proliferative index of malignant myeloid cells. This method allows identification of chromosomal aberrations involved in MM by conventional cytogenetic and/or classical I-FISH methods may be limited by low proliferative index of plasma cells. The sensitivity and specificity of I-FISH analysis may significantly increase previous immunofluorescent labeling of malignant myeloma cells. This method allows identification of chromosomal changes even in cases with low bone marrow infiltration. Aims. The aim of the study was to assess the frequency of the most significant chromosomal abnormalities (abnormalities of IgH gene, deletions of RB1 gene) in a series of MM patients with advanced immunofluorescently labeled non-dividing plasma cells of patients with MM by I-FISH and to evaluate their prognostic significance. Methods. I-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). For I-FISH directly marked locus specific DNA probes (Abbott-Vysis) were used. Detection of deletion/monosomy of chromosome 13 was performed by LSI 13q14 (RB1) and LSI 13q34 DNA probes. Aberrations of 14q32 region were proved by LSI IgH rearrangement probe. For detection of specific IgH translocations LSI IgH/CCND1 and/or LSI IgH/FGFR3 probes were used. Cytogenetic findings were correlated with different clinical and laboratory parameters. Results. Altogether 114 newly diagnosed MM patients were examined by I-FISH. Deletion of RB-1 gene was found in 22 (19%) patients and monosomy 13 was identified in other 34 (30%) of them. Combination of both aberrations was proved in 6 (5%) cases. Aberration of IgH gene was found in 60 (57%) from 106 evaluated patients (deletions, partial trisomies and monosomies and numerical changes involving chromosome 14 were also found). Sixteen out of 33 cases (48%) evaluated for t(11;14)(q13;g32) were positive. Another six patients were examined for t(4;14)(p16;q32) and translocation was proved in four of them. Patients with aberration of 13q had significantly shorter event-free survival (EFS). Strong association with advanced clinical stages was also proved. Between IgH positive and IgH negative cases, difference in EFS was not statistically significant due to heterogeneity of IgH positive patients. In most cases t(11;14) is associated with other chromosomal aberrations and prognostic relevance of these find-
**0595**

**T(5;12)(Q23-31;P13) WITH ETV6-ACSL6 GENE FUSION IN POLYCYTHEMIA VERA**

A. Murati,1 J. Adlade,1 V. Gelsi-Boyer,2 A. Etienne,2 D. Sainty,1 L. Xerri,1 N. Vey,3 H. Fezoui,4 M. Chaffanet,4 J. Duszenko,5 M. Mozziconacci1 1Institut Paoli-Calmettes, MARSEILLE, France; 2Institut Paoli-Calmettes, MARSEILLE, France; 3Hopital Font Pr, TOULON, France

**Background.** Myeloproliferative disorders (MPD) are chronic clonal proliferations of haematopoietic progenitors. Typical MPDs include chronic myeloid leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET) and idiopathic myelofibrosis (IF). Oncogenic alterations identified so far in MPDs target tyrosine kinases, result from chromosomal translocations or gene mutations and lead to constitutive activation of survival and proliferation pathways. Reciprocal translocations lead to gene fusion and production of chimeric proteins such as BCR-ABL, ETV6-PDGFRB or PCM1-JAK2. Point mutations of the JAK2 kinase occur in almost all PV, and in around half of ET and IF. JAK2 functions downstream of membrane receptors, including cytokine receptors such as IL3 receptors. Overproduction of IL3 has been reported in atypical CML following rearrangements of the IL3 gene upstream region in cells from patients with t(5;12)(q31;q22) translocation and ETV6-ACSL6 fusion. **Aims.** We report here two cases of t(5;12)(q23;31)p13) translocation with ETV6-ACSL6 rearrangement in PV patients. **Methods and results.** Cytogenetic analysis with R-banding technique on BM cells detected a t(5;12)(q23;31)p13) translocation. We demonstrated the involvement of ETV6 and the 5′ region of ACSL6 (previously ACS2) in the translocation by using dual-color fluorescence in situ hybridization (FISH) on metaphases of MPD cells from one patient, using labeled-BAC clones. The 5′ breakpoint was characterised by using two contiguous RAC clones, from centromere to telomere and results showed that the breakpoint was located in the 5′ region of ACSL6. We determined the status of the JAK2 kinase in this patient by sequencing DNA from peripheral blood cells. The Val617Phe mutation was not found. Due to the orientation of the rearranged genes on the der(12), it is probable that the cause of this upregulation is a chromatin conformation change rather than an ETV6 promoter effect. In one of the patients the sequencing of blood cells DNA failed to show a JAK2 mutation. Two explanations may be proposed. First, because the t(5;12) is only present in 20% of mitoses, the JAK2 mutation could be present in the same clone but not detected by our DNA analysis although its measured sensitivity was below this range; in this case, the translocation would appear as a secondary event. Eosinophilia was detected in one patient with M2 subtype of AML at the second relapse, concomitantly with the occurrence of the t(5;12). Alternatively, increased level of IL3 due to the rearrangement may enhance non-mutated JAK2 activity and trigger PV, eosinophilia and basophilia, and may eventually lead to acute transformation of the translocated clone. If this is the case, genomic events such as a t(5;12) rearrangement may account for few JAK2-negative PV.

**0596**

**ADDITIONAL CHROMOSOMAL ABNORMALITIES IN PH-POSITIVE CML DE NOVO: A MULTICENTER STUDY**

Z. Salamanczuk,1 B. Pienkowska-Grela,1 E. Wawrzyniak,1 I. Kardas,1 M. Iliszko,2 B. Mucha,3 M. Jakbczyk,1 E. Duszko,2 O. Haus2 1Medical College, Jagiellonian University, KRAKOW, Poland; 2Centre of Oncology, WARSAW, Poland; 3Department of Haematology, LODZ, Poland; 4Department of Biology&Genetics, CDANSK, Poland; 5Department of Clinical Genetics, BÝDGOSZCZ, Poland

Chronic myeloid leukemia (CML) is a clonal disorder of multipotent haematopoietic cells associated with specific cytogenetic changes involving a translocation t(9;22)(q34;q11) resulting in Ph chromosome occurrence. Cytogenetic investigations revealed that Ph chromosome appeared most often as a sole karyotype aberration during the chronic phase, whereas additional changes frequently accompanied or preceded a transformation to the advanced stages of CML (50-80%). However, the additional cytogenetic changes are found in 10-20% of CML patients at diagnosis and their prognostic impact is still difficult to assess unequivocally. Cytogenetic study as a part of Polish national program of Development of standard operating procedures for the diagnosis and follow up of treatment of CML in 2005 was performed to evaluate the appearance and the frequency of additional cytogenetic changes in patients with de novo CML. Material and Methods. A total number of 206 newly diagnosed Ph-positive CML patients investigated in 6 genetic centers formed the subjects of this study. All cases were analyzed by routine cytogenetic techniques on unstimulated and/or stimulated bone marrow samples according to standard protocols. Karyotypes were described in accordance to the ISCN1995. Additionally, nearly all cases underwent FISH, RT-PCR, and RQ-PCR analysis for BCR/ABL. Results: 40 of 206 patients (19.42%) showed aberrations different from simple t(9;22). Constitutional changes (inv(9)(p13q13)) were found only in 2 patients. In the remaining patients, karyotypes showed rearrangements related to leukemia. Three-way Ph translocations were observed in 13 cases (52.5%), involving nonrandom chromosome bands, such as t(1q21, 2p15, 3p21, 3q21, 10q25, 10q2), t(7;17)(q32;q25), inv(16)(p11.2q24), del(17)(p13), add(21)(p11), 17q24. Other structural changes not related to Ph translocation, accounted for 30% of cases (12/40): add(1)(p36), t(1;19)(q32;p13), t(1;17)(p32;p13), add(2)(qter), t(4;22)(p16;q11), t(4;22)(q31;q13), del(6)(q21), t(7;17)(q32;q25), inv(16)(p11.2q24), del(17)(p13), add(21)(p11), add(18)(p11). Numerical aberrations were also observed in 30% of cases (12/40). Beside the most common trisomy/tetrasomy 8 and +Ph, the aberrations: +X, +Y, +15, +17, +19, +21, +21, were disclosed. Few unidentified markers were also revealed. Conclusions. Among described aberrations, we found significant part of nonrandom cytogenetic changes described previously, but some of them, to our knowledge, are described for the first time in relation with CML. For all patients with additional aberrations further observations and at least 1 year follow-up are needed to evaluate their prognostic significance based on clinical stage, prognostic scores and response to treatment.
Genomics and proteomics

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FUNCTIONAL ANALYSIS OF CANDIDATE GENES LOCALISED IN 13q14.3, A REGION COMMONLY AFFECTED IN B-CLL
C. Tschuch, A. Pscherer, S. Wolf, M. Hahn, P. Lichter, D. Mertens
German Cancer Research Center, HEIDELBERG, Germany

Background. Genomic material from chromosomal band 13q14.3 is lost in a variety of neoplasms. Thus, a tumor suppressor mechanism distinct from the RB1 gene has been postulated in this region. In B-cell Chronic Lymphocytic Leukemia (B-CLL), the most common leukemia in the Western world, deletion within chromosomal band 1q14.3 is the most frequent genomic imbalance. However, the pathomechanism in the critical region has not yet been defined. Characterisation of the function of genes in the critical region will allow identification of the most likely tumor suppressor candidate genes and their role in tumor pathomechanism. Therefore, we analysed the function of different candidate genes localised in 13q14.5. Aim. The aim of this project is functional analysis of candidate genes localised in 13q14 such as RFP2, C13orf1, DLEU2/LEU2/BCMSUN/DLB2 and KPNAs. To this end, expression levels of those genes were modulated by knock down and overexpression followed by subsequent analysis of transcriptome changes. Methods. Gene expression was modulated by overexpression using cDNA plasmids and knock down using RNAi. A combined lipofection and electroporation technology was used in order to obtain sufficiently high transfection efficiency in cell lines with loss of 13q14 (Granta 519). RNA was isolated from cells after 4, 7 and 12 hours to check genome wide gene expression levels via oligonucleotide arrays. In a second strategy, we used RNai technology to knock down RFP2, C13orf1, DLEU2 and KPNAs in human embryonic kidney cells (HEK-293) and HELA cells. RNA was isolated after 48h to identify effects in downstream target genes via expression profiling. Differentially expressed genes of both strategies were verified using Real-Time-PCR. Results. To analyse phenotypic changes in transfected cells, a significant overexpression of knockdowninduced down-regulated genes was essential. For RFP2 and C13orf1, we could achieve an overexpression of over 90 fold compared to the transfection of empty vector. Overexpression of DLEU2 was only possible up to 12 fold. In HELA cells, the knock down of C13orf1 and KPNAs was over 70%. For RFP2, a 60% knock down could be achieved and for DLEU2, the knock down was between 40 and 50%. Downregulation of KPNAs by RNAi was also shown on protein level. Using RNAi in mammalian cells specificity of knock down has to be shown. There exists an interferon response mechanism in case double stranded RNA is injected into the cell for example by a virus. To be sure not to induce the interferon response in our siRNA transfected cells, we checked expression level of a marker gene for interferon response (OASI). Using both strategic approaches, we could identify a number of gene products which are affected by modulation of expression of candidate genes of B-CLL. Summary. Candidate genes of B-CLL were modulated in their expression levels and phenotypic effects were analysed by expression profiling genome-wide. Our results suggest involvement of different signalling pathways in the pathomechanism residing in 13q14.

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VALUE OF PROTEOMIC SCREENING FOR PREDICTION OF GRAY VERSUS HOST DISEASE AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
E.M. Weissinger,¹ H. Zakaria,¹ E. Schiffer,¹ H. Mischak,¹ B. Hertenstein,¹ A. Ganser²
¹Hannover Medical School, HANNOVER, Germany; ²Mosaiques-Diagnostik GmbH, HANNOVER, Germany; ³Klinikum Bremen Mitte, BREMEN, Germany

Background. We have recently published a polypeptide pattern specific for the early diagnosis of acute GvHD (aGvHD), based on the application of capillary electrophoresis (CE) and mass spectrometry (MS). Aims. Here we report the application of an aGvHD-specific pattern to prospectively and blindly collected samples from 76 patients (33 AML, 10 sAML, 13 ALL, 2 MDS, 6 PCT, 5 SAA, 3 CLL, 2 OME; 2 Hodgkin Lymphoma, 1 CML, 1 NHL[mh]). Fifty patients were transplanted from matched unrelated donors (MUD), 27 received stem cells from matched related donors (MRD), 2 from haplo-identical donors. In the majority of the patients the GvHD prophylaxis was methotrexat (59) or mycophenolate(24) and cyclosporin A, 2 patients received T-cell depleted grafts and 4 received steroids instead of MTX. Methods. Urine samples were collected on ice prior to conditioning, weekly until discharge from the ward and monthly thereafter. Immediate freezing of the samples avoids degradation of the proteins/peptides. After thawing and removal of confounding materials, like salts, or molecules larger than 30 kDa, the samples were loaded onto the CE, separated according to their charge and, after ionization, directly analyzed in an electrospray ionization time of flight (ESI-TOF)/MS. This lead to the detection of 500 up to 2500 peptides and proteins in individual samples. Results. The polypeptide patterns specific for detection of acute GvHD grade II or greater were applied to the data from the prospectively and blindly collected samples. A total of 760 samples were evaluated using this set up. In general between 4 and 10 samples were collected and screened after HSCT, whereas the control groups (other diseases) contain only 1 sample per patient. Taken together, 800 samples from patients after HSCT have been prospectively evaluated. The sensitivity was 92.3% with a positive prediction value of development of aGvHD of 83% and the specificity was 94% with a negative prediction value of more than 98%. Conclusion. We have shown that the application of a peptide pattern, consisting of several differentially excerted peptides allows prediction of the development of aGvHD. Especially patients developing steroid resistant aGvHD show the aGvHD-specific changes very early (about 10 days) prior to clinical symptoms. Taken together, a therapeutic strategy using the aGvHD pattern as guidance for an early start of immunosuppression seems justified.

0599
FINE-MAPPING THE HISTONE CODE AT 13q43.3, A CRITICAL REGION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA
M. Ruppel, D. Mertens, P. Lichter
German Cancer Research Center, HEIDELBERG, Germany

Background. B-cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia among adults in the Western world. The most recurrent genomic aberration is deletion of a critical region in chromosomal band 12q14.3, which is found in over 50% of cases (Stilgenbauer et al. 1998). So far, no tumor suppressor gene has been identified. The minimal deleted region of B-CLL harbor protein-coding genes (RFP2, KCNRG and FAM10A4), two long non-coding RNA genes (BCMS and DleU2/BCMSUN) and two microRNA genes (mir-15a and mir-16-1). In B-CLL patients, RFP2 is most significantly down-regulated (Mertens et al. 2002). Current evidence indicates that the region is regulated by an epigenetic mechanism. In order to identify this epigenetic pathomechanism, we analyzed the histone code, which epigenetically marks genes as being active or inactive, and which could explain their differential expression. Aim. In this study we fine-mapped the chromatin of the region 13q14.3 by analyzing DNA- and histone-modifications in order to identify the epigenetic mechanism responsible for the transcriptional deregulation of 13q14.3. Methods. We analyzed the chromatin modifications of the region by chromatin immunoprecipitation (ChIP) using antibodies specific for different histone modifications. Specificity was verified by Western blot analysis of precipitated chromatin and the precipitated CpG-islands localized in 13q14.3 were quantified with Q-PCR. Results. Chromatin immunoprecipitations showed enrichment of methylated lysine 4 of histone H3 (H3K4) at CpG-islands in the vicinity of the two non-coding RNA genes of 13q14.3. Between the different CpG-islands in the region enrichment of methylated H3K4 differed. Surprisingly, histone modifications also concentrated to specific regions within one CpG-island. Conclusions. The CpG-islands localized in the vicinity of one non-coding RNA gene of 13q14.3 harbor a specific histone code. Interestingly, we find differences between histone modifications and the distribution of DNA-methylation. These differences indicate a regulatory mechanism involving epigenetic modifications that could explain expression patterns of candidate genes in normal and in tumor cells. Our systematic fine-mapping of the histone code in the critical region will show, whether this region is uniformly marked by different histone modifications and to which extent there are differences between the seven CpG-islands in the region.

References
XLI PREDISPOSES TO MYELOID MALIGNANCIES BUT GAIN OF FUNCTION WASP MUTATIONS ARE NOT FOUND IN MYELODYSPLASIA OR AML

K.A. Beel, E. Schollen, A. Uyttebroeck, G. Verhoef, H. Demuynder, K. Devriendt, P. Vandenberghe

KULeuven, LEUVEN, Belgium; H.Hart Hospital, ROESLARE, Belgium

Background. X-linked neutropenia (XLN), described in 2001 in a three generation family with 5 affected men, is caused by a T843C WAS mutation, resulting in a L270P gain-of-function mutation GTPase binding domain (GBD) of the Wiskott-Aldrich-syndrome protein (WASP) (Nat Genet 2001,27,313). XLN is characterized by recurrent bacterial infections, severe neutropenia and monocytopenia, decreased CD4/CD8+ ratios, and bone marrow maturation arrest at the promyelocyte/maturemyelocyte stage. The mutation disrupts the auto-inhibitory domain of WASP, causing constitutive activation, and leads to neutropenia by an unknown mechanism. Since our original report, only one more case, originally diagnosed as myelodysplasia, has been identified, with an I294T mutation (Blood 2001,98,439a). Aims. 1) to extend the description of the XLN phenotype, including 2 cases of myelodysplasia 2) to investigate whether patients with GB mutations can present with myelodysplasia, AML or monosomy 7. Methods. We investigated 206 cases with myelodysplasia (n=49) or acute myeloid leukemia (n=157). There were 21 cases with median age 12y, range 2m-20y. 56 Cases had monosomy -7. Male/female ratio was 1.35. There were two brothers with monosomy 7, one with AML M5 and the other with secondary ALL after MDS. Exons 7-10, encoding the GTPase Binding Domain (residues 230-381), were amplified and screened for mutations using dHPLC. DNA from case IV2 (Nat genet, 2001, 27, 313) was used as positive control. Results. We observed 2 myeloid malignancies among the 5 affected members in this family. Patient III5 developed a myelodysplastic syndrome (MDS-RAEB) at age 65, while under hematopoietic growth factor support. He achieved stable remission after three courses of decitabine, but died after another course. Patient III6 succumbed to refractory MDS RAEB, rapidly evolving to MDS-RAEB at age 38. In both cases, monosomy 7 was identified in the leukemic cells. Although immunoglobulin levels are normal in adults, case IV1 had IgA deficiency at age 12mo. At the age of 12 years, he developed auto-immune adrenalitis. Together with the inverted CD4/CD8 ratios that were observed in 5/5 tested cases, this further supports immune dysregulation as part of the phenotype. Reasoning that the maturation arrest at the promyelocyte/maturemyelocyte stage can masquerade as myelodysplasia and that XLN can evolve to MDS/AML, we investigated whether GB mutations occur in myelodysplasias. In 206 samples from patients with MDS or AML, with or without monosomy 7, no intronic mutations were found in the exons 7-10 of the WAS gene. One mutation was found in intron 6 in a male patient with AML and monosomy 7. The mutation did not influence splicing of the exon and was thus considered irrelevant. Conclusions. Immune dysregulation and increased risk of MDS seem to be part of the XLN phenotype. However, GB mutations could not be identified in these patients, and thus the XLN-phenotype is not associated with GB mutations.

INTRONIC MICRO RNA ANALYSIS IN LEUKEMIA REVEALS COORDINATED EXPRESSION WITH HOST GENES

S. San-Marina, F. Suarez Saiz, M. Minden
Ontario Cancer Institute, TORONTO, Canada

In leukemia, the integrity of the transcriptome is altered by chromosomal translocations, deletions, duplications, as well as by epigenetic changes in chromatin structure. By targeting miRNAs for translational repression or RNAse-dependent hydrolysis (AU-rich miRNAs or shRNA-like effects), the micro RNA (miRNA) component of the transcriptome is estimated to regulate expression of up to 50% of all proteins. Yet the causes and role of deregulated miRNA expression in malignancy are largely unknown, in part because promoter events are not characterized. Since more than one-third of all known mammalian miRNA genes are encoded in the introns of protein-coding genes they may be regulated by the same promoter events that regulate host-gene miRNA expression. To identify such regulatory events, we performed expression profiling and analysis of miRNAs and their host genes we compared Affymetrix U133A gene expression data for the promyelocytic NB4 and acute myelogenous leukemia AML2 cell lines with the expression of miRNA precursors. We found similar patterns of host gene expression in the two cell lines and a good correlation with the expression of miRNA precursors in NB4 cells (r=0.464, N=30 miRNAs, p<0.016). To further demonstrate that host gene miRNAs and miRNAs are expressed from common transcripts, we activated promoter events by enforcing the expression of Lyl1a basic helix-loop-helix transcription factor that is often over-expressed in AML. This resulted in a greater than 2-fold increase in hsa-mir-126, -102b, 184b, -102a, -102c, -102, -102-3p and a corresponding increase in host gene expression. Meta-analysis of microarray data across many experiments and platforms (available through Oncomine.org) has been used to study the cancer transcriptome. To help determine if intrinsic miRNAs play a substantial role in malignancy, we correlated host gene expression data with the expression of selected miRNA precursors. Less than 20% of all differentially expressed genes in leukemia and lymphoma were predicted targets, compared to 68% in breast cancer. Since the Gene Ontology term on immune response is most commonly associated with miRNA host genes, the data suggest that this cancer module is relatively inactive in leukemia and lymphoma, compared to breast cancer. Gene cluster analysis of a leukemia data set using only miRNA host gene expression was able to classify patients into similar (but not identical) subsets as did an analysis based on over 20,000 transcripts. To further demonstrate that miRNAs and their host genes are expressed from the same transcription unit, we correlated the expression of miRNA precursors with the expression of genes that are either hosts for miRNAs or are situated several kilobases downstream of a miRNA, and thus belong to different transcription units. We applied this analysis to a subset of 81 AML patients that presented a normal karyotype and found significant negative correlations (p<0.01) between the levels of host genes for hsa-mir-15b, -103-1, and -125b and the expression of their predicted gene targets, but no statistically significant correlation between non-host genes and targets for up-stream miRNAs. These data demonstrate co-regulated expression of host genes and intrinsic miRNAs and the usefulness of intrinsic miRNAs in cancer profiling.

IN VITRO AND IN VIVO VALPROIC ACID RESPONSE SIGNATURES IN ADULT ACUTE MYELOID LEUKEMIA

1University of Ulm, ULM, Germany; 2Stanford University, STANFORD, United States of America

Background. Patients with acute myeloid leukemia (AML) show a poor response to standard chemotherapy, with 50% of patients dying within 100 days of diagnosis. In vitro AML cell lines are useful models to study the mechanism of therapeutics, and to induce tumor-selective cytoxicity against blasts from patients with acute myeloid leukemia (AML). While there exist many known mechanisms involved in AML pathophysiology, there remain many unsolved questions like e.g. what factors determine whether a cancer cell undergoes cell cycle arrest, differentiation, or death in response to HDACIs. In addition, most studies evaluated HDACIs as single agents in vitro. The question arises whether the efficacy of single HDACIs is still limited as a result of the emergence of drug resistance in vivo, as well as HDACIs function in combination with standard induction chemotherapy. Aims. Following determination of VPA response signatures in different myeloid leukemia cell lines and in primary AML blasts in vitro, we next sought to analyze in vivo VPA effects in blasts from adult de novo AML patients, entered within two randomized multicenter treatment trials of the German-Austrian AML Study Group. Methods. Using DNA Microarray technology, we profiled gene expression in order to determine VPA in vitro response signatures following 48 hours VPA treatment of myeloid leukemia cell lines (HL-60, NB-4, HEL-1, CMK, and K-562) and primary AML blasts. Next, in an initial attempt we evaluated the VPA effects on gene expression in AML samples (n=15) collected within the AMLSG 07-04 trial for younger (age<60yrs) and older adults (age>60yrs), and within the AMLSG 06-04 trial for older adults (age>60yrs), in which patients are randomized to receive standard induction chemotherapy (idarubicine, cytarabine, and etoposide = ICE) with or without concomitant VPA.Gene expression was profiled in AML blasts collected at the time of diagnosis and following 48 hours of treatment with ICE or ICE/VPA. Results. In accordance with previous studies in vitro VPA treatment of myeloid cell lines induced the expression of the cyclin-dependent kinase inhibitors CDKNA and CDKN2D coding for p21 and p19, respectively. In general, supervised analyses using SAM (Significance Analysis of Microarrays) revealed many gene signatures to be associated with a G1 arrest. In all cell lines except for CMK we examined an up-regulation of TNFSF10 coding for TRAIL, as well as differential regulation of other genes involved in apoptosis. Furthermore, gene set enrich-
ment analyses showed a significant down-regulation of genes involved in DNA metabolism and DNA repair. First results from our ongoing analysis of in vivo VPA treated samples are encouraging as we e.g. also see an induction of CDKN1A expression. However, the picture observed is less homogenous as concomitant administration of ICE, as well as other factors like e.g. VPA serum levels might substantially influence the in vivo VPA response. Analysis of expression profiles of MKs and EBs that were then directly compared using cDNA microarrays containing 15,000 features (Sanger Hver2.1.1.). Twenty hybrids were performed, representing five biologically paired comparisons, each with four technical replicates. Statistical analysis of this data identified 658 features that were upregulated in MKs (>2-fold, \( p<0.05 \)), and 219 features in EBs. Known lineage specific markers, such as PTGASB (CD74), GP9 (CD11b) and GP1B (CD42b), were upregulated in MKs relative to EBs as expected. In addition, a number of novel transmembrane proteins were also identified as upregulated in MKs. RT-PCR was used to validate the differential expression of both known and novel transcripts and to investigate the expression of these genes in other haematopoietic lineages. The presence of selected transmembrane proteins on platelets and MKs was confirmed using murine anti-sera generated against recombinant, E. coli expressed protein. Gene silencing experiments in Zebrafish and human MKs are in progress to determine the biological role of these novel proteins.

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**0606**

**EXPRESSION SIGNATURE OF GENES ASSOCIATED WITH TELOMERE-TELOMERASE COMPLEX IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE LEUKAEMIA: TEP1 GENE IS SURPRISINGLY UPREGULATED IN PROGRESSION OF MDS AND IN LEUKEMIC CELLS**

H. Zizkova,¹ S. Vcelikova,¹ J. Cermak,¹ J. Maaloufova,¹ R. Neuviertova,¹ Z. Sieglova¹

¹Inst.of Hematology and Blood Transfusion, PRAGUE, Czech Republic; First Medical Clinic Charles University, PRAGUE, Czech Republic

**Background.** Knowledge of dynamics of telomere-telomerase complex brings important sign into molecular background of leukemogenesis. Misbalance initiated by erosion of telomeres may affect also expression level of genes encoded in regulation of telomere length and telomerase activity. Thus, data on expression profiles of associated genes: hTERT encoding catalytic sub-unit of telomerase, the tankyrase (TNKS), TRF1 (Telomeric Repeat binding Factor 1), POT1 (Protection Of Telomeres 1), TEP1 encoding telomere associated protein, and myc may be valuable from the viewpoint of disease prognosis and monitoring of therapy effectiveness. **Aims.** To ascertain expression variations of genes involved in regulation of telomere-telomerase complex in patients with myelodysplastic syndromes (MDS), acute myelogenous leukemia (AML) from MDS, and primary untreated AML with the aim to evaluate their significance as prognostic factors of MDS evolution towards overt leukemia and markers of leukemic cells. **Methods.** The study was done on mononuclear bone marrow (BM) or peripheral blood (PB) samples from 42 patients with MDS, AML from MDS and untreated primary AML divided into subgroups according to the PAB criteria. Mononuclear cells of 14 healthy BM or PB progenitor cells healthy donors served as normal controls. RNA was extracted using modified method of Chomczynski. Relative expressions of hTERT, TNKS, TRF1, POT1, TEP1, and myc RNA were assayed by real-time RTPCR with specific Taq-Man probes in RotorGene 3000/60A (Corbett Research) in comparison to expression of the housekeeping gene. Results with the ratio more than mean + 2 s.d. of healthy controls were postulated as cases with positive gene expression. Expression signatures were discussed together with telomere length, telomerase activity and clinical features: proportion of blast cells, results of the DFS analysis and also with individual patients risk score established for MDS according to the International Prognostic Scoring System (IPSS). **Results.** Notable increase of expression of hTERT, TEP1, and POT1 genes was observed in patients with advanced forms of MDS (RAEB and RAEB-t) in contrast to insignificant changes of telomerase activity representing a later event in misbalance of telomere-telomerase complex. Significant correlation between individual values of POT1 gene expression and telomerase activity confirmed in MDS and AML patients (p < 0,007) supports role of the POT1 gene as positive molecular regulator of telomerase. On the other hand, no relationships were found between POT1 expression and the IPSS risk score of MDS patients on one side and portion of blast cells in BM/PB both in MDS and AML on the other side. **Summary/Conclusion.** We showed that hTERT and POT1 genes up-regulated already in early forms of MDS and its expression has increasing trend with disease progression. Significantly increased expression of these genes is also feature of mononuclear BM/PB cells of majority of patients at diagnosis of primary AML. These observations predestine POT1 and hTERT genes at least as additional prognostic factors of MDS and molecular markers of AML. High TEP1 expression in patients with advanced forms of MDS and AML indicates on its more active role in signaling of telomere-telomerase complex as it has been supposed.

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**0607**

**TEN NOVEL MUTATIONS IN THE HMBS GENE RESPONSIBLE FOR ACUTE INTERMITTENT PORPHYRIA**

E. Di Pierro, V. Besana, V. Brancaleoni, S. Ausenda, D. Tavazzi, M.D. Cappellini

University of Milan-Policlinico Hospital, MILAN, Italy

**Background.** Acute intermittent porphyria (AIP) is an autosomal dominant disorder caused by a partial deficiency of hydroxymethylbilane synthase (HMBS), the third enzyme in the heme biosynthetic pathway. Clinical features of the disease are intermittent attacks of neurological dysfunction and abdominal pain with or without signs of acute intermittent porphyria. Most of the affected individuals remain asymptomatic throughout their life but 10-20% presenting severe acute attacks. Diagnosis of AIP is often difficult if based on urinary overproduction of porphyrin precursors ALA and PBG only. The erythrocyte HMBS gene activity is not extensively available and not always informative because of the overlap between the normal and carriers range. The molecular analysis of HMBS gene represents the most reliable diagnostic tool for AIP. The human HMBS gene maps to chromosome 11q24.1-q24.2 with a total of 15 exons. Two distinct promoters direct housekeeping and erythroid specific mRNAs by alternative splicing. So far, more than 210 different mutations have been identified worldwide in the HMBS gene as responsible for AIP, showing a high genetic heterogeneity. Most of the reported mutations have been detected only in single families, however a prevalence of specific mutations in different geographic areas has been reported. Only preliminary data are available for the Italian population. **Aims and Patients.** In this study we searched for molecular defects in HMBS gene, in order to define the most common HMBS mutations in Italian subjects affected by AIP. We investigated twelve unrelated patients and their relatives. The diagnosis was based on clinical manifestations, elevated urinary excretion and reduced erythrocyte HMBS activity. **Methods.** The promoters, the entire coding region and the intron-exon boundaries of HMBS gene has been amplified by polymerase chain reaction and submitted to direct automated sequencing. Restriction fragment length polymorphisms, poly-acrylamide gel electrophoresis and XL PCR were performed to confirm the presence of putative mutations. **Results.** Twelve different molecular defects in HMBS gene have been identified. Two mutations (7G>A and 962G>A) were previously reported and ten mutations are new findings: five deletion, one insertion, one splicing defect, one nonsense and two missense. The 447-467del21bp causes the loss in frame of seven aminocids in the exon 9 and the 13890bp deletion causes the loss of the entire HMBS gene. The 181delG, the 418-419delAA, the 468-470delAAA and 852insG mutations cause frameshift in the exon encoding the last exon and 3′UTR respectively. The loss of exon 6 is due to the IVSS 1G>A splicing defect; the nonsense mutation (940C>T) in exon 15 is responsible for creation of a stop codon at aminoacid 314; two missense mutation (242T>C and 1075 G>A) in exon 6 and 15 result in a Leu 81Pro and Asp 559Asn amino acid substitution respectively. **Summary.** These results allowed the identification of ten novel HMBS mutations. In a previous work, we have identified other 11 new molecular defects for a total of 21 new different mutations restricted to the Italian population. This study confirmed the high heterogeneity of molecular abnormalities responsible for AIP phenotype and the presence of clusters of mutations in particular geographic areas.

**0608**

**ASSOCIATION OF HUMAN PLATELET ALLOAGGENTS, 1, HP2, HP3, HP4, AND HP5 ALLELES AND GENOTYPES WITH SICKLE CELL ANAEMIA**

W.Y. Almawi,¹ A.M. Al-Sabae,¹ N.A. Fawaz,¹ K.E. Al-Abi,¹ S. Saidi,¹ N. Mahdi,¹ K. Al-Ola¹

¹Arabian Gulf University, MANAMA, Bahrain; ²King Faisal University, DAMMAM, Saudi Arabia; ³University of Monastir, MONASTIR, Tunisia; ⁴Salhaniya Medical Complex, MANAMA, Bahrain

**Background.** Insofar as sickle cell anaemia (SCA) was described as a hereditary state where cases were autosomal recessive (OVC) and progression to stroke is frequently seen, polymorphisms of human platelet alloantigen (HPA) were reported as risk factors for several vascular abnormalities, including stroke. With the exception of a lone report documenting association of HPA-5b with SCA OVC, studies on potential association of HPA1 through HPAS with SCA are lacking. **Aims.** This study investigated the prevalence of HPA1, HP2, HP3, HP4, and HP5 alleles and genotypes among Bahraini SCA patients and control subjects. Linkage disequilibrium analysis will be used to investigate the disease association of the these polymorphisms. **Method.** This was a case control study. Study subjects comprised 135 SCA patients (mean age 15.6±9.8) and 157 healthy controls (mean age 27.8±15.1); all were Bahraini nationals. Mutation analysis was assessed by PCR-SSP analysis. Statistical analysis was performed on SPSS v. 13.0 statistics software, significance being set at p < 0.05. **Results.** The distribution of HPA2 (p=0.225) and HPA4 (p=0.075) genotypes were comparable between SCA patients and controls. In contrast, higher frequencies of HPA1a (p < 0.001), HPASa (p=0.007) were found among controls, while HPASb (p=0.034) and HPASa (p < 0.001) alleles were more frequent in patients. Whereas HPA 3a/3a (p=0.068; RR = 0.463) and HPA 5b/5b (p<0.001; RR = 0.182) were more prevalent among controls, HPA 1b/1b (p<0.001; RR = 19.935), HPA 5b/b (p=0.042; RR = 1.734), and HPA 5a/5b (p < 0.001; RR = 3.078) were significantly higher among SCA patients. Significant linkage disequilibrium were noted between HPA alleles, with the strongest occurring between HPA1b and HPASa (p = 0.119; p<0.001).

**Summary/Conclusion.** Differential association of HPA polymorphism with
SCA was noted among Bahraini patients, with HPA1, HPA3, and HPA5 representing genetic risk factors of SCA. In view of the reported link between HPA polymorphism and OVC, our results serve a diagnostic/prognostic role in identifying SCA patients with possible OVC complications, as well in the development of future therapeutic regimens.

**0609**

**GENOTYPE AND CLONAL EVOLUTION IN CHILDHOOD ACUTE LEUKEMIA CASES**

S. Bungaro,1 M. Raghavan,1 J. Irving,1 R. Mura,1 B.D. Young,2 A. Hall,1 A. Biondi,1 G. Cazzaniga1

1Centro Ricerche Tettamanti, MONZA, Italy; 2Barts & the London School of Medicine, LONDON, United Kingdom; 3Divisione di Emato-Oncologia Pediatrica, CAGLIARI, Italy; 4Northern Institute for Cancer Research, NEWCASTLE UPON TYNE, United Kingdom

**Background.** Leukemia is the phenotypic result of multiple events, which can accumulate in a pre-leukemic clone and whose origin can be either pre-natal or post-natal in different leukemia subtypes. The understanding of consequential events is important for the comprehension of the pathogenesis of pediatric acute leukemia. The aim of our study was to search for hidden genetic aberrations by detecting genome-wide loss of heterozygosity (LOH) and genes copy number variation (CNV) with single nucleotide polymorphism (SNP) arrays. Here we report the detection of genotype and clonal evolution in two cases of childhood leukemia, a twin pair affected by TEL-AML1 positive ALL and a FLT3-ITD positive AML patient, as a paradigm for describing different mechanisms of clonal evolution. Results: the TEL-AML1 positive monoyzogotic twins with concordant ALL were classified as pre-B ALL and common-ALL. They shared one common Ig/TcR rearrangement amongst others. Both the diagnosis and remission samples analyzed by GeneChip Mapping 10K SNP array showed LOH at the 2q13-14.3 region in both twins, while deletion of the normal TEL allele was only found in twin 2. LOH was not associated with CNV, implying a recombination event resulting in uniparental disomy (UPD). Further analyses are necessary to understand the functional implications of this chromosomal abnormality. One hypothesis could be that the twins were born with a genetic predisposition to develop leukemia, along with the t(12;21) and additional events were then responsible for the overt leukemia. UPD of this region has not been reported in other tumors or in remission samples of leukemia. Moreover, this is the first report on constitutional UPD in leukemia patients. We also studied clonal evolution in a FLT3-ITD positive childhood AML patient, who experienced two relapses and for whom we had the availability of the cord blood (CB) sample. The patient was diagnosed at 6 years of age with AML-M1, a normal karyotype and FLT3-ITD mutation. The same FLT3-ITD clone re-emerged at relapse, three months after auto-BMT. The DNA from CB was negative for the FLT3-ITD RQ-PCR, consistent with the well-established hypothesis that FLT3-ITD mutations represent post-natal events. The GeneChip Mapping 10K SNP array analysis on DNA from the first relapse showed deletion of the long arm of chromosome 9, and LOH on the whole chromosome 13 not associated with CNV. This latter is consistent with UPD, so either non-disjunction or somatic recombination has led to the homozygosity of FLT3-ITD at 13q14. This is emerging as a frequent event of disease progression, subsequent to FLT3-ITD heterozygous mutation. 10K SNP array analysis did not reveal LOH or copy number changes in the diagnostic and in CB samples. Other methods must be applied to find the primary event(s) giving rise to leukemia in association with FLT3-ITD mutation. Conclusions: despite the heterogeneity of the cases presented here, this study shows that genome-wide LOH analysis by SNP arrays represents a powerful tool to unravel genetic aberrations and to better understand the genetic events cooperating in clonal evolution.

**0610**

**IDENTIFICATION OF NOVEL THERAPEUTIC TARGETS IN ACUTE MYELOID LEUKAEMIA USING A PHOSPHOPROTEOMIC STRATEGY**

C. Craddock,1 M. Griffiths,2 M. Wakelam1

1Queen Elizabeth Hospital, BIRMINGHAM, United Kingdom; 2West Midlands Genetics Laboratory, BIRMINGHAM, United Kingdom; 3University of Birmingham, BIRMINGHAM, United Kingdom

**Background.** Pharmacological inhibition of the dysregulated Bcr-abl tyrosine kinase has emerged as an effective therapeutic strategy in chronic myeloid leukaemia. Although accumulating evidence demonstrates the importance of the constitutive activation of signalling pathways in acute myeloid leukaemia (AML), the development of targeted therapies has been compromised by our limited understanding of the identity of the dysregulated tyrosine kinases in AML. Aims: Reasoning that since protein tyrosine phosphorylation is an important mechanism mediating the transduction of proliferative and survival signals we have utilised a phosphoproteomic strategy to identify dysregulated phosphoproteins in myeloblasts from patients with AML. Methods: Using an anti-phosphotyrosine antibody we have immunoprecipitated proteins from AML blasts, separated proteins by SDS PAGE and identified proteins within distinct bands by mass spectrometry. Results in primary AML blasts have been compared with CD34+ progenitors from G-CSF mobilised normal donors. The methodology was validated using vanadate-stimulated HL60 cells. Results: 10 patients with a median age of 51 (range 16-90) were studied. 6 patients had a normal karyotype, one good risk cytogenetics and three adverse risk cytogenetics. Mutations in the fli-3 tyrosine kinase gene were present in four patients. Myeloblasts from every patient demonstrated phosphorylation of MAP kinase (MAPK) implying activation of the ras-MAPK cascade. In contrast CD34+ cells isolated from normal donors demonstrated weak or no MAPK phosphorylation. Since each patient demonstrated MAPK phosphorylation irrespective of fli-3 status we next examined their phosphotyrosine-protocome. This identified a number of phosphorylated and non-phosphorylated proteins in signalling complexes in anti-phosphotyrosine immunoprecipitates present in AML blasts but not normal CD34+ progenitors. These included receptors (ephrin type A-5, interleukin-13), signalling intermediates (TAPP1, RASA1, LARG, cortactin, CD-2 associated protein) and transcription factors (ELK-1, HFK-1). The phosphorylation of a number of the identified proteins was confirmed immunochromatically. These proteins identify three functionally distinct groups in AML blasts that were not detected in CD34+ progenitors:(i)intermediates in PtdIns-3-kinase-mediated signalling presumably suppressing apoptosis (ii) intermediates of a tyrosine kinase-mediated ras-MAPK signalling proliferative cascade and (iii) regulators of the actin cytoskeleton and thus cell movement. This strategy also identified a novel pattern of phosphorylation of the S-HT5A receptor in AML blasts raising the possibility that this protein plays a role in the pathogenesis of AML. Summary: Adoption of a phosphoproteomic methodology has identified novel phosphoproteins in AML which require further validation. Intriguingly our data also point to a common intracellular signalling pathway (ras-MAPK) in AML. These observations provide information for the rational development of targeted therapies in AML.
**0611**

**EXPRESSION OF TGFβ GENES AND THEIR RECEPTOR TYPES I, II AND III IN LYMPH NODES FROM PATIENTS WITH NON-HODGKIN'S LYMPHOMAS**


Medical University of Silesia, KATOWICE, Poland

**Background.** Transforming growth factor β (TGFβ) is a multifunctional cytokine triggering different physiological situations: cell-cycle control, hematopoiesis control, cell differentiation, angiogenesis, immunological functions control, apoptosis induction as well as extracellular matrix formation. The TGFβ activity depends on the presence of three specific cell surface receptors involved in a variety of important cellular functions. A consequence of TGFβ prevalence in an organism is its significant influence on the majority of physiological and pathological processes, including the development of malignant diseases where the lack of TGFβ dependent growth control may be responsible for oncogenesis. Recently there has been a growing number of reports on increased expression of TGFβ genes in certain tumors. TGFβ1 is most commonly present in humans. The aim of our study was to compare the expression of TGFβ1 and its I, II and III receptors in the lymph nodes of 47 never-treated Non-Hodgkin's lymphoma patients in different stages of the disease (15 of these patients were diagnosed with the aggressive lymphoma, 8 with the mantle cell lymphoma and the remaining 24 with the indolent lymphoma; the control group consisted of 7 patients with persistent chronic lymph node enlargement). **Methods.** The QRT-PCR method was employed to assess the activity of TGFβ1 and its receptor types I, II and III. **Results.** The expression of TGFβ1 differed between the mantle cell lymphoma and the indolent lymphoma groups (p=0.05019). No difference in the expression of TGFβ1 was found between any other groups. Also no difference in the expression of genes for Tbr1 and TbrII was found in any of the groups, including the control group. A difference in the expression of genes for TbrII was found between the mantle cell lymphoma and the indolent lymphoma groups (p=0.050187). Strong positive correlations were found within the studied subgroups for all the examined parameters for the mantle cell lymphoma and the indolent lymphoma subgroups. In the aggressive lymphoma group we found no positive correlation only for TGFβ1 and TbrI as well as TbrI and TbrII. In the control group we found no correlation for TGFβ1 and TbrI as well as TGFβ1 and TbrII. For all the remaining parameters strong positive correlations were found. **Conclusions.** Where expressed, ThrIII is the most abundant TGFβ receptor and classically functions by binding the TGFβ ligand and transferring it into its signaling receptors, TbrI and TbrII. TbrI initiates intracellular signaling by phosphorylating a family of transcription factors, the Smads. In this respect, the lack of positive correlation between TGFβ1 and TbrI as well as TGFβ1 and TbrII, for all the remaining parameters strong positive correlations were found. Where expressed, ThrIII is the most abundant TGFβ receptor and classically functions by binding the TGFβ ligand and transferring it into its signaling receptors, TbrI and TbrII. TbrI initiates intracellular signaling by phosphorylating a family of transcription factors, the Smads.

**0612**

**GENE EXPRESSION PROFILING OF PRIMARY HODGKIN/REED-STERNBERG CELLS AND THEIR RELATIONSHIP WITH PRIMARY MEDIASTINAL B-CELL LYMPHOMA**

E. Tiacci,1 V. Brune,1 S. Eckerle,2 G. Mechtersheimer,3 A. Bruninger,3 M.L. Hansmann,4 R. Küppers4

1Tumor Research, ESSEN, Germany; 2Institutes for Cell Biology & Pathology, ESSENFRANKFURT, Germany; 3Institute of Pathology, FRANKFURT, Germany; 4Inst. for Cell Biology, ESSEN, Germany

**Background.** Classical Hodgkin lymphoma (cHL) and PMBCL share some similarities in terms of clinical presentation, histological and immunophenotypic picture, and genetic and pathogenetic features. So far, genome-wide expression profiling studies have compared whole biopsies of PMBCL and other diffuse large B-cell lymphomas (DLCLs) to cHL cell lines, owing to the rarity of primary HRS cells in lymph nodes involved by cHL. These studies reported for PMBCL a partial molecular overlap with cHL cell lines, which was more pronounced than that with other DLCLs. However, cultured HRS cells most likely do not reflect primary HRS cells in all their important features, as they were derived from patients with end-stage disease and from sites (e.g. pleural effusions, peripheral blood) which are very rarely involved by cHL and in which the dependence on the prominent inflammatory background typically surrounding primary HRS cells in the lymph node is lost. **Aims.** To investigate the genome-wide expression profile of primary HRS cells and its relationship with that of other lymphomas (including PMBCL) and of normal peripheral B-cell subsets. **Methods.** A total of 2000-2000 HRS cells were laser-microdissected from H&E-stained frozen sections of cHL samples. After two rounds of in vitro linear amplification, RNA is hybridized to Affymetrix HG-U133 Plus 2.0 chips (interrogating ~54000 probe sets corresponding to ~30,000 genes). Expression profiles are also generated from similar cell numbers of: i) neoplastic cells FACS-sorted from HCL cell lines or microdissected from various non-Hodgkin lymphomas, including PMBCL, and from lymphocyte-predominant HLRPLH) cases; and ii) normal mature B-cell subsets (plasma cells and naive, memory and germinal center B cells) that are MACS/FACS-sorted from tonsil or peripheral blood of healthy donors. **Results.** A supervised comparison of primary HRS with HL cell lines shows a tending to form discrete sub-clusters) and T-cell rich B-cell lymphomas. The further branching of the dendrogram shows that each of the four B-cell subsets tend to form discrete clusters, and that, among tumor samples, cell lines grouped apart from primary cases. The latter further split in two sub-branches: one with PMBCLs, Burkitt lymphomas, follicular lymphomas (each of the three forming its own sub-cluster) and DLCLs, and the other branch mainly comprising HLLs (with both CHLs and LPHLs tending to form discrete sub-clusters) and T-cell rich B-cell lymphomas. A supervised comparison of primary HRS with HL cell lines shows a highly differential expression (>4fold change) of ~1200 genes, including...
ing many involved in intercellular signaling, chemotaxis, and immune/in-
flammatory response. Conclusion. These preliminary results suggest that expression profiles can be reliably generated from small numbers of microdissected cells, and that primary HRS cells and HL cell lines seem to differ in various biological features. A more complete analysis (e.g. relatedness of HL with other lymphomas, including PMBLC, and with normal B-cells) will be presented at the Meeting.
E. Taccioli is supported by a fellowship (F05/04) from the Deutsche José Car-erras Leukämie-Stiftung.

0613
PERIFOSINE, AN ORAL BIOACTIVE NOVEL AKT INHIBITOR, INDUCES ANTITUMOR ACTIVITY IN WALDENSTRÖM MACROGLOBULINEMIA
Dana-Farber Cancer Institute, BOSTON, USA

Background. Waldenström’s Macroglobulinemia (WM) is an incurable low-grade lymphoplasmacytic lymphoma. The PI3K/AKT pathway is a critical regulator of cell survival by stimulating cell proliferation and inhibiting apoptosis. Our previous studies using proteomic analysis have demonstrated upregulation of members of the PI3K/AKT pathway in WM. The new AKT inhibitor, perifosine (NSC 639966; Keryx Biopharmaceuticals, NY) has demonstrated activity in other B-cell malignancies. Aims. We hypothesized that the AKT inhibitor, perifosine will induce cytotoxicity in WM. Methods. WM cell lines (BCWM1 and WSU-WM) were used. Primary WM patient cells were obtained from patients after informed consent. Inhibition of proliferation was measured using the MTT proliferation assay. DNA synthesis was measured using the thyminidine uptake assay. Apoptosis was determined using Apos. Flow cytometry analysis (Beckman Coulter Inc., CA). Bone marrow (BM) stromal cells confer growth and resistance to conventional treatments. Therefore, tested the effect of perifosine on WM cells co-cultured with BM stromal cells. Cell cycle analysis was performed using flow cytometry with PI staining (Molecular Probes, Oregon). IgM secretion was tested using ELISA assay (Immu-no-tek, NY). Immunoblotting for pAKT, pERK1/2 and pJNK was performed at 6 hrs of treatment. A two-sided t-test was used to determine differences in response. Results. Perifosine induced significant cytotoxicity and inhibition of DNA synthesis in WM cell lines with an IC50 of 5-20uM in all cell lines tested. Similar effects were demonstrated in 3 primary CD19+ WM cells obtained from patients’ bone marrow. Cell cycle analysis demonstrated G1 arrest at 24hs. The effects of perifosine were significant even in the presence of BM stromal cells that induce resistance. Perifosine did not induce cyto-

171 and anti-tumor activity was assessed in immunocompromised mice bearing established human tumor xenografts. Results. PR-171 irreversibly inhibits the chymotryptic subunit of the 20S proteasome and is >50-
fold selective over the other proteasome catalytic activities. PR-171 potency for this proteasomal subunit in tumor cells correlates with its cytotoxic potential resulting in IC50 values <10 nM for both activities in multiple cell lines. Furthermore, the in vitro cytotoxic activity of PR-171 is retained in tumor cells resistant bortezomib. In experimental ani-

0615
ANALYSIS OF DELTA AND T-CELL RECEPTOR GENES IN MYCOSES FUNGOIDES AND SZARY SYNDROME
S. Pulini,1 G. Goteri,1 A.R. Scorteccini,1 S. Pulini,2 R. Capretti,1 A. Tassetti,1 A. Stronati,1 S. Mulattieri,2 A. Filosa,2 D. Stramazzotti,2 R. Ranaldi,2 G. Fabris,2 P. Leoni2
1‘Clinic of Hematology, ANCONA, Italy; 2Institute of Pathology, ANCONA, Italy

Background. Demonstration of a dominant T-cell clone in Mycosis Fungoides (MF) and Sézary Syndrome (SS) is usually made with probes and rarely with Δ probes. Aims. We studied T-cell clonality for TCR Δ and γ chain gene in the peripheral blood (PB), bone marrow (BM) samples and cutaneous lesions of 14 patients with early-stage MF and 10 patients with advanced-stage MF and SS. Activity for four specimens were analysed: 11 skin biopsies and 14 PB cells from patients with early-stage MF; 4 skin biopsies, 28 PB cells and 7 BM samples from patients with advanced-stage MF and SS. PCR for TCR Δ gene rearrange-

Summary. These studies demonstrate the tolerability, anti-tumor activity and dosing flexibility of PR-171 and provide validation for the clinical testing of PR-171 in the treatment of hematologic malignancies utilizing dose adaptive schedules.
ing and cutaneous T-cell clones in MF/SS 2) In comparison with PAG, the TCR expression using a PCR-based approach allowed a more precise assessment of the molecular pattern at diagnosis and during follow-up; all TCR Δarrangements were Vα3-Jα11 and Vα1-Jα11, often integrated in their mono-oligoclonal expression. Our finding of a restricted pattern of rearrangements support the suggestion of a specific antigen in MF/SS; moreover it is intriguing that some Authors consider the clonotypic TCR as a source of tumor-specific antigens and a possible target for recognition by CD8+ CTLs.

0616 IMMUNOGLOBULIN V GENES IN WALDENSTROM'S MACROGLOBULINEMIA REVEAL ECTOPTIC SOMATIC MUTATIONS BUT NO GLYCOSYLATION MOTIFS

S. Sahota,1 I. Hamid,1 G. Babbage,1 M. Townsend,1 F. Forconi,2 E.K. Stevenson1 1University of Southampton, SOUTHAMPTON, United Kingdom; 2Universita di Siena, SIENA, Italy

Background and Aims. In Waldenstrom’s macroglobulinemia (WM), current evidence from Ig VH gene analysis indicates heterogeneous disease origins. Most cases appear to derive from post-follicular B-cells with somatically mutated (MUT) VH genes, but a few WM display unmutated VH genes, consistent with pre-germinal center (GC), naive B-cells. In MUT WM, expression of CD27, regarded as a marker of post-GC memory B-cells also appears to be variable, with tumor cells apparently identifiable in both CD27+ve and CD27−ve fractions, raising further questions about the cell of origin. Here, we re-evaluate V gene mutational features in MUT WM. Results and Discussion. In MUT WM cases, we used single cell analysis to reveal intraclonal variability in CD27 expression. Failure to express CD27 could be disease-associated or may reveal an unusual cell of origin. Interestingly, both CD27+ and CD27− tumor cells unexpectedly showed evidence for low level ongoing somatic mutation in VH (D)J-Cmu sequences. These data suggest that ectic, continuing mutational events occur in WM in the bone marrow. To probe this further, we examined the expression of activation induced cytidine deaminase (AID), a pre-requisite for mutational activity. AID transcripts were identifiable in single CD27+ and CD27− cells, albeit at a low frequency. A striking feature of lymphoma cells that undergo continual somatic mutation, and remain in the GC site, is their ability to generate novel glycosylation motifs via mutated nucleotides in V genes. These are functional and in follicular lymphoma appear mandatory, suggesting a role in tumor-stroma interactions. Given that localised mutations can be identified in MUT WM, we examined a series of these cases for such sites, using paired VH and VL analysis in 14 cases. In these, and in a further 7 WM VH genes, the incidence of glycosylation sites was at a low level. Somatic mutations in MUT WM therefore appear not to lead to acquisition of novel glycosylation sites. Such modifications are also absent in MUT chronic lymphocytic leukemia (CLL). These findings indicate no relationship between WM and GC lymphoma tumors. Instead, location in bone marrow and heterogeneity in somatic mutational activity point to a closer similarity to CLL of tumor behaviour at this site.

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0617 IDENTIFICATION OF POTENTIAL PROGNOSTIC MICRONAS IN DIFFUSE LARGE B CELL LYMPHOMA BY EXPRESSION PROFILING

C.H. Lawrie,1 S. Soneti,1 C.D.O. Cooper,1 C.S.R. Hatton2 1University of Oxford, OXFORD, United Kingdom; 2John Radcliffe Hospital, OXFORD, United Kingdom

Background. MicroRNAs (miRNAs) are a recently discovered class of naturally occurring short non-coding RNA molecules that regulate gene expression through translational repression. They have been shown to play a critical role in many biological functions and there is emerging evidence to suggest that dysfunctional expression of miRNAs is a common feature of malignancy. The identity of lymphoma-associated miRNAs however remains poorly defined. Diffuse large B cell lymphoma (DLBCL) is an aggressive disease accounting for nearly 40% of all lymphoid tumours. DLBCL is characterized by marked clinical and pathological heterogeneity that is reflected at the molecular level. DLBCL can be divided at least into prognostically distinct molecular subtypes by gene expression profiling those that are germinal centre B cell like (GCB) and those that are activated B cell like (ABC). It is not known whether similar heterogeneity is also present at the miRNA level. Results. We used microarrays to show that the miRNA expression profiles of ABC (OCI-Ly3 and OCI-Ly10) and GCB (SUP-HL4, SUP-HL5 and SUP-HL10) prototypes DLBCL cells were distinct, and that expression profiles of all cell lines were distinct from normal lymphocyte populations. We validated the microarray data by RNase-protection assay and identified miRNAs expressed exclusively in either ABC (miR-221, miR-222, miR-21 and miR-125) or GCB (miR-142 and miR-101a) cell lines. Using RNase-protection analysis we showed that miR-101a was highly expressed in peripheral blood B cells but less so in T cells, whilst the converse was true for miR-342. Both miR-342 and miR-181a were expressed in naive but not GC or memory B cells. miR-21 and miR-155 were not expressed in lymphocyte populations and miR-221 was expressed but showed no differential expression between populations. Although clearly heterogeneously expressed, there was no association between the pattern of expression of these miRNAs and ABC/GCB immunophenotype in twenty-eight cases of DLBCL we examined by RNase-protection assay. Interestingly, miRNA expression patterns were linked with clinical characteristics of the cases. High miR-101a expression was associated with patients that had undergone high grade transformation from follicular lymphoma (p<0.01) and miR-221 expression with the presence of extranodal disease (p<0.05). Moreover, patients with high levels of miR-155 expression had shorter relapse-free survival times (RFS) (p=0.01), whilst those that expressed miR-342 had longer RFS times (p=0.05). Summary. These results raise the possibility that miRNAs could be useful molecular diagnostic and prognostic indicators for this heterogeneous disease and probably for haematological malignancies in general.

0618 EVIDENCE FOR A PATHOPHYSIOLOGICAL ROLE OF CYSTEINYL LEUKOTRIENES IN HODGKIN LYMPHOMA

N.O. Sjöberg,1 E. Schain,1 Y. Tryselius,1 L. Backman,1 M. Malec,1 A. Porwit-Mac Donald,1 M. Björkholm,1 H.E. Claesson1 1Karolinska University Hospital and Insti, STOCKHOLM, Sweden; 2Biolipox AB, STOCKHOLM, Sweden

Background. Classical Hodgkin Lymphoma (cHL) is a predominantly B lymphocyte-derived neoplasia characterized by a minority of malignant cells, the so-called Hodgkin- and Reed Sternberg (H-RS) cells, surrounded by inflammatory cells such as eosinophils and mast cells. In contrast to other tumours, the malignant H-RS cells constitute only a few percent of the total cells in the affected tissue. Therefore, it is generally believed that various compounds released by H-RS cells are of great importance in the pathophysiology of cHL disease. Aim. To characterise the expression of cysteinyl leukotriene 1 receptors (CysLT1R) in cHL. Methods and Results. We have identified functional CysLT1R in a HL cell line as shown by increased intracellular calcium release upon leukotriene (LT) D4 stimulation (100-500 nM). This response was completely blocked after addition of zafirlukast, a specific CysLT1R antagonist. Immunohistochemical studies of paraffin embedded cHL tissue showed H-RS cells positive for CysLT1R in 12 of 16 cHL tumours. The HL cell line was cultured in the presence of LT(D4) (100 nM) to investigate the effects of CysLT signaing in H-RS cells. Real-time RT-PCR analysis showed up-regulation of TNF-α, interleukin (IL)-6, IL-8 and IL-13 mRNA after stimulation with LT(D4). Furthermore, the effects of LT(D4) on cytokine protein secretion by the HL cells were studied by flow cytometry. The results showed a markedly increased secretion of TNF-α, IL-6 and IL-8 upon LT(D4) stimulation. Conclusion. Since H-RS cells are surrounded by cysteinyl leukotriene producing cells (eosinophils and mast cells), these results indicate that CysLT signaling could be of importance in the pathogenesis of cHL by contributing to the disturbed cytokine features of this tumour.

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0619 HUMAX-CD20, A NOVEL FULLY HUMAN ANTI-CD20 MONOCLONAL ANTIBODY: BIOLOGICAL PROPERTIES

W.J.M. Mackus,1 J.L. Teeling,1 J.H.N. van den Brakel,1 L.J.M. Wiegman,1 W.K. Bleeker,1 T. Vink,1 M.E. Monk,2 M. Folden Flensburg,1 J.W. Snoostra,1 M.J. Glennie,1 E.W.H.I. Parren,1 J.G.J. van de Winkel1 1Genmab BV, UTRECHT, Netherlands; 2Tenovus Research Laboratory, SOUTHAMPTON, United Kingdom; 3Genmab A/S, COPENHAGEN, Denmark; 4Pepscan Systems B.V., LEYSTAD, Netherlands; 5Immunotherapy Laboratory, UMCU, UTRECHT, Netherlands

Background. Monoclonal antibodies (mAb) play a crucial role in host immune defense and are increasingly recognized as effective therapeu-
tics in a range of conditions. The CD20 molecule expressed on B cells is the best validated therapeutic target for B-malignancies. Using human Ig transgenic mice, we have generated a panel of fully human CD20 mAb (HuMax) directed against the human CD20 molecule. Characterization of these antibodies revealed that two types of CD20 specific antibodies exist: type I CD20 mAb, exhibiting similar characteristics to the chimeric mAb rituximab, and type II CD20 mAb, being functionally equivalent to the murine anti-CD20 mAb, Tositumomab (B1). Aim: The biological property of one of the type I mAb, HuMax-CD20, has been evaluated performing in vitro and in vivo experiments. Methods and Results. In vitro experiments showed that HuMax-CD20 has an unusually slow off-rate, and induced rapid translocation of CD20 into lipid rafts. Analysis of its CDC potential showed that HuMax-CD20 recruited C1q to the surface of CD20-positive cells and mediated tumor cell lysis via activation of the classical pathway of complement. Importantly, HuMax-CD20 was exceptionally active in CDC. In the presence of human plasma or whole blood, being able to lyse a range of rituximab-resistant targets, such as CD20-low expressing CLL. This CDC potency appeared to be related to the unusually slow off-rate of these human antibodies. Our current data on epitope mapping indicated that the CDC potency might be influenced by the region of the CD20 recognized by HuMax-CD20. Binding by rituximab and mouse CD20 mAb, had an absolute requirement for alanine, and proline at positions 170, and 172, respectively, within the large extracellular loop of CD20. Epitope mapping studies, using both mutagenesis studies and overlapping 15-mer peptides of the extracellular loops of CD20, revealed a novel binding site required for binding of HuMax-CD20. The HuMax-CD20 binding epitope is located amino terminally of the binding site for rituximab and is also located in the extracellular loop of CD20. Thus, while off-rate may influence biological activity of mAb, the most critical factor determining CDC potency by CD20 mAb, appears to be the region within the target molecule they recognize. In vivo experiments showed that HuMax-CD20 increased survival in a SCID xenograft model. I.v. infusion of HuMax-CD20 in cynomolgus monkeys lead to a profound, long lasting B cell depletion, which recovered after the last dose. HuMax-CD20 has been selected for further clinical development. HuMax-CD20 is currently in phase I/II trials for follicular lymphoma, and B-CLL, and in a phase II trial in Rheumatoid Arthritis. Conclusion. These results indicate that HuMax-CD20 holds considerable promise for improved clinical activity and may represent an attractive candidate to treat patients with B-cell malignancies and autoimmune disease.

0620

THE INFLUENCE OF INTERLEUKIN-10 PROMOTER GENE POLYMORPHISM ON THE OCCURRENCE OF NON HODGKIN LYMPHOMA IN SUBJECTS INFECTED WITH HEPATITIS-C VIRUS

A. De Renzo,1 M. Capasso,1 P. Ferna,1 E. Persico,1 C. Marzocchella,1 A. Iolasco,1 M. Persico1

1University of Naples, NAPLES, Italy; 2Second University, NAPLES, Italy

Background. HCV along with chronic liver disease is also considered a causative agent of other clinical pathological conditions which testify the possible direct pathogenic role of the virus in several different cell types including hepatocytes and leukocytes. Prevalence of HCV is significantly higher also in patients suffering with NHL and, all around the world, it was confirmed except for patients studied in North Europe and some areas of North America. In Italy, different groups showed prevalence ranging from 15 to 30%. Aim. The goal of this paper is to establish if a polymorphic gene encoding for cytokine could be a predisposing factor for this condition. Methods. To do this, we analyzed the distribution of the polymorphism of IL-10-1082 G/A in 63 patients, not infected with HCV, with Non Hodgkin Lymphoma (NHL/HCV) and in 50 patients, infected with HCV, with chronic active hepatitis, with Non Hodgkin Lymphoma, (NHL/HCV+). Results. In this study, for the first time we show that irregardless of age, sex, virus genotype and/or severity of chronic liver disease a significant prevalence of IL-10-1082 GG genotype seems to influence the occurrence of NHL in HCV infected patients. In fact the distribution of the IL-10-1082 G/A polymorphism was different between NHL/HCV+ and NHL/HCV- patients (p=0.028). The frequency of the IL-10-1082 G allele (p=0.019) and the frequency of the IL-10-1082 GG genotype against overall genotypes (IL-10-1082 GA/AA) were significantly lower in NHL/HCV+ patients as compared with NHL/HCV- patients (p=0.014).
Lenalidomide inhibited proliferation of these cells
in vitro. Lenalidomide inhibited proliferation of these cells in vitro with a few cells exhibiting ca. 50 copies of EBV, whereas the rest of the cell lines were EBV-negative by FISH. Some cell lines show a small fraction of lytic EBV infected cells which could be increased by treatment with the phorbol ester TPA. The appearance of lytic phase cells corresponds to the demonstration of linear EBV bands applying in situ hybridization analysis. On the contrary, for most of the cell lines with lytic phase cells, no immediate early protein BZLF1 could be detected by Western blotting. Metaphase FISH revealed that most of the cell lines which harbored EBV genomes contained integrated copies as deduced from paired signals as well as episcopal DNA. The number of integrated sequences range from 1 (cell line NAMALWA, no episomes; cell line CI-1, several episomes) to about 100 integration sites (cell line RAJI, many episomes). In summary, we could show that FISH can be used to recognize lytic phase cells in cell cultures and to determine the amount of EBV genomes per cell and the integration status of the EBV genome.

0623
DETERMINATION OF LYTIC PHASE CELLS AND INTEGRATION STATUS OF EBV-INFECTED CELLS LINES
C. Uphoff, R.A.F. MacLeod, S.A. Denkmann, M. Kaufmann, H.G. Drexler

DSMZ - German Collect. of Microorganisms, BRAUNSCHWEIG, Germany

Epstein-Barr virus (EBV, human herpesvirus type 4) is ubiquitously distributed in all human populations, reaching infection rates of more than 90%. EBV is known to infect B-lymphocytes and mucosal epithelium cells and to establish latent or productive infections. The virus is the causative agent of infectious mononucleosis and closely associated with the endemic form of Burkitt lymphoma. EBV has also been associated with various lymphoid and epithelial malignancies, such as Hodgkin, T-cell, and AIDS-related lymphomas, and lymphoepithelioma-like carcinomas of several organs. In vitro, B-lymphocytes are transformed by EBV into permanent lymphoblastoid cell lines (B-LCL). Active EBV particles contain linear double-stranded genomes, which are circu-
larized intracellularly. The episomes persist in the nucleus and can be integrated into the eukaryotic genomes. We determined the EBV infection status of a panel of 421 primate cell lines by PCR (417 human, 4 monkey). 59 cell lines (38 human, 1 monkey) contained EBV sequences. All cell lines were established from B-lineage primary cells. No cell lines from other tissues were found to be EBV+. To investigate the number and the integration status of the EBV genomes and the ratio of lytic phase cells in a cell culture population, we established a fluorescence in situ hybridization (FISH) method on cytospin preparations of untreated cells and on metaphase spreads of colcemid treated cells. Hybridization was performed with biotinylated probe Vysis Cy5 and Spectrum Red/Green labeled indi-
vidual clones encompassing almost all of the EBV genome. The B9-5 cell line, which was described to produce active EBV particles was used as positive control. The number of EBV copies varied highly among the cell lines. This was demonstrated by FISH and by Southern blotting. The number of episomes was not evenly distributed among the individual cells of a cell line, but the proportion of the cells contained many fewer copies of EBV. At one end of the spectrum is the cell line DOHH-2 with a few cells exhibiting ca. 50 copies of EBV, whereas the rest of the cells are EBV-negative by FISH. Some cell lines show a small fraction of lytic EBV infected cells which could be increased by treatment with the phorbol ester TPA. The appearance of lytic phase cells corresponds to the demonstration of linear EBV bands applying in situ hybridization analysis. On the contrary, for most of the cell lines with lytic phase cells, no immediate early protein BZLF1 could be detected by Western blotting. Metaphase FISH revealed that most of the cell lines which harbored EBV genomes contained integrated copies as deduced from paired signals as well as episcopal DNA. The number of integrated sequences range from 1 (cell line NAMALWA, no episomes; cell line CI-1, several episomes) to about 100 integration sites (cell line RAJI, many episomes).

Table 1. Discrepancy cases when FCM, cytology, and/or histology results were compared (n=29).

<table>
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<th>Age/Sex</th>
<th>FCM</th>
<th>Cytology Diagnosis</th>
<th>Histopathology</th>
<th>PCR/SD</th>
<th>Final Diagnosis</th>
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<td>RP</td>
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<td>POLYSDRAL</td>
<td>RP</td>
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<td>RP</td>
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<td>PCO</td>
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*Female: M, male; NNL, non-nodular lymphoma; PCO, Pharynx or Oropharynx; RS, reactive process, ST, solid tumor; HL, Hodgkin's lymphoma; DCL, Dendritic cell leukemia, AMI, Acute myeloid leukemia; PCO, Polycythemic.
In 69 out of the 228 samples (30%), the final diagnosis was compatible with B-non-Hodgkin Lymphoma (B-NHL) (53 cases Igkappa+, 15 Iglambda+ and one case IgGama), 113 cases (49%) were considered as reactive processes (RP; n=97) or Hodgkin disease (HD; n=16), confirmed by histology and in 27 (12%) infiltration by non-hematopoietic cells was detected (solid tumor -ST- by histology). In the remaining 19 cases (8%), the diagnoses corresponded to: plasmacytoma (n=7), T-cell CLPD (n=6), T-acute lymphoblastic leukemia (T-ALL; n=5) and acute myeloid leukemia (AML; n=1). Discrepant samples (n=35) corresponding to 29 cases are shown in Table 1a and 1b. In 14 out of these 29 cases (48%) the final diagnosis was concordant with that provided by FCM, while in 3 (10%) cytology gave the correct diagnosis, in the remaining 12 cases (41%) the diagnosis was not conclusive, mainly due to low cellularity or peripheral blood contamination. The sensitivity and specificity of FCM in diagnosing the different CLPD ranged between 94-100% and 88-100%, respectively. Overall, in B-NHL the percent of clonal B-cells was significantly higher than in the other groups (59±25% vs 8%), all these cases showed an imbalance in the k-λ ratio. Also, a significantly higher percentage of T-cells was found in T-NHL as compared to the others.

**Aims.** The aim of our study was to analyze the clinical and pathological correlation of 45 cases of T and null ALCL. Methods. This is a multicentric retrospective clinical and pathological study of 45 cases diagnosed in out hospitals between 1997-2004; a lot of monoclonal antibodies were utilized for phenotypic evaluation by immunohistochemical techniques. Results. The incidence of ALCL was low (only 2.13% from all NHL), but they represented 1/4 of T-NHL. Although it was a large diversity of pathological aspects, about 80% of the cases were classified as common ALCL. Phenotype, especially T-lineage, was more frequent. The malignant cells constantly expressed CD30; 88% of cases were EMA positive, and 56.5% ALK positive. The bimodal age distribution of patients was evident in ALK positive cases without correlation with the T/null phenotype and the male predominance more evident in cases with T phenotype and respectively ALK positive. Approximately 2/3 of patients were diagnosed in advanced stages of disease, 82% presented B symptoms at presentation. 42% presented extranodal disease especially cutaneous and bone involvement. Because of the young age and good performance index, most patients were classified in accordance with IPI in low and intermediate low risk. Even the therapeutically approach was diverse, in most cases were used CHOP protocols; autologous bone marrow transplantation was performed only in two cases, with complete remission in one case. The major predictive factors correlated with a favorable prognosis were IPI score ≤ 2 and ALK expression by malignant cells. Conclusions. In the majority of cases the ALCL diagnosis is rather difficult; the young age predominance and the aggressiveness of the disease justify the need for a new therapeutically targets correlated with the phenotypic expression of the malignant cells.

**Splenectomy zone lymphoma: one or more entities? A histological, immunohistochemical and molecular study of 42 cases**

T. Papadaki,1 K. Stamatopoulos,2 C. Belessi,1 E. Pouliou,2 P. Paras1, V. Douka,1 A. Hadzidimitriou,1 N. Laoutaris,1 A. Fassas,1 A. Anagnostopoulou,2 D. Anagnostou1

1Evangelismos Hospital, ATHENS, Greece; 2G. Papanicolaou Hospital, THES-SALONIKI, Greece; 3Nikea General Hospital, PIRAEUS, Greece

The histogenesis of splenic marginal zone lymphoma (SMZL) is currently unknown. We conducted a detailed histological, immunohistochemical and genotypic analysis in a series of 42 SMZL cases, diagnosed on splenectomy specimens after established WHO criteria. A broad spectrum of monoclonal antibodies was used in order to exclude other medium-small B cell malignancies mimicking SMZL. In this study we compared CLL, mantle cell lymphoma, follicular lymphoma, lymphoplasmacytoma. The aim of our study was to correlate phenotypic and molecular findings (IG heavy/light chain repertoire and somatic mutations) so as to gain insight into SMZL immunopathogenesis. A predominantly nodular growth pattern was observed in 24 cases; the cells showed predominantly low (11/42) or exclusively (7/42) diffuse infiltration. Twenty-one cases showed the ‘classical’ biphase appearance. The remaining cases (21/42) were monomorphous: 13/21 cases exhibited marginal-zone morphology, while 8/21 cases were composed predominantly of small cells. Five cases demonstrated plasmacytoid differentiation (CD21+/CD38+); 3/5 CD27-negative cases were SIgD-positive. CD27 staining was observed in 17/42 cases, generally in a concordant fashion. Residual FDC meshworks were detected by CD21 and/or CD35 staining in all cases. DBA-44, traditionally known as a marker of HCL and normal mantle cells, was detected in 24/42 cases. Seventeen out of 37 cases were SIgD-positive; 12/22 cases expressed SIgM/SlgD, 7/22 expressed SIgM, while one case expressed SlgD only; 5/36 cases were SlgD-positive. CD27 staining was observed in 22/35 cases; 8/19 CD27-positive cases also expressed SlgD; 3/5 CD27-negative cases were SlgD-positive. CD5 was detected in 4/42 cases. Forty IGHV-D-J rearrangements were amplified in 34/42 cases. Among six cases with double rearrangements, three carried double in-frame rearrangements. The most frequent IGHV gene was IGHV4-34 followed by IGHV1-2, IGHV1-18, and IGHV3-30. Two IGHV1-69/IGHD3-16/IGHJ3 rearrangements with identical HCDR3 sequences were identified; a similar, restricted HCDR3 sequence has been reported in CLL cases. Twenty-six IGKV rearrangements were amplified in both kappa and lambda-expressing cases; IGKV rearrangements using six different germline IGV genes were amplified in 8 lambda-SMZL cases. Using the 98% homology cut-off value, 22/40 IGHV sequences (55%) and 15/34 IGKV sequences (44%) were considered as ‘mutated’. Nine out of eleven cases (82%) with monomorphous, marginal-zone morphology carried IGHV4-mutated genes. In contrast, 4/6 cases with plasmacytoid, small-cell morphology carried IGHV4 genes. Six out of seven cases expressing IGHV1 subgroup genes had biphASIC morphology. In contrast, 5/9 IGHV3-expressing cases had monomorphous, marginal-zone morphology; 3/9 cases expressing unmutated IGHV4 genes had monomorphous small-cell morphology. All IGHV1-expressing cases were CD21-negative. Most cases (12/19; 63%) with high density by lymphocytes, monocytes/macrophages, eosinophils, thymocytes and macrophages, but is absent in granulocytes, platelets, red cells, and bone marrow stem cells. CAMPATH-1H or Alemtuzumab is a genetically reshaped human IgG1 monoclonal antibody against CD52. It has been shown to be effective in T-cell malignancies, particularly in T-cell prolymphocytic leukemia and cutaneous T-cell lymphoma (TCL). It is also a powerful agent with high toxicity when given in the “fludarabine plus cyclophosphamide” (FC) regimen which had been heavily pretreated with conventional chemotherapy, although it is associated with significant hematologic toxicity and infectious complications. To date, there have been very limited studies focusing on the expression of CD52 in TCL. Aims. To investigate CD52 expression in TCLs. Methods. Immunohistochemical study using anti-CD52 (MA1642, Serotec, Oxford, U.K) in 4/17 cases. Results. In 35 (86%) cases including 10/14 (71%) angioimmunoblastic T-cell lymphomas (AITLs), 15/34 (44%) unspecified PTCLs, 4/17 (24%) NK/T cell lymphomas, 5/16 (31%) anaplastic large cell lymphomas, 1/10 (10%) T-cell lymphoblastic lymphomas, 1/1 panleucocytic TCL, 1/1 adult T-cell leukemia/lymphoma, but not in one hepatosplenic TCL. Summary/Conclusions. Our results show that one third of T-cell malignancies express CD52 with various frequency among various subtypes and suggest that AITL and unspecified PTCL are better candidates than other subtypes for CAMPATH-1H treatment. It might be advisable to perform CD52 immunostaining before starting CAMPATH-1H treatment.
IGHV-mutated genes were SlgD-negative; in contrast, IGHV-unmutated cases expressed SlgD (7/12, 58%). CD27 was detected at a similar frequency in either the IGHV-mutated or IGHV-unmutated subgroups (11/16 and 8/12 cases, respectively). Six out of 10 CD27-negative cases carried IGHV-mutated genes; all six CD27-negative/IGHV-mutated cases expressed DBA 44. These results confirm the considerable histological, immunohistochemical and molecular heterogeneity of SMZL and indicate that assessment of the expression of the differential markers may be relevant in the diagnosis of normal SMZ. Furthermore, they indicate a role for selective antigentic pressures in the pathogenesis of at least a subset of SMZL cases.

0628

REAL-TIME QUANTITATIVE PCR AND FOUR COLOUR FLOW CYTOMETRY FOR MINIMAL RESIDUAL DISEASE ASSESSMENT OF MANTLE CELL LYMPHOMA

P. Gameiro,1 M. Sebastião,1 P. Lucto,1 T. Faria,1 J. Cabeçadas,1 A. Parreira,1 M.G. Silva2

1Portuguese Institute of Oncology, LISBON, Portugal; 2Hematology Department-IPOLFG, LISBON, Portugal

Background. MCL is an aggressive disease and few patients reach long-term survival. The impact of tumour load estimation and MRD quantification in these patients is unclear. We analysed peripheral blood (PB) and bone marrow (BM) disease levels obtained by both FC and PCR. PB and BM were obtained at diagnosis and after therapy (4/31) 1st line therapy or during complete remission (2/31). RQ-PCR monitoring was performed using the following panel: anti CD19, CD20, CD22, CD23, CD10, FMC7, CD5, CD45, K. B cells were considered neoplastic if they were CD19+/CD5+ and exhibited light chain restriction; CD23 and CD10 were always negative. 20000-150000 events were acquired per sample. Results: At diagnosis (N=15) FC sensitivity between 10-4 and 10-5 and PCR sensitivity between 2x10^-10 and 10^-10) demonstrated tumour infiltration in all samples analysed. Importantly, in 4 BM samples without histological infiltration, FC and RQ-PCR detected disease levels between 1.2x10^-2 and 2.6x10^-9, respectively. Overall we observed concordant results between RQ-PCR and FC in 83% of the cases. Disparities occurred in 2 BM samples and resulted in 2 RQ-PCR+FC- and 1 RQ-PCR+FC- obtained after and during 1st line treatment, respectively. The former can be false positive FC results and the latter can be attributed to the higher sensitivity of the RQ-PCR. Nevertheless, sampling effect can not be excluded. In 5/15 follow-up samples with MRD levels quantified by FC and RQ-PCR (results within the quantitative range), we found a positive correlation between the two techniques: p<0.001, Pearson correlation r=0.979. There was no significant association between disease levels at diagnosis (n=12) and clinical status at end of 1st line treatment: complete remission (n=5), median quantity (MQ)=2.6x10^-5, range 0-6.9x10^-4. Partial remission (n=4) MQ=17.57% (5.3-49.13), progression (n=3) MQ=43.75% (29.8-100). Conclusions. FC and RQ-PCR tumour burden estimation and MRD quantification correlate well in MCL. Nevertheless, discrepant results can be obtained and both approaches should be used to improve assessment of tumour dissemination. At diagnosis FC and RQ-PCR can detect low levels of disease in patients without histological BM involvement and contribute to a more accurate clinical staging. Longer follow-up studies, based on FC and RQ-PCR are needed to clarify the association between minimal disease levels and clinical outcome.

0629

PHARMACOGENOMIC APPROACH IN WALDENSTRÖM’S MACROGLOBULINEMIA AND SMALL LYMPHOCYTIC LYMPHOMA PATIENTS: hCNT1 EXPRESSION AS A POSSIBLE PREDICTIVE BIOMARKER OF 2-CHLORO-2’-DEOXYADENOSINE CLINICAL ACTIVITY

C. Basascio,1 D. Laszlo,1 L. Saronni,1 V. Raia,1 F. Bertolini,1 P. Antoniotti,1 A. Pinto,1 F. Frigeni,1 A. Fabbrì,1 L. Righacci,1 A. Billio,1 G. Martellì1

1European Institute of Oncology, MILAN, Italy; 2Istituto Nazionale dei Tumori, NAPOLI, Italy; 3Università degli Studi, SIENA, Italy; 4Polichroni Careggi, FIRENZE, Italy; 5Ospedale di Boltazo, BOLZANO, Italy

Background. Pharmacogenomic can be used to identify genetic factors that influence drug response, such as single nucleotide polymorphism, RNA splicing, gene expression and transcription. 2-chloro-2’-deoxyadenosine (2-CDA) undergoes complex intracellular metabolism and cell resistance mechanism to 2-CDA is not completely known. Aim. In this study we used a pharmacogenomic approach to identify genetic factors that could influence 2-CDA clinical response. Method. Using ABI PRISM 7000 Real Time platform we amplified seven genes, encoding for equilibrative and concentrative nucleoside transporter (hENT1, hCNT1), deoxyctydine and deoxyguanosine kinase (dCK, dGKR), S-nucleotidase (5’NT), ribonuclease reductase catalytic and regulator (RR1, RR2) subunits, in the bone marrow at diagnosis of 27 patients with Waldenström’s Macroglobulinemia or Small Lymphocytic Lymphoma. All patients were treated with 4 courses of combination therapy. 2-CDA 0.1 mg/kg for 5 days sc injection and Rituximab at standard schedule. Quantitation was performed using the Delta CT calculation: the value of gene expression was normalised to the calibrator (healthy tissue controls). Results. hCNT1, RR2, 5’NT gene expression analysis has shown lower values in patients than in healthy tissue controls. Patients who achieved clinical partial remission (PR) presented 100 times lower hCNT1 levels (median 3x10^-4, range 0.6x10^-9 to 10^-10) than patients (n=12) in complete remission or very good partial remission (median 2x10^-4, range 0.1x10^-9 to 0.03). Three patients showed drug toxicity; 2 of them (with very low hCNT1 levels) interrupted 2-CDA treatment. Acomplete the therapy and now is in follow-up, his basal hCNT1 level was similar to the expression of patients who obtained a complete remission. Conclusion. hCNT1 seems to be an important gene involved in 2-CDA clinical activity and its expression may correlate with prognosis. Compared to controls, the low RNA level of hCNT1 level was exhibited in patients that doesn’t seem to be predictive of lack of clinical activity of 2-CDA. However the lower hCNT1 expression detected in patients who achieved only PR could suggest a possible relationship between reduced hCNT1 expression and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing a quantitative method in order to identify a threshold value which could be predictive of drug resistance.

0630

LYMPHOMA CELLS MICRODISSECTED FROM PRIMARY SPLENIC LOW GRADe NON-HODGKIN’S LYMPHOMA BUT NOT NORMAL SPLENIC CELLS CONTAIN HCV GENOME

A. de Renzo,1 B. De Angelis,1 P. Marcilli,1 C. Quintarelli,1 M. Picardi,1 R. Ciancia,1 M. Persico,1 B. Rotoli,1 F. Pane1

1University of Naples, NAPLES, Italy; 2Second University, NAPLES, Italy

The role of HCV infection in the pathogenesis of various types of B cell Non-Hodgkin’s lymphomas (B-NHL) has been suggested from epidemiological studies, however molecular mechanisms accounting the neoplastic transformation have been to be determined. To investigate the relationship between HCV infection and B-NHL we studied spleen paraffin-embedded sections of 12 patients with primary splenic B-NHL. 8/12 patients were affected from a high grade B-NHL, 4/12 from a low grade B-NHL. Normal and neoplastic cells were individually microdissected as pure cells populations from tissue sections by using a Laser-assisted microdissection apparatus. The presence of HCV genomes was investigated by RT-PCR in RNA extracted from these two cell populations of each patient. While the HCV genome was found both in neoplastic and in normal cells in all the eight patients with high grade disease, the four low grade B-NHL samples showed the presence of the HCV genome only in the lymphoma cells and not in the normal cells. These results suggested a direct role of the virus in the low grade lymphoma transformation. In addition, to investigate the molecular pathways involved in the neoplastic transformation, we evaluated, by quantitative real time PCR, BCL2 expression in the cell populations microdissected from the patients. Interestingly, lymphoma cells isolated from low grade NHL patients showed very low expression of the BCL2 gene thus suggesting that molecular mechanisms of neoplastic transformation in these patients is not BCL-2 mediated as in the other low grade NHLs. In conclusion, our results indicate that at least in low grade NHLs, the HCV might directly involved in the neoplastic transformation, and that the molecular mechanisms may be different respect the other types of low grade NHLs.
Acute myeloid leukemia III

0631
APPLICATION OF EXTENDED COX PROPORTIONAL HAZARD MODELS ON THE DATA SETS OF THE ORIGINAL DATA BASED META-ANALYSIS ON PATIENTS 18 TO 60 YEARS OF AGE WITH CHROMOSOME-FORMING ACUTE MYELOID LEUKEMIA OF THE GERMAN AML-INTERGROUP

R.F. Schlenk,1 K. Döhner,2 M. Kerz,3 B. Heydrich,1 A. Glimmer,1 H. Fraile,1 K. Götsz,4 E. Koller,2 W. Frieder,2 D. Haase,4 M. Theobald,2 D. Nachbaur,1 A. Ganser,1 H. Döhner2
1University of Ulm, ULM, Germany; University of Kiel, KIEL, Germany; University of Bonn, BONN, Germany; University of Giessen, GIESSEN, Germany; University of Munich, MUNICH, Germany; University of Hamburg, HAMBURG, Germany; University of Göttlingen, GÖTTINGEN, Germany; University of Mainz, MAINZ, Germany; University of Innsbruck, INNSBRUCK, Germany; Hannover Medical School, HANNOVER, Germany

Background. There is increasing interest to apply survival analysis to data sets with multiple events per subject including on the one hand multiple events of the same type and on the other hand events of different types. In our meta-analysis on CBF-AML (Schlenk et al. JCO 2004, 22:3741) we used preponderantly time to first event approaches like relapse free survival. However, such approaches neglect the multiplicity of events and especially the influence of the number of treatment failures on the impact of different strategies in remission. We now report a meta-analysis of extended Cox proportional hazard models to assess the impact of different treatment strategies during first remission (CR) and after relapse in a unique model. Methods. We used three approaches of extended Cox models for the re-analysis: i) the Andersen-Gill (AG) model assuming independence of events in the different time periods first CR and after relapse, ii) the Prentice-Williams-Peto (PWP) model assuming that a patient can only be at risk for the second time period after relapse until he underwent an event in the first time period first CR, iii) the Wei-Lin-Weissfeld (WLW) model allowing a separate underlying hazard for each event. The different models were compared by explained variation using the Brier-Score. The dataset were restricted to full-set records for patients achieving a first CR (inv(16) n=158, t(8;21) n=149). The variables in the models were the different treatment strategies [allogeneic transplantation from matched-related donor (ALLO-SCT), autologous transplantation (AUTO-SCT), chemotherapy (CHEMO), allogeneic transplantation from matched-unrelated donor (MUD-SCT), intensive high-dose cytarabine based chemotherapy (HIGH), cytogenetic subtypes of AML].

Results. A total of 948 patients were included in the meta-analysis with a median follow-up time of 11.3 months. The impact of different treatment strategies during first remission (CR) and after relapse in a unique model.

CR
GO-A-HAM 26 (53%) 40 (34%) 5 (23%) 3 (14%)
P 6 (12%) 33 (28%) 5 (23%) 4 (19%)
RD 15 (31%) 36 (31%) 12 (54%) 12 (57%)
death 2 (4%) 8 (7%) 0 (0%) 2 (10%)

No CTC-grade 3-5 liver toxicity was seen in patients receiving GO-A-HAM. Logistic regression on the achievement of CR after salvage therapy revealed that regimens containing ATRA (odds ratio 2.1, p=0.05) and GO (odds ratio 2.2, p=0.02) were associated with response. 142 of 255 patients have received stem cell transplantation. One (4%, 95%-CI 0.002-0.018) case of severe veno occlusive disease was seen in 28 so far transplanted patients who have had GO-A-HAM. Median survival was 11.3 months. Conclusions. Although retrospective in nature our study suggests that ATRA and GO as adjunct to salvage chemotherapy in primary refractory AML patients improves CR rates.

0633
GENETIC CHARACTERIZATION OF PATIENTS WITH AML-M2. THE JAK2 V617F ACTIVATING MUTATION IS FREQUENTLY FOUND IN CASES WITH NORMAL KARYOTYPE

A. Conchillo,1 I. Vazquez,1 C. Vicente,1 N. Martocci,2 G. Rivell,3 C. Carranza,2 E. Bandres,1 X. Aguirre,1 M.J. Larrayoz,2 M.J. Calasanza,1 I. Lahortiga,1 M.D. Odero2
1CIMA, University of Navarra, PAMPLONA, Spain; Genetics, CIMA, University of Navarra, PAMPLONA, Spain; 2Genetics, University of Navarra, PAMPLONA, Spain

The characterization of genetic and molecular aberrations in AML has substantially improved our understanding of the pathogenesis of this disease. The activating V617F mutation of JAK2 has been recently described as a common event in MFD. The same mutation was also found in a small number of patients with either AML or MDS. However, there are few data about the frequency of JAK2 V617F in specific subtypes of AML. We investigated the incidence of this mutation in 10 cell lines and 331 well characterized AML patients, and its association with other factors with a prognosis meaning. V617F genotyping was performed by ARMS as previously described (Jones et al., 2005). All cas-

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The Wnt pathway is activated frequently in acute leukemia. A total of 76 bone marrow (BM) and peripheral blood (PB) samples from 32 fresh AML patients were collected after obtaining informed consent. Separately, 5 normal hematopoietic stem cell transplantation donors were used as a control. Two major methods were used as a control. The initial investigation of the patient’s FLIP expression level was examined compared to the levels of normal population.

Clinical profiles and results of RQ-PCR during treatment were compared. Overall, AML patients, specifically at initial diagnosis, showed relatively higher FLIP levels of expression both in the BM and in the PB. BM showed relatively higher expressions than the PB in AML.

Two significant values in association with clinical outcome were the levels of expression of FLIP at initial diagnosis and on day 7 BM/PB during IC. The most reliable one was at day 7 PB (p=0.02) and patients with complete remission (CRs) showed comparable expressions with those of normal controls. An unexpected finding was that the expressions after immediate recovery had no correlations with outcome. The maximum expression difference of FLIP between CRs and poor responders was 1.2 logs before RQ-PCR. Conclusions. Although further investigations with more patients are needed to verify the exact role of FLIP in a minor cell population as shown in this study, specifically at day 7 during IC, FLIP expression might be an important factor for predicting IC and in the treatment of AML patients, based on expressions of FLIP RQ-PCR.

EXPRESSION OF THE BRAIN AND ACUTE LEUKEMIA CYTOPLASMIC GENE IN ACUTE MYELOID LEUKEMIA

P. Bernasconi,1 S. Calatroni,2 B. Rocca,1 M. Boni,2 R. Zappatore,1 P.M. Cavigliano,1 I. Giardini,1 I. Dambrusso,1 M. Lunghi,1 M. Caesarna,1 C. Antoni,1 C. Castagnola,1 M. Lazzarino1

1IRCCS Policlinico San Matteo, PAVIA, Italy; 2Division of Hematology, PAVIA, Italy

Clonal chromosome abnormalities play a major role in de novo AML being absolutely required to make a correct diagnosis and an accurate prognostic stratification. However, about 45% of AML patients are karyotypically normal and are supposed to have an intermediate prognosis even though only 40% of them are long-term survivors. Therefore, in patients with new molecular markers of prognostic significance that have been actively searched and recent evidence suggests that BAALC gene expression levels are one of the most relevant. In the present study we determined BAALC expression in the pre-treatment bone marrow samples of 25 adult AMLs (9 M0-M1, 3 M2, 14 M4-M5 and 1 M6), 6 females and 19 males (median age 68 years; range 27-75), which showed a normal karyotype, 2 a del(7)(q31q35), 2 a del(9)(p22q34) [one with +8], a complex karyotype (33 abnormalities), 5 an inv(16)(p13q22) [two with +8], and 3 miscellaneous defects. The study was aimed at detecting the incidence of high BAALC expression and at correlating BAALC expression levels with clinical/biological parameters and outcome. Statistical analysis were carried out by applying the method of Paffili HW & Dempfle L (Nucleic Acids Res 2002;30:536). BAALC relative quantification was achieved with real-time PCR using SybrGreen I as a double-stranded DNA-binding fluorescent dye. The forward and reverse primers used were those already published (Baldus et al., JCO 2006;24:790). Standard curve for real-time quantification was obtained by serial dilution of total RNA from an AML patient exhibiting an elevated BAALC expression. Quantification was achieved by applying the DDCt method. BAALC expression was normalized to ABL1 gene and calibrated on a normal control sample. A reference interval for BAALC expression quantification was fixed at 0.69 [mean expression ± 3 times the standard deviation (0.13)] after having analysed 12 normal controls. BAALC expression was low in 11 patients (median ± SD = 0.158±0.561) and high in 14 (median ± SD = 5.427±11.691) with a statistically significant difference (p=0.001). No difference between the two groups was noted in pre-treatment age, sex, white blood cell count and percentage of bone marrow blasts. High BAALC expressers were predominantly of M4-M5 cytotype. Six of the 11 chromosomally normal patients were low expressers, whereas the 3 patients harbouring a single inv(16) and all the 3 with +8 were high expressers. Nine low expressers received induction...
chemotherapy; 6 achieved a complete remission (CR) and 3 did not respond. Five of the 6 CRs relapsed and were unable to achieve a new CR. Eleven high expressers received induction chemotherapy; 8 achieved CR and 3 did not respond. Four of the 8 CRs relapsed but succeeded in achieving a second CR. In Conclusion, 56% of our AML patients presented a BAALC expression significantly higher than that of the remaining 11 patients; a high BAALC expression correlated with +8 and inv(16) (p13q22); high BAALC expressers showed a CR duration and an overall survival longer than those of low expressers perhaps because of the higher occurrence of +8 and inv(16) in the first patient group.

0637
THE OUTCOME OF POSTREMISSION TREATMENT FOR AML WITH FAVORABLE CYTOGENETICS IN FIRST REMISSION
H.-J. Shin,1 J.S. Chung,2 Y.J. Choi,1 G.J. Cho,1 H.-J. Kim,1 Y.-K. Kim,1 D.-H. Yang,1 S.K. Sohn,1 J.G. Kim1
1Pusan National University Hospital, BUSAN, South-Korea; 2Chonnam National University Hospital, HWASUN, South-Korea; Kyungpook National University Hospital, DAEGU, South Korea

Background. The beneficial impact of high-dose cytarabine (HDAC)-based consolidation chemotherapy in acute myeloid leukaemia (AML) is much greater in patients with favorable cytogenetics (t(8;21), inv(16) and t(16;16)) than in those with normal karyotypes. However, in MRC AML 10 study, patients with favorable cytogenetics who received autologous stem cell transplantation (SCT) had a markedly lower relapse rate than those who did not receive autologous SCT, although a high procedural mortality rate in adults resulted in being ultimately no difference in the overall survival (OS). Allogeneic SCT have not been recommended as standard therapy for AML with favorable cytogenetics due to relatively high remission related mortality (TRM). However, progress in SCT and supportive care over the past decades have led to gradual improvement in the TRM after allogeneic SCT. Aims. We try to compare the outcome of allogeneic SCT with HDAC during the first remission of AML with favorable cytogenetics. Methods. 80 AML patients with favorable cytogenetics (excluded AML, M3) who entered complete remission (CR) between March 1997 and July 2005 at three centers were reviewed retrospectively. Among 50 patients, 13 patients who relapsed or died during consolidation chemotherapy, received less than three cycles of consolidation chemotherapy or underwent autologous SCT in first remission were excluded. Overall, 87 AML patients over the 18 years with favorable cytogenetics who underwent allogeneic SCT or received three/four cycles of HDAC consolidation chemotherapy in first CR could be analyzed. Results. The median follow up duration was 48 months. The 5-year probability of disease free survival (DFS) and OS were 50.3% and 31.6%, respectively. The estimated 5-year probability of DFS (73.2% vs 21.0%) (p=0.005) and OS (71.9% vs 28.9%) (p=0.03) were significantly better in the patients who underwent allogeneic SCT than in those who received HDAC. The cumulative incidence of TRM and relapse rate were 9.5% and 18.6%, respectively. In the subset analysis, OS was better in the allogeneic SCT group than in the HDAC group in the setting of age < 55 years (5-year estimated OS: 100% vs 33.5%) (p=0.0054), but not different in age ≥ 55 years (p=0.54). The OS was statistically superior for allogeneic SCT group versus HDAC group in the setting of chromosomal abnormalities ≥ 2 (5-year estimated OS: 72.9% vs 41.7%) (p=0.007), but not in chromosomal abnormalities < 2 (p=0.38). Conclusions. In AML patients with favorable cytogenetics (especially younger age) who have a matched related donor, allogeneic SCT can be option. Especially those who have more than 2 chromosomal abnormalities should undergo allogeneic SCT with matched related donor or unrelated donor. It is needed that AML patients with favorable cytogenetics who have sibling matched donor are assigned to allogeneic SCT and remaining to HDAC or autologous SCT are randomly assigned.

0638
ARE FLT3 ITD AND D835 MUTATIONS SUFFICIENT INDICATORS FOR ALLOGENEIC TRANSPLANTATION IN ACUTE MYELOID LEUKAEMIA? AN ANALYSIS OF PATIENTS FROM THE CZECH ACUTE LEUKAEMIA CLINICAL REGISTER (ALERT)
M. Doubek,1 J. Muzik,2 T. Szotkowski,3 V. Koza,4 P. Cetkovsky,5 T. Kozak,6 J. Voglova,1 L. Dusek,1 K. Indrak7
1University Hospital, BRNO, Czech Republic; 2Masaryk University, BRNO, Czech Republic; 3Palacky University Hospital, OLOMOUC, Czech Republic; 4Charles University Hospital, PLZEN, Czech Republic; Institute of Hematology, PRAGUE, Czech Republic; 5University Hospital Krakovske Vinohrady, PRAGUE, Czech Republic

Background. FMS-like tyrosin kinase 3 (FLT3) is preferentially expressed on acute myeloid leukemia (AML) hematopoietic progenitor cells and mediates stem cell differentiation and proliferation. Two types of activating FLT3 mutations have been described in acute myeloid leukemia (AML): internal tandem duplication (ITD) of the FLT3 gene and point mutation within the activation loop of the tyrosin kinase domain, which mostly affects asparagine 835 (D835). Many studies have shown that presence of FLT3 ITD correlates with poor outcome of AML patients. The prognostic relevance of D835 mutation is less clear, although most likely it also has a negative prognostic effect on the patients with AML. So far it is not clear how to treat the patients with FLT3 ITD and D835 mutations compare to patients without these mutations and whether these patients benefit from allogeneic transplantation. We tried to compare these mutations and their impact on AML patients. To assess the prognostic relevance of activating mutations of FLT3 gene on outcome of allogeneic transplantsations in AML patients, we performed an analysis of all patients with FLT3 mutations registered in the Czech Acute Leukemia Clinical Register (ALERT) from 2003 till the end of 2005. ALERT registers all adult patients diagnosed in 6 main haematology centres in the Czech Republic. Results. Within the mentioned period 170 patients with AML of median age 59 years (in total) were investigated for FLT3 mutation, within them 37 cases (22%; 19 men and 18 women) with FLT3 mutations (33 FLT3 ITD and 4 FLT3 D835) were found. 33 patients were suitable for analysis. 15 of these patients had allogeneic transplantation, 20 patients with mutations of FLT3 were treated with chemotherapy without transplantation. Results of the treatment of these patients were compared with the results of the group of patients without FLT3 mutation, which was according to other characteristics identical with the group of patients with FLT3 mutations (n=125). Results. Median overall survival (OS) of patients with mutations of FLT3 who had allogeneic transplantation was 42.5 weeks, median survival of patients with mutations of FLT3 treated only with chemotherapy was 29.6 weeks (p=0.4). Median disease free survival (DFS) of the same patients was 32.1 weeks in transplanted patients and 24.3 weeks in patients treated only with chemotherapy (p=0.6). OS of patients with mutations of FLT3 was significantly better in patients without mutation FLT3 than in patients with mutation FLT3 (28.2 weeks compared to 50.2 weeks; p=0.05). Conclusions. Our results suggest that at present there is no strong evidence that FLT3 status alone should influence the decision to proceed to allogeneic transplantation in AML patients. Decision to proceed to allogeneic transplantation should not be based on the FLT3 status only, but it should also consider other prognostic factors. Although the mutations FLT3 mean higher risk of relapses, according to our analysis they do not significantly influence the OS of AML patients.

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0639
OPTIMISATION OF A 48HOUR IN VITRO CHEMOSENSITIVITY ASSAY FOR CD34+CD38- CD123+ LEUKAEMIC STEM AND PROGENITOR CELLS
M. Pallis,1 U. Mony,2 N. Russel1
1Nottingham City Hospital, NOTTINGHAM, United Kingdom; 2University of Nottingham, NOTTINGHAM, United Kingdom

Background. The majority of AML patients respond to remission-induction chemotherapy, but the relapse rate is high. Relapse is underpinned by outgrowth of leukemic stem and progenitor cells (LSPC). There is a need to develop chemosensitivity assays for LSPC. Aim. We aimed to establish a methodology to distinguish normal from leukemic NPC, to optimise maintenance of the LSPC phenotype in 48 hour culture and to quantify viable LSPC following culture with and without drugs. Methods and Results. The CD34+CD38-CD123+high phenotype was used to optimise a 48 hour in vitro chemosensitivity assay for CD34+CD38- CD123+ leukemia stem and progenitor cells. The assay was validated using a panel of drugs and cell lines and found to accurately quantify drug response.
distinguish LSPC. CD123 fluorescence intensity was measured using fluorescence standards. LSPC were enriched from presentation samples using Miltenyi CD133-coated beads. The concentration of LSPC at the start of 48 hr culture ranged from 0.2-16×10^4/mL (median 3.4×10^4/mL). We compared various culture conditions aimed at maintaining these cells in culture without differentiation (i.e. without loss of phenotype), including serum, immobilized fibronectin, SCF, IL-3, IL-6, GM-CSF and angiopoietin. Two flow cytometric assays in parallel for analysis of LSPC survival: in the first assay, viable cells are enumerated using the dye 7-AAD along with an internal standard for cell counting. In the second assay, cells are labelled with CD34FITC, CD123PE, 7-AAD and CD38APC in order to quantiﬁy LSPC as a percentage of viable cells. We found that serum-free culture medium, ﬁbronectin-coated wells, and a cocktail of SCF, IL-3, IL-6 and angiopoietin 1 was the most successful at maintaining the concentration of CD34+CD38-CD123+ cells in culture for 48 hours, (median 0% change), although there was considerable variation between samples. We examined the effect of these niche conditions on the sensitivity of LSPC to cytokine arabinoside (ara-C). One-month primary data on 5 samples indicates that 500 mL ara-C reduced the LSPC number to 15% of untreated control values in the absence of ﬁbronectin and cytokines, whereas 50% of ara-C-treated LSPC were still viable in the presence of ﬁbronectin and cytokines. Conclusion. We have deﬁned a system for assessing the in vitro chemosensitivity of LSPC suitable for the study of anti-leukaemic agents in primary AML samples.

**0640**

**EXPRESSION OF P73 AND P53 PROTEINS IN LEUKEMIC CELLS AND SURVIVAL OF ACUTE MYELOID LEUKEMIA PATIENTS**

A. Pluta, P. Smolewski, B. Cebula, K. Jamroziak, A. Wierzbow ska, A. Wrzesien-Kus, T. Robak

Medical University of Lodz, LODZ, Poland

Background. Prognostic significance of apoptosis-regulating proteins, especially recently discovered p73, is not clearly determined in acute myeloid leukemia (AML). The p73 protein is a new member of p53 family implicated in the regulation of cell cycle, apoptosis and development. Overexpression of p73 protein, with prevalence of short TAp73 isoforms, has recently been described in patients with AML. Aims. The main objective of this study was to verify whether expression of p73 and p53 proteins, pro- and anti-apoptotic members of the Bcl-2 family and caspase 3 has a prognostic impact on response to induction chemotherapy and overall survival (OS) of adult patients with AML. Additionally, we aimed to compare the expression of these apoptosis-regulating proteins between normal CD34+ and leukemic cells. Material and Methods. Intracellular expression of p73 protein in leukemic blasts isolated from bone marrow or peripheral blood was examined in 50 AML patients (36 de novo, 14 refractory/relapsed) of median age 55 years (range 28-78). In parallel, expression of other apoptosis-regulating proteins including p53, Bcl-2, Bax, as well as the cleaved form of caspase-3 as a marker of apoptosis, was studied. The control constituted CD34+ cells isolated from 10 Hodgkin lymphoma patients without bone marrow involvement. All measurements were performed using multi-color flow cytometry. Protein expression was expressed by both percentage of positive cells and mean fluorescence intensity. Results. Thirty (56%) patients achieved complete response (CR) after induction chemotherapy, 20 (56%) patients did not respond and 3 (8%) patients died in the early post-induction period. The median time of the follow up reached 5 months (range 0.1-27). Comparing to normal CD34+ cells, AML blasts had higher expression of p73 and Bax proteins as well as cleaved caspase-3 (p<0.007, p<0.001 and p<0.0001, respectively), while no significant differences were noted regarding p53 and Bcl-2. None of the analysed proteins showed predictive impact on probability of CR achievement after induction regimen. However, we found that AML patients with higher expression of p53 protein had significantly better OS as compared to other patients (14 vs 6 months, p=0.044). Moreover, similar trend towards longer OS was observed for the patients with higher expression of p73 protein (p=0.061). Interestingly, simultaneous high expression of both p53 and p73 proteins correlated better overall OS of our AML cohort, as confirmed by univariate and multivariate analyses (p=0.012 and p=0.059, respectively). Conclusions. These data indicate that high expression of p73 protein in leukemic blasts, especially when co-expressed with p53 protein, favourably correlates with survival of adult AML patients. Furthermore, AML blasts may have increased proclivity to spontaneously undergo apoptosis comparing to normal CD34+ cells, with overexpression of proapoptotic p73 and Bax proteins.

**0641**

**GENE EXPRESSION PROFILE OF ACUTE MYELOID LEUKEMIA WITH MULTILINEAGE DYSPLASIA CONFIRMS ITS BIOLOGICAL HETEROGENEITY**


1 Hospital Clinic, BARCELONA, Spain; 2 Hematopathology Unit, Hospital Clinic, BARCELONA, Spain

Background. Acute myeloid leukemia with multilineage dysplasia (AML-MD), recognized in the WHO classification as a major AML category, is usually related to myelodysplasia and considered a poor-prognosis disease. However, the biology of this condition has not been extensively assessed, and previous studies suggest that cytogenetics define different pathogenic and prognostic AML-MD subgroups. Aim. To analyze the gene expression profile of a series of AML with multilineage dysplasia (AML-MD), Patients and Methods. Nineteen patients (median age: 71, 30-93; 58% female) diagnosed with AML-MD in a single institution were included in the study. The gene expression profile of these cases at diagnosis was examined with oligonucleotide HG-U133 Plus 2.0 arrays (Affymetrix). Expression measures were summarized using RMA methodology from the Affy package of the Bioconductor project. Unsupervised two-dimensional cluster analysis of high variability genes was done with dChip v1.3. In addition, a supervised analysis to identify genes with significant differential expression according to cytogenetic category was based on Limma package from Bioconductor which employs Bayesian statistics adjusted for multiple testing. Results. The unsupervised hierarchical cluster analysis identified two main groups of cases, which differentiated mainly according to cytogenetic risk category: group 1, (n=10), including 9 (90%) AML cases with intermediate-risk cytogenetics, and group 2 (n=9), with predominance of high-risk cytogenetics (78%, p=0.0045). Genes found overexpressed in group 1 included FLT-3 and several homeobox (HOXAS HOXAS, HOX7, HOXA9, HOXA10, HOXAI1, HOXBI2, HOXB3, HOXB5, HOXB6, HOXB7, HOXB8 and HOXB9) genes. On the contrary, relevant genes such as MLL, MLL3, CEBPD and EVI1 were overexpressed in group 2. The supervised analysis allowed the identification of a cluster of 92 genes differentially expressed according to cytogenetic category. Thus, genes found overexpressed in AML-MD with intermediate risk cytogenetics included ribosomal constituents and genes involved in translation (RPS20, LOC200916, LOC400085, EIF5S5), while diverse membrane-receptor genes, including genes involved in the immune response (FCGR3A, FCGR3B, IL1R2, PLXNC1, FCAR, CLEC4D, CLEC4E, TNFRSF10C, CSF1R), were overexpressed in AML-MD associated with high-risk cytogenetics. The survival analysis of patients receiving intensive chemotherapy identified only cytogenetics, and not gene expression profile categories, as prognostically significant (figure). Conclusions. Gene expression profiling herein described supports the biological diversity of AML-MD, which seems to be related to the underlying cytogenetic abnormalities. Further studies in larger series of patients are warranted to gain insight into the biological diversity of this disease and clinical implications.

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Background. Cardiotoxicity is a relatively frequent and potentially serious complication of hematopoietic treatment. Anthracyclines (ANT) represent the greatest risk. Cardiotoxicity of ANT may develop during the treatment (acute cardiotoxicity) and during the follow-up (chronic and late cardiotoxicity). Various methods including biochemical markers have been recommended for monitoring of cardiotoxicity of treatment in hematopoietic treatment. Aims. Monitoring of cardiotoxicity of ANT in patients treated for acute leukemia with biochemical markers ‘N-tertiary pro brain natural peptide (NT-proBNP), cardiac troponin T (cTnT); echocardiography (ECHO) and electrocardiography (ECG). Methods. 26 adult acute leukemia patients (mean age 46.2±12.4 years, 15 males) treated with 26 cycles of ANT-based chemotherapy (CT) were studied. Cardiac evaluation was performed at the baseline (before CT), after first CT (cumulative ANT dose 160.5±28.3 mg/m²), after last CT (cumulative ANT dose 464.5±117.5 mg/m²) and circa 6 months after completion of CT (6 Mo after CT). Results. The results are summarized in the Table. Six months after CT, NT-proBNP concentrations correlated with systolic and diastolic LV dysfunction on ECHO (r=0.514; p<0.01 and r=0.587; p<0.01). Decreased QRS voltage on ECG correlated with systolic and diastolic LV dysfunction on ECHO (r=0.606; p<0.001) and (r=0.592; p<0.001). Our results demonstrate acute and chronic cardiotoxicity of ANT. Clinical manifestation of cardiotoxicity in terms of heart failure developed in 2 (7.7%) patients. In asymptomatic patients, abnormal cardiac findings represent subclinical cardiotoxicity, which indicates a risk for development of heart failure (NT-proBNP elevations, diastolic LV dysfunction) and malignant ventricular arrhythmias (QTc prolongation). In regard of late ANT cardiotoxicity, further cardiology follow-up is warranted in all acute leukemia survivors.

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Acute myeloid leukemia (AML) is a heterogeneous group of disease and AML patients may have distinct morphologic, cytochemical, immunophenotypic and clinical characteristics. Extramedullary infiltration (EMI) of malignant myeloid precursor cells may occasionally be a presenting clinical symptom at onset and may develop at any site in the body but most commonly in the gum, skin, central nervous system (CNS) and soft tissue. There is controversy about the prognostic significance of extramedullary disease in AML, while in some reports it confers a poorer prognosis other studies do not report a prognostic significance. Aim. The present study examines the incidence, biological features and prognostic significance of EMI at diagnosis in adult patients with AML. Methods. From January 1997 to December 2004, 213 untreated patients with de novo AML were studied; patients with APL were excluded. Results. Of 213 cases with de novo AML, 29 (13.6%) had EMI at diagnosis. Ten patients (48.4%) had skin infiltrates, 12 (41.4%) had gum hypertrophy, 5 (17.2%) had CNS involvement and 2 (6.9%) had soft tissue infiltration. No significant differences in terms of sex, age, median Hb level and platelets count were found between patients with EMI and patients without EMI. The patients with EMI had higher median WBC counts (27 x 109/L) than patients without EMI (8.5 x 109/L) (p=0.05). The patients with EMI had a higher incidence of the M4/M5 FAB subtype (62%) than patients without EMI (27.4%) (p=0.005). Cytogenetic analysis was performed in patients with and without EMI; none of the abnormal cytogenetic findings was associated with EMI. We evaluated the relationship between the AML blasts surface antigen expression, and EMI: the association between CD56/CD4 and CD56/CD14 was more significantly expressed in patients with EMI (35% and 29%, respectively) than without EMI (10.4% and 6.9%, respectively) (p=0.004, p=0.003). All patients had been treated with induction therapy according to the GIMEMA Protocols including Ara-C, etoposide and cytarabine (EMI) and soft tissue infiltration. No significant differences in terms of sex, age, median Hb level and platelets count were found between patients with EMI and patients without EMI. The patients with EMI had higher median WBC counts (27 x 109/L) than patients without EMI (8.5 x 109/L) (p=0.05). The patients with EMI had a higher incidence of the M4/M5 FAB subtype (62%) than patients without EMI (27.4%) (p=0.005). Cytogenetic analysis was performed in patients with and without EMI; none of the abnormal cytogenetic findings was associated with EMI. We evaluated the relationship between the AML blasts surface antigen expression, and EMI: the association between CD56/CD4 and CD56/CD14 was more significantly expressed in patients with EMI (35% and 29%, respectively) than without EMI (10.4% and 6.9%, respectively) (p=0.004, p=0.003). All patients had been treated with induction therapy according to the GIMEMA Protocols including Ara-C, etoposide and idarubicin (15 pts), mitoxantrone (15 pts) or daunorubicin (185 pts). The overall CR rate was 65%; the CR rate was lower in patients with EMI (46.2%) than those without (76.1%) (p=0.001) and their disease free survival was also shorter (p=0.017): the median duration of CR was 10 and 25 months (range 1-96) in the EMI and no EMI group, respectively. Conclusions. Our data demonstrate that a high WBC count, M4/M5 subtype, CD56/CD4 and CD56/CD14 expression are associated with extramedullary infiltrates of AML at diagnosis; the presence of EMI adversely affects the complete response rate to induction chemotherapy and the OS rate. Analysis of the clinical and biologic features in a larger series of adult AML patients is needed to evaluate the allocation of this subgroup to a different or more intensive treatment arm. Patients with EMI may warrant alternative therapy to improve their clinical outcome.

Background. Acute myeloid leukemia (AML) has been proposed to arise from the collaboration of various chromosomal abnormalities. These chromosomal abnormalities found in AML frequently target the transcription factor genes which can control important biological processes, including cellular proliferation, differentiation, transformation and apoptosis. Aims. We selected 15 SNPs (single nucleotide polymorphisms) of transcription factor genes to test whether they are associated with increased susceptibility to patients with AML. Methods. This study analyzed the frequencies of 15 SNPs of transcription factor genes in 269 de novo AML patients and age- and sex-matched controls. These 15 SNPs were selected from 339 SNPs' analysis in previous study which were confirmed to be more than 15-20% in minor allele frequency in 120 normal Korean population. Genotyping method is pyrosequencing using genomic DNA from peripheral blood or bone marrow samples. Results. ETS2 rs530 (T1874A, OR: 1.929, range: 1.391-2.663), rs711 (G1+1655A, OR: 2.208, 1.596-3.504) and ELFI rs7799 (A173G, OR: 1.949, 1.925-2.867) were found to be significantly higher frequencies of each mutant genotype and allele in AML patients than in control. On the other hand, ELFI rs1058281 (A1107T), ZNF42 rs4756 (A531G) and FLI1 gchrd03-024 (C1-1014A) showed higher frequency of mutant genotype in AML than in control but there was no significant difference in mutant allele frequencies. Conclusions. This study showed the association between specific ETS family genes such as ETS2 and ELFI transcription factors and AML prevalence. Therefore, it suggests these specific ETS gene abnormalities as a susceptibility gene in AML, and proposes a number of molecular strategies for targeting these genetic abnormalities for therapeutic intervention.

Characterization of AML-M0: A Search for Tumor Suppressor Genes and Oncogenes

F.E. Silva, I. Almeida, M. van Velzen, A. Lind, B. Morolli, F. de Zwart, Y. Atyurek, S. White, H. Wessels, F. Valk, W.R. Sperr, M.A. van Putten, J. Schrijvers, A. Westerveld, G. van Dongen, K. de Rooij, F. P.G. Silva, I. Almeida, M. van Velzen, A. Lind, B. Morolli, F. de Zwart, Y. Atyurek, S. White, H. Wessels, F. Valk, W.R. Sperr, W.A.F. Manji, H.C. Klun-Nelemans, W. Ludwig, M. Giphart-Gassler, L. Leiden University Medical Centre, LEIDEN, Netherlands; Eramus Medical Center, ROTTERDAM, Netherlands; Medical University of Vienna, VIENNA, Austria; University Medical Center Groningen, GRONINGEN, Netherlands; Humboldt University of Berlin, BERLIN, Germany

Acute Myeloid Leukemias (AML) form a heterogeneous group of hematologic malignancies partly characterized by specific translocations. Several oncogenes and only a few tumor suppressor genes (TSG) have been associated with AML. The search for TSG in leukemias has been to a certain extent neglected. In this study, we aim to better characterize the minimally differentiated AML subgroup (AML-M0). AML-M0 do not present specific cytogenetic abnormalities and generally have a poor prognosis. We studied cryopreserved cells from 52 AML-M0 patients. From this material we expanded T-cells to be used as control cells and sorted the leukemic blasts to obtain a pure tumor cell fraction. To find new TSG, we have used Affymetrix 10K SNP-microarrays to compare the DNA of the blasts with that of the control material. We searched for regions of loss of heterozygosity (LOH), as LOH is frequently the second hit resulting in the total loss of function of a TSG. Furthermore, we have screened the patients for mutations in known oncoproteins such as FLT3, KIT, NPM1, NRAS, KRAS, PTPN11 and JAK2 and genes with dominant negative effect such as CEBPA. We found 16 patients with LOH of chromosome 21. Chromosome 21 harbor RUNX1, a well-known TSG frequently mutated in AML. We sequenced exons 3, 4 and 5 containing the Runt domain of RUNX1. 13 out of the 16 patients (81%) with LOH presented either a point mutation or deletion of RUNX1. Two other patients showed heterozygous mutations and another bi-allelic insertions. Thus in total 16 out of 52 patients (31%) showed mutation of RUNX1. We found 9 internal tandem duplications and 2 DEK-CAN1 activation loop mutations in FLT3 (19% of patients). From these patients only 2 cases had a RUNX1 mutation. From the 52 patients only one showed an insertion in exon 12 of NPM1. All other oncogenes mentioned above are currently being screened. Areas in chromosomes 3, 4, 7, 8 and 17 show LOH of DNA regions of less than 2 Mb and candidates TSG in these regions are currently under investigation. We hope that the characterization of AML-M0 will provide a better understanding and eventually a more favorable treatment of this disease, and will give insight into the pathogenesis of other subgroups of AML.
With modern chemotherapy, a complete remission (CR) is achieved in up to 90% of younger (<60 years) adult myeloid leukemia patients, but the majority of patients relapse due to persistence of minimal residual disease (MRD). Treatment strategies based on MRD status have not been established.

**Aim.** The aim of the study was to evaluate the prognostic significance of multi-parameter flow cytometry (FCM) based MRD monitoring in relation to stem cell transplantation (SCT) in younger adult AML patients Methods. Between July 1994 and June 2001, 62 younger adult patients (<60 years) were diagnosed with non-promyelocytic AML at Karolinska University Hospital Solna (in Stockholm). Morphological CR was achieved in 53 of 62 patients (85%). Follow-up MRD information was available in 45 CR patients (23 males and 22 females). The diagnostic flow cytometry panel included membrane CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD65, CD117, HLA-DR, and intracellular CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD65, CD117, HLA-DR, and intracellular CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD65, CD117, HLA-DR, and intracellular CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD65, CD117, HLA-DR, and intracellular CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15.

**Results.** Detectable MRD (1) did not predict relapse-free survival (RFS) and overall survival (OS) though there was a trend for longer RFS in MRD (2) negative patients (p<0.061). Improved RFS and OS were predicted only by SCT (p<0.001 and p= 0.001, respectively). To analyze in detail the impact of SCT on patient outcome, MRD positive patients were divided into 3 groups according to type of post-remission therapy: a) conventional chemotherapy, b) auto-SCT and c) allo-SCT. MRD (1) and/or (2) positive patients subjected to allo/auto-SCT had significantly better RFS and OS than patients who received only conventional chemotherapy (Figure 1). However, patients who underwent allo-SCT had a significantly better prognosis than patients who relapsed post-auto-SCT. At time-point (2) 5-year RFS was 80%, 53% and 0% in allo-SCT, auto-SCT and no transplantation groups, respectively (p=0.003).

**Conclusions.** Younger adult AML patients who have detectable MRD at the end of post-remission chemotherapy have a dismal prognosis and for these patients allo-SCT, auto-SCT or innovative new treatment strategies should be strongly considered.
Acute lymphoblastic leukemia

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SPECIFIC INTENSIVE CHEMOTHERAPY PLUS RITUXIMAB FOR Burkitt’s lymphoma or leukemia in HIV-positive and negative adult patients
A.O. Oriol,1 J.M. Ribera,2 J. Bergua,2 E. Giménez-Mesa,1 C. Grande,1 J. Esteve,3 S. Brunet,4 M.J. Moreno,5 L. Escoda,1 J.M. Hernández-Rivas,2 E. Felix2
1ICO-Hospital Germans Trias i Pujol, BADALONA, Spain; 2Pethema Group, SAN PEDRO DE ALCANTARA, Spain

Background. A previous PETHEMA protocol (PETHEMA ALL3/97) proved that HIV-positive patients with Burkitt’s lymphoma (BL) and Burkitt-like acute lymphoblastic leukemia (ALL3) had similar outcome than HIV-negative patients. Aims. To study the impact of the addition of rituximab to our previous protocol in terms of toxicity and efficacy, with special attention to HIV-positive patients. Patients and Methods. All consecutive patients diagnosed with BL/ALL3 between July 2003 and January 2006 received induction therapy including a pre-phase with cyclophosphamide (CPM) and prednisone (PND), followed by cycle A (rituximab, ifosfamide, VCR, dexamethasone –DMX-, HD-MTX, ARA-C and VM-26), cycle B (rituximab, VCR, HD-MTX, CPM, DMX and doxorubicin) an cycle C (rituximab, DMX, VDN, HD-MTX, HD-ARAC and VP-16). Patients with BL in stages I or II received 4 cycles (A1,B1,C1, A1) whereas those with BL in stages III or IV or with ALL3 received six cycles (A1,B1,C1,A2,B2,C2) followed by two additional rituximab doses. CNS prophylaxis consisted of IT MTX-ARA-C-DMX given in each cycle for a total of 8 doses. Results. 31 adult patients (20 HIV-negative and 11 HIV-positive) were included. Both groups of patients were comparable for age, gender, ECOG score, BM and CNS involvement, bulky disease, LDH and albumin serum levels. Twenty-two patients had BL and 9 ALL3. Three out of 11 HIV positive patients began treatment with HAART at the time of diagnosis and 8 were already under treatment. Median follow-up was 7 months (range 1-30). Main results of therapy are summarized in Table 1. No significant differences in CR, DFS or OS were observed between BL and ALL3 or between HIV-positive and negative patients. Grade 4 neutropenia and thrombocytopenia were constant and lasted a median of 7 days (range 2-31). Other frequent grade 3-4 toxicities were hepatic (8% of cycles), mucositis (18%) and infectious (18%). Episodes of grade 3-4 extrahematological toxicity were more frequent in HIV-positive patients (65% of mucositas, p=0.04; 65% of infections, p=0.04 and 62% of hepatic toxicities, p=NS).

Conclusions. Preliminary results suggest that the addition of rituximab to a specific BL/ALL3 treatment is also feasible for HIV-positive patients with similar results to HIV-negative patients in terms of efficacy although with higher toxicity.

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0651
MINIMAL RESIDUAL DISEASE ANALYSIS IN NON-MRD BASED TREATMENT PROTOCOL FOR CHILDHOOD ALL: LOW RISK FEATURES TOGETHER WITH FAST MORPHOLOGICAL RESPONSE FAIL TO IDENTIFY SLOW-RESPONDERS WITHIN THE ALL IC-BFM 2002 STANDARD RISK GROUP
E. Fronkova,1 S. Avigad,1 K.W. Chik,1 L. Castillo,1 S. Manor,2 L. Reznickova,3 E. Mejtrikova,3 M. Schrappe,4 V. Conter,5 S. Izraeli,1 B. Stark,1 J. Stary,5 J. Trka5
12nd Medical School, Charles University, PRAGUE, Czech Republic; 2Schneider Children’s Medical Center, PETAH TIKVA, Israel; 3Chinese University of Hong Kong, HONG KONG, Hongkong (China); 4Centro Hospitalario Pereira Rossell, MONTEVIDEO, Uruguay; 5Charles University, PRAGUE, Czech Republic; 6University Hospital Schleswig-Holstein, KIEL, Germany; 7University of Milan, MONZA, Italy; 8Sheba Medical Center, TEL-HASHOMER, Israel; 92nd Medical School, Charles’ University, PRAGUE, Czech Republic

Since 2000, minimal residual disease (MRD) information at week 5 and 12 of therapy has been used for the treatment stratification in childhood ALL-BFM 2000 trial. In parallel, ALL IC-BFM 2002 has been designed by the International-BFM Group to test the morphological assessment of the early treatment response. Patients are stratified according to the blast proportion in peripheral blood (PB) at day 8 and in bone marrow (BM) at day 15 and 33 of therapy together with the age, initial WBC and the presence of BCR/ABL and MLL/AF4 fusion. Aims. One of the research questions of the ALL IC-BFM 2002 study is the comparison of this risk group assessment to the MRD-based criteria used in ALL-BFM 2000. Methods. MRD in BM and PB samples was assessed by patient-specific RQ-PCR for clonal immunoglobulin and T-cell receptor (ig/TcR) gene rearrangements. Results. In total 184 patients treated according to ALL IC-BFM 2002 in the Czech Republic, Israel, Hong Kong and Uruguay were investigated for the presence of clonal ig/TcR rearrangements. At least one patient-specific RQ-PCR target with minimal sensitivity of 10(-4) was designed for 161 patients. In these patients, MRD in BM at several time-points of therapy (including mandatory points at weeks 5 and 12) was evaluated; the PB specimens of Czech T-ALL patients were tested simultaneously. In total, 621 follow-up BM specimens and 80 PB samples were tested. The results showed separation of MRD levels between standard-risk group (SRG) and intermediate-risk group (IRG) stratified patients at day 33 (p=0.005). However, in 21 of 66 SRG patients (31.8%), MRD positivity at week 5 and/or at week 12 was observed (ranging from the positivity below QR to 1.5x10(-2)), thus identifying patients who would not qualify to the MRD-based SRG in ALL-BFM 2000 despite the identical induction regimen. Conversely, 24% of IRG patients showed MRD negativity by two independent Ig/TcR targets at both critical time-points, thus accomplishing the ALL-BFM 2000 SRG criterion. As expected, high-risk group (HRG) patients showed significantly slower molecular response than other groups. Taken together, patients with BCP ALL had significantly lower MRD levels at day 15 (p=0.005) and at day 33 (p=0.004) than T-ALL patients. There was no significant difference in MRD levels between the two groups at week 12. In 80 follow-up T-ALL samples, MRD levels in PB clearly paralleled those in BM.

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0652
MULTIVARIATE ANALYSIS INCLUDING MTHFR GENOTYPES IN A COHORT OF ALL PATIENTS
Università Cattolica del Sacro Cuore, ROME, Italy

Background. Several prognostic factors have been used to stratify ALL patients’ risk. These prognostic factors include clinical and biological characteristics (age, WBC, count, cytogenetic or molecular aberration and, more recently the level of early response to treatment). One of the most important being the influence of polymorphisms of different genes involved in metabolism of chemotherapeutic agents have been studied especially in childhood ALL. Methylene tetrahydrofolate reductase (MTHFR) catalyzes conversion of 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate in the folate pathway, a limiting step for one-carbon metabolic pathways. MTHFR C677T polymorphism is a common genetic variation in this gene that is associated with decreased enzyme activity. Since 2007, the role of MTHFR C677T polymorphism in ALL has been studied extensively and has been associated with poorer clinical outcomes. In this study, we analyzed the association of MTHFR C677T polymorphism with the clinical outcomes of children with ALL.

Supported by MSM0021630813, Israel Cancer Association, Children’s Cancer Foundation Hong Kong and 62/2004 GAUK CR.
The genotypes frequencies were i) I-FISH in combination with CC is a C. Castagnola, S. Calatroni, M. Lazzarino, I. Giardini, G. Martinelli, B. Rocca, M. Lunghi, M. Caresana, Dasatinib (D) (BMS-354825) is a multikinase inhibitor of primary endpoint was confirmed (sustained for at least 4 weeks) major Abl mutations were assessed at baseline and at time of progression. The hematologic response rates (MaHR).

Results. The genotypes frequencies were consistent with previous published reports. The polymorphisms’ distribution among different karyotype groups was homogeneous. On univariate analysis, pts with the MTHR C677T and A1298C variant alleles did not experience significantly increased relapse and mortality risk (chi-square test p=0.02 and p=0.59 for 677 and p=0.06 and p=0.72 for 1298). Comparison of RFS and EFS between homzygous wild type and variant patients in both 677 and 1298 polymorphisms was not significant by the log rank test (p=0.79, p=0.53 and p=0.3, p=0.37 respectively), while RFS and EFS were significantly decreased in the presence of high risk karyotype and age >54y (p<0.0001 and p=0.03 respectively). The Cox regression analysis containing gender, age, WBC, karyotype, phenotype and MTHR genotypes showed an increased hazard ratio (HR) relapse and mortality in patients with high risk karyotype (p<0.001 and HR 4.35 and p<=0.0001 and HR 3.67 respectively); an increased HR mortality evaluated in pts older than 24 years (p=0.003 and HR 0.415). Regarding WBC count at diagnosis there was no significant correlation between WBC>10x10^9/L and outcome whilst we found an increased risk of mortality among patients with WBC<5x10^9/L (chi-square test p=0.006). Conclusions. In our study we did not observe any association between MTHR polymorphisms and relapse and survival rate in a group of almost adult ALL patients. Our data are in contrast with those from other groups which evaluated the influence of these two polymorphisms in pediatric standard risk patients. Due to the higher frequency of molecular alterations (9,22 and t4,11) in our context MTHR polymorphisms per se has not enough power to influence DFS and EFS, when compared to classical risk factors like karyotype alterations, WBC at diagnosis and age influencing prognosis.

DASATINIB IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN LYMPHOID BLAST CRISIS OR PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOPROLIFERATIVE LEUKEMIA THAT IS IMATINIB-RESISTANT OR INOTOLERANT: THE CAL980015 START-L STUDY

O. Ottmann, G. Martinelli, H. Dombret, B. Simonsson, G. Saglio, A. Gollerker, A. Apanovicth, P. Erben, S. Coutre, H. Kantarjian

Johann Wolfgang Goethe Universitat, FRANKFURT, Germany; University of Bologna, BOLOGNA, Italy; Hoiptal Saint-Louis, PARIS, France; University Hospital, Uppsala, UPPSALA, Sweden; Azienda Ospedaliera S. Luigi, ORBA-ALI, Italy; Bispo-Ayala Hospital, SEVILLE, Spain; University of California, STANFORD, USA; M.D. Anderson Cancer Center, HOUSTON, USA

Background. Dasatinib (D) (BMS-354825) is a multitkine inhibitor of Bcr-Abl and SRC. In a phase I study, hematologic responses were achieved with D in pts with LB-CML and Ph+ALL. Aim. To estimate the major hematologic response (MaHR) rates to D in IM-R and IM-1 patients with LB-CML and Ph+ALL. Methods. START L is an open label phase II study of D in IM-R or IM-1 pts with LB-CML and Ph+ALL which was conducted in 42 centers worldwide. D was given orally, 70 mg twice daily (bid), with escalation to 100 mg bid for inadequate initial response or up to 21 months (range 1-190). Results. The most frequent D-related non-hematologic toxicities were diarrhea (28%), nausea (20%), fatigue (19%), rash (17%) and pleural effusion (13%). Conclusion. D has substantial activity in heavily pretreated LB-CML and Ph+ALL pts. Data on all 94 pts will be presented at the meeting including an analysis of the molecular response and Bcr-Abl mutations.

CRYPTIC KARYOTYPE DEFECTS ARE DISCOVERED BY INTERPHASE FLOURESCENCE IN SITU HYBRIDIZATION (I-FISH) IN CHROMOSOMALLY NORMAL ADULT B-CELL ACUTE LYMPHOPROLIFERATIVE LEUKEMIA (B-ALL)


IRCCS Policlinico San Matteo, PAVIA, Italy; Division of Hematology, PAVIA, Italy

In adult B-ALL the chromosome pattern plays a pivotal role in the prognostic stratification of patients and in driving therapeutic decisions. Unfortunately, conventional cytogenetics (CC) is not always informative since it is often hampered by either the absence of mitotic cells or by the bad quality of metaphases. Thus, FISH, which can be performed on mitotic as well as on quiescent cells, has progressively been used in addition to CC to unmask chromosomal changes and cryptic defects in B-ALL. We have applied I-FISH to analyze 58 adult B-ALLs who showed a hemiz/monosomized LB-CML on G- and Q-banded metaphases. Our study was aimed at establishing the true incidence of BCR-ABL, ETV6-AML1, MLL rearrangements and p16/INK4A deletion in chromosomally normal B-ALL and at correlating our findings with clinical parameters and outcomes. The 58 patients examined, 22 females and 16 males with a median age of 41 years (range 16-75), were part of a large series of 263 consecutive adult B-ALLs who came to our observation in a ten years period (1994-2005). Within this series 56 patients (21.2%) did not yield metaphases and 73 (27.7%) presented a normal chromosome pattern. I-FISH was carried out with the following commercial probes: LSI BCR/ABL1 dual color single fusion, LSI TEL/AML1 ES, LSI MLL and LSI ETV6-AML1, p16/INK4A, p15/INK4B and D9S1748, p15(INK4B) and D9S1752 (Vysis, Downers Grove, IL, USA). Hybridization procedures were carried out according to manufacturers’ guidelines. Cut-off values were determined after having analyzed two-hundred cells from ten normal controls and using a one-sided binomial distribution with a 95% confidence interval. So, the cut-off values were fixed at 10% and 6% for the BCR/ABL1 and MLL probes and at 3% for both the ETV6-AML1 and the LSI p16 (9p21)/CEP 9 probes. I-FISH discovered clonal chromosome defects in a total of 17/38 (44.7%) patients. The loss of either one or two red spots corresponding to the LSI p16 (9p21)/CEP 9 dual color probe was the most common cryptic abnormality, being observed in 10 patients (26.3%). No patient presented a cryptic BCR-ABL or ETV6-AML1 rearrangement. The amplification of the AML1 gene and the monosomy of the ETV6 gene were observed in 11-15% cells from 3 and 2 patients, while the monosomy and the amplification of the MLL gene in one patient each. The 2 patients with p16 nullisomy were unresponsive to chemotherapy and survived four and six months; those with p16 monosomy were too few to obtain any prognostic information. Conclusion. i) I-FISH in combination with CC is a very useful tool to unmask cryptic defects in B-ALL since it readily discovered genetic aberrations in 44% of our chromosomally normal patients, ii) p16/INK4A monosomy is the most common cryptic chromosome defect not only in T-ALL but also in B-ALL, iii) ETV6-AML1 and MLL rearrangements are extremely rare in adult B-ALL, iii) CC is very effective in detecting Ph positive cells although the clonal cell population is more accurately defined by I-FISH.
WILMS TUMOR GENE 1 EXPRESSION IN CHILDHOOD ACUTE LYMPHOBlastic LEUKEMIA

Institute of Molecular Medicine, DUBLIN, Ireland; 2nd Medical School, Charles' University, PRAGUE, Czech Republic; Our Lady's Hospital for Sick Children, DUBLIN, Ireland

Background. Wilms' tumor gene 1 (WT1), located on chromosome 11p13, encodes a zinc-finger transcription factor with important roles in embryogenesis and oncogenesis. WT1 is supposed to be overexpressed in the majority (70-90%) of acute leukemias and has been identified as an independent prognostic factor for MRD in pediatric ALL, AML, and T-ALL. WT1 is considered to be a useful marker for MRD detection in childhood ALL, however, its role is controversial. Many of the discrepancies could be attributed to the non-standardized techniques of WT1 detection and quantification, different patients' characteristics and limited number of samples and controls investigated. Objectives. The aim of this study was to establish reproducible PCR assays for WT1 detection and evaluate WT1 expression in a representative group of childhood ALL patients. Methods. RT-PCR and Q-PCR enabling absolute quantification of total WT1 and its four main isoforms (variants A, B, C and D) were designed, optimized and validated according to BIOMED-1 Concerted Action [van Dongen et al. Leukemia 1999] and Europe Against Cancer Program [Gabert et al., Leukemia 2005] recommendations, respectively. With these methods we evaluated WT1 in diagnostic bone marrow (BM) samples of 125 consecutively enrolled childhood ALL patients (106 BCP-ALL, 19 T-ALL), normal peripheral blood (PB) and BM, and regenerating MRD negative BM were used as controls. Results. Low WT1 expression of a uniform pattern was present in all control samples. In BCP-ALL, we detected a wide range of WT1 levels (5 logs) with median close to that of normal BM; WT1 expression in T-ALL was significantly higher (p<0.001). Patients with MLL-AF4 translocation showed considerable WT1 overexpression (p<0.01) compared to other patients. Older children expressed higher WT1 levels than children under 10 years of age (p<0.001), while there was no difference between patients with WBC over 5x10^9/L and lower. There was also no correlation between WT1 and CD34 expression. Analysis of relapsed cases (14/125) indicated that abnormal increase or decrease in WT1 expression was associated with significantly increased risk of relapse (p=0.001), and this prognostic impact of WT1 was independent of other main risk factors (p=0.0012). All four WT1 isoforms were detected in normal controls and ALL samples. Preliminary results did not show a significant difference in WT1 exon5[+] and WT1/KIT[+]/ ratio in ALL patients compared to normal BM. Conclusion. In summary, WT1 expression in childhood ALL is variable and much lower than in AML or adult ALL. WT1 thus will not be a useful marker for MRD detection in childhood ALL, however, it does represent a potential independent risk factor in childhood ALL. Interleukin-6 and tumor necrosis factor (TNF) - dependent cytokines are expressed at low levels below the normal physiological bone marrow WT1 expression, and this reduced WT1 expression also appears to be associated with a higher risk of relapse. The designed RT-PCR and Q-PCR assays for detection of WT1 and its main isoforms could be considered as standards for future reference and use.

CELL BIOLOGICAL FEATURES OF BLASTS PersistING AT DAY 8 OF INDUCTION THERAPY IN CHILDHOOD PRECURSOR B-CELL ACUTE LYMPHOBlastic LEUKEMIA

P. Rhein, S. Scheid, R. Ratej, C. Hagermeier, K. Seeger, R. Kirchner-Schwabe, M. Schrapp, R. Spang, W.D. Ludwig, I. Karawajew
Robert-Rosse-Clinic, HELIOS Klinikum, BERLIN, Germany; MPI for Molecular Genetics, BERLIN, Germany; Charité Medical School, BERLIN, Germany; University Hospital Schleswig-Holstein, KIEL, Germany

In childhood acute lymphoblastic leukemia (ALL), persistence of leukemic blasts during therapy is of crucial prognostic significance. In the frontline ALL-BFM (Berlin-Frankfurt-Münster) trial, treatment stratification is based on blast count estimation in peripheral blood at day 8 of induction prephase with prednimusone and one intrathecal dose of methotrexate. Recently, we investigated genome-wide gene expression of blasts persisting after one week of induction therapy (day 8 blasts). The observed expression changes in day 8 blasts as compared with blasts at initial diagnosis (day 0 blasts) included key regulators of the cell cycle and genes encoding for B-cell differentiation markers. Furthermore, we observed an induction increase of inflammatory response genes and a decrease of BCL2, the prototypic member of the anti-apoptotic BCL2 subfamily. In the current study we analyzed day 8 blasts at protein and cellular levels. Firstly, we isolated the day 0 and day 8 blasts of 13 patients by flow sorting and measured the cell cycle distributions at both days. As a result, mean percentage of cycling (S, G2/M-phases) cells in the blast subpopulations significantly decreased from 5.1% (range: 0.2-22%) at day 0 to 1.2% (range: 0.1-5.1%) at day 8 (p=0.014). In a total series of 56 patients, flow cytometric analysis confirmed expression changes of the B-cell differentiation markers CD10 (decrease by 1.4-fold), CD20 (increase by 2.4-fold), CD34 (decrease by 1.3-fold) and TdT (decrease by 2.4-fold) (p<0.005). Moreover, we were able to confirm the expression increase of the inflammatory response molecules CD11b (5.1-fold, p=0.001, n=15) and IFNGR (2.2-fold, p<0.001, n=15), and the decrease of the BCL2 protein (1.5-fold, p<0.001, n=29). Taken together, the cell biological characterization of ALL cells persisting during induction therapy demonstrated an inhibited cell proliferation and an overall gene expression shift towards resting mature B cells. Furthermore, expression decrease of BCL2 in the day 8 blasts points to the involvement of this anti-apoptotic protein in the molecular mechanism of action of glucocorticoids in childhood ALL.

MINIMAL RESIDUAL DISEASE MONITORING OF BCR-ABL POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA USING QUANTITATIVE REAL-TIME PCR: PRELIMINARY RESULTS FROM THE MRC-UKALLXII TRIAL

G. Gerrard, W. Mitchell, B. Patel, A. Goldstone, L. Foroni
Royal Free & UCMS, LONDON, United Kingdom

Background. Adult ALL carrying the BCR-ABL fusion-gene is associated with a dismal prognosis. Minimal residual disease (MRD) is a significant tool for monitoring disease progression and outcome and BCR-ABL offers a convenient target for molecular MRD monitoring by QRT-PCR. In 2003, the MRC UKALLXII trial was amended to incorporate the addition of the tyrosine-kinase inhibitor Imatinib Mesylate for BCR-ABL+ patients during intensification. 25-30% of adult ALL patients exhibit BCR-ABL and the p190BCR-ABL isoform is more common than the p210BCR-ABL. It remains to be seen whether isoform type carries clinical significance. Aims. To investigate the efficacy of monitoring response to induction therapy and Imatinib in adult ALL patients enrolled on the MRC-UKALLXII trial using BCR-ABL as a target for molecular MRD. Methods. MRD analysis was performed using Roche LightCycler 1.2 with SYBR-Green fluorescent technology and primers for ABL, p210BCR-ABL and p190BCR-ABL. Statistical analysis of median BCR-ABL expression showed a significant drop in BCR-ABL levels between each time point (p<0.0005, p=0.0221) and that p210BCR-ABL patients exhibited significantly higher levels of BCR-ABL than p190BCR-ABL patients at each time point (p=0.0108, p=0.0019). Summary. BCR-ABL isoforms were found to be equivalent in sensitivity, except for the pre-Imatinib samples, where BM was found to offer significantly higher sensitivity (p=0.0252). Although BCR-ABL levels decreased in response to induction therapy and in response to Imatinib, patients with the P210 isoform had consistently higher transcript levels than patients with the P190 isoform. MRD in adult ALL is shown to be a significant indicator of disease progression and as such may be used to implement treatment stratification.
0658
SHOULD HYPERCVAD / METHOTREXATE-CYTARABINE BE CONSIDERED A STANDARD TREATMENT IN ACUTE LYMPHOBlastic LEUKEMIA? A BRAZILIAN EXPERIENCE


Background. Adult ALL is traditionally treated by a Vincristine and Prednisone protocol with the addition of an Anthracycline. Early dose intensification as described by the MD Anderson group with the hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone/high dose methotrexate-cytarabine regimen has shown in there experience an improvement in disease free survival. There are few data about the experience with this high dose protocol from other groups.

Methods and Results. We analysed retrospectively 65 patients treated between 1994 and 2005 in 3 brazilian hospitals. Median age was 21 years; only 3 patients were > 60 years. The incidence of Philadelphia positive ALL was 6% and T-ALL 23%. According to age, leucocyte count, SNC disease at diagnosis, remission after first cycle, patients were classified as high risk (69.5%) or low risk (30.5%). Overall 54 (84%) patients achieved complete remission after the first chemotherapy cycle. The median time between treatment cycles was 56 days (range 27 – 83) mainly because of severe infections. Recurrence of the disease occurred in 5 patients (7%) during induction. Forty patients (61%) were able to complete the 8 induction courses. Twenty three (53%) patients died during induction and 2 patients changed chemotherapy protocol because of severe toxicities. Infection was the most common cause of death. From the 40 patients who finished the induction courses only 25 were able to receive maintenance. Of the remaining 15 patients, 10 relapsed before maintenance and 5 died because of infectious complication. At a median follow up of 12 months, only 22 (34%) of the patients are alive. Three more patients relapsed during maintenance. Ninety nine of the patients (99.5%) were in CR. The median time to complete the 8 induction courses was 3 months (range 1 – 10). All patients achieved complete remission after the first chemotherapy cycle. The median time between treatment cycles was 56 days (range 27 – 83) mainly because of severe infections. Recurrence of the disease occurred in 5 patients (7%) during induction. Forty patients (61%) were able to complete the 8 induction courses. Twenty three (53%) patients died during induction and 2 patients changed chemotherapy protocol because of severe toxicities. Infection was the most common cause of death. From the 40 patients who finished the induction courses only 25 were able to receive maintenance. Of the remaining 15 patients, 10 relapsed before maintenance and 5 died because of infectious complication. At a median follow up of 12 months, only 22 (34%) of the patients are alive. Three more patients relapsed during maintenance. Ninety nine of the patients (99.5%) were in CR. The median time to complete the 8 induction courses was 3 months (range 1 – 10). All patients achieved complete remission after the first chemotherapy cycle.

Conclusions. Adult ALL is traditionally treated by a Vincristine and Prednisone protocol with the addition of an Anthracycline. Early dose intensification as described by the MD Anderson group with the hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone/high dose methotrexate-cytarabine regimen has shown in there experience an improvement in disease free survival. There are few data about the experience with this high dose protocol from other groups. The median time between treatment cycles was 56 days (range 27 – 83) mainly because of severe infections. Recurrence of the disease occurred in 5 patients (7%) during induction. Forty patients (61%) were able to complete the 8 induction courses. Twenty three (33%) patients died during induction and 2 patients changed chemotherapy protocol because of severe toxicities. Infection was the most common cause of death. From the 40 patients who finished the induction courses only 25 were able to receive maintenance. Of the remaining 15 patients, 10 relapsed before maintenance and 5 died because of infectious complication. At a median follow up of 12 months, only 22 (34%) of the patients are alive. Three more patients relapsed during maintenance. Ninety nine of the patients (99.5%) were in CR. The median time to complete the 8 induction courses was 3 months (range 1 – 10). All patients achieved complete remission after the first chemotherapy cycle. The median time between treatment cycles was 56 days (range 27 – 83) mainly because of severe infections. Recurrence of the disease occurred in 5 patients (7%) during induction. Forty patients (61%) were able to complete the 8 induction courses. Twenty three (33%) patients died during induction and 2 patients changed chemotherapy protocol because of severe toxicities. Infection was the most common cause of death. From the 40 patients who finished the induction courses only 25 were able to receive maintenance. Of the remaining 15 patients, 10 relapsed before maintenance and 5 died because of infectious complication. At a median follow up of 12 months, only 22 (34%) of the patients are alive. Three more patients relapsed during maintenance. Ninety nine of the patients (99.5%) were in CR. The median time to complete the 8 induction courses was 3 months (range 1 – 10). All patients achieved complete remission after the first chemotherapy cycle.

0659
IMATINIB COMBINED TO INDUCTION OR CONSOLIDATION CHEMOTHERAPY IN YOUNGER PATIENTS WITH DE NOVO PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBlastic LEUKEMIA RESULTS OF THE GRAAPH-2003 STUDY


Background. Methylation of CpG islands in the 5′ gene region is associated with transcriptional silencing of gene expression. The hypermethylation of tumor suppressor genes has been described in gastric and pancreatic cancer, as well as in acute myeloid leukemia, suggesting its potential role in neoplasia. Among the three members of the Kip/Cip family of cyclin-dependent kinase inhibitors (CDK) p21, p27 and p28, little is known about their methylation status in hematological malignancies.

Contrasting studies, have been reported on the role of p21 hypermethylation in acute lymphoblastic leukemia (ALL). Aims. To analyze p21 gene methylation status and protein expression in primary blasts from adult ALL enrolled in the GIMEMA protocol LALL2000. Methods. Human leukemic cell lines, normal peripheral blood lymphocytes (PBL) and 89 primary samples from untreated ALL patients were evaluated in this study. The p21 gene methylation status was investigated using a widely accepted method based on bisulfite modification of DNA, followed by the use of methylation-specific PCR assay (MSP). This assay was further validated in vitro by SSI methylation. The p21 protein expression was analyzed by Western blot using the p21-WAF1 MoAb (Santa Cruz, CA). Results. The human lymphoblastic cell lines RPMI8866 and CEM, the myeloid cell line OCI-AML3 and normal PBL from 10 healthy donors were characterized by a consistent p21 promoter unmethylation. The Raji and Jurkat cell lines, while the Rael (Burkitt’s lymphoma) cell line was strongly methylated (positive controls). In addition, p21 protein expression was found in the OCI-AML3, Raji and RPMI8866 cell lines, while it proved negative in the Jurkat and Rael cell lines, and in normal PBL. Sixty primary ALL cases evaluated for p21 methylation status showed a consistent unmethylation in all samples, while the p21 protein expression was found in 26/89 cases (29.2%). A significant correlation (p=0.010) was observed between p21 protein expression and immunophenotype, 37.5% of B lineage ALL compared to 8.3% of T lineage ALL. In addition, a trend was found between p21 expression and age. Achievement of CR was observed in 65.4% and 79.4% of p21 positive and negative cases, respectively. Summary. While p21 gene methylation does not appear to play a pathogenetic mechanism in adult ALL, p21 protein expression is found in one third of these patients suggesting a role in the disease.
**0661**

PROGNOSTIC VALUE OF HOX11L2/TXL3 AND TAL1/SCL EXPRESSION IN CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: RESULTS OF THE FRALLE 93 PROTOCOL


Hôpital Armand Trousseau, PARIS, France; HämatoLOGIE Biologique St Louis, PARIS, France; HämatoLOGIE Biologique Hôpital Necker, PARIS, France; DPIM Hôpital Tenon, PARIS, France; Département d'Hématologie St Louis, PARIS, France; Hôpital Trousseau, PARIS, France; CHU Toulouse, Toulouse, France; CHU Rennes, France; Service d'Hématologie Hôpital St Louis, PARIS, France; Centre for Human Genetics, University of LEUVEN, Belgium; Onco-Hämato, Hôpital Trousseau, PARIS, France; Hôpital Saint-Louis, PARIS, France; Hôpital Trousseau, PARIS, France

**Background and aim of the study.** The most frequent oncogenic activation events characterized in childhood T acute lymphoblastic leukemia (T-ALL) result in the transcriptional activation of genes coding for transcription factors. The main genes are TAL1/SCL, a member of the basic region helix-loop-helix gene family, HOX11L2/TXL3 a member of the homeobox-containing protein family. Confirming results have been reported concerning molecular epidemiology and prognostic values of these markers. We therefore analysed retrospectively 200 patients treated in the French protocol FRALLE 93 for T-ALL between 11/93 and 12/99. Methods. Patients were stratified according to prednisone response at D8 (good or poor) as GPR or PPR and bone marrow at D21. Pts with D8 PPR or M3 received an intensified treatment with genoidentical or autologous bone marrow transplantation (BMT). Molecular constitution analysis was done in 120/200 T-ALL samples. Results. Clinical characteristics were not significantly different between population with or without molecular analysis: male (n=121) (69% vs 70%); median age 8.4 (range 1.1-19.5) vs 9.2y; median leucocytosis 140.109 (0.6-7.36) vs 171.915 109 (<20=53, 20-99=6, >100=46), mediastinal involvement 72% vs 71%, CNS 4% vs 5%, CD 10 neg 54% vs 52%. Steroid response PPR n=37/73, GPR n=36/73, CR n=105/118 pts (90%) after first induction therapy and 2 deaths occurs during induction treatment. With a median follow-up of 63 months (2-125), S y OS, EFS and DFS is 62% 9 and 54% 10. SIL-CHU, RENNES, France; Onco-Haematologie, Hôpital Necker, PARIS, France; Hôpital Trousseau, PARIS, France; Centre for Human Genetics, University of LEUVEN, Belgium; Onco-Hämato, Hôpital Trousseau, PARIS, France; Hôpital Saint-Louis, PARIS, France; Hôpital Trousseau, PARIS, France

**Conclusions/Perspectives.** Measurement of DEVDase activity by ZVAD-fmk in primary leukemia cells. However, cell death was largely unaffected by caspase inhibition, suggesting that caspase independent cell death mechanisms are operative in drug induced leukemia cell apoptosis in vitro. In a xenotransplant model for human leukemia, cells were analyzed for chemotherapy induced cell death and caspase activity. Cytarabine induced caspase activity is not inhibited by ZVAD-fmk and cell death is even higher than in the mice without ZVAD-fmk treatment. In an in vivo experiment with the CEM cell line, ZVAD-fmk was able to inhibit caspase activity in drug treated control-mice whereas Cytarabine induced caspase activity was not affected by the pan-caspase inhibitor. Conclusions/Perspectives. Measurement of DEVDase cleavage in primary leukemia cells permits detection and quantification of chemotherapy induced caspase activation in vitro and in vivo. Quantification of cellular caspase activation reveals differential induction of caspase activation by cytarabine and Cyclophosphamide. Induction of cell death by drug treatment in vitro is independent of caspase-3 activation. The marked heterogeneity of drug induced apoptosis signaling in primary leukemia cells permits further studies on its prognostic value and its use as treatment stratification.

**0663**

RESULTS OF THE PETHEMA ALL-96 TRIAL IN ELDERLY PATIENTS WITH PHILADELPHIA CHROMOSOME-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA


Pethema Group, BADALONA, Spain

**Background and Aim.** The incidence of ALL in the elderly is low and the outcomes of these patients remain poor. Only 20-30% of elderly patients are enrolled in clinical trials due to the presence of co-morbid disorders or poor performance status. For that reasons the number of published trials in elderly ALL is scarce. We present the results of treatment of Philadelphia chromosomes-negative (Ph-) ALL patients ‘55 years treated within the PETHEMA ALL-96 trial. Patients and therapy: From 1996 to 2005, 31 patients with B or T precursor Ph- ALL ’55 years were included in the PETHEMA ALL-96 trial. Induction therapy consisted of VCR, DNR (30 mg/m² d 1, 8, 15, 22), PDN, E. coli ASP (10,000 IU/ m² d 1, 2, 29) and cell death is even higher than in the mice without ZVAD-fmk treatment. Induction of cell death by drug treatment in vitro is independent of caspase-3 activation. The marked heterogeneity of drug induced apoptosis signaling in primary leukemia cells permits further studies on its prognostic value and its use as treatment stratification.
a trend for increase in CR rate and OS probability (38% vs. 67% and 15% vs. 40%, p=0.063 and p=0.075, respectively) in those patients not receiving ASP and CPM for remission induction. Conclusions. Even excluding Philadelphia chromosome-positive patients, the prognosis of elderly ALL patients is poor. In our study, removal of CPM and ASP from induction therapy resulted in a significant decrease of early death and a trend to improved CNS complications. Small deletions of (or 1q/m 1q/664 and 1q21) and loss of heterozygosity of the chromosome bands 1q21.3, 1q21.2 and 1q21.1 are frequent events in pediatric ALL, and might have a significant impact on the prognosis of the underlying disease as suggested in this study. It is therefore mandatory to include new techniques such as matrix CGH into the diagnostic toolbox, which could change the stratification strategy of risk groups in adult ALL.

0665
SMALL 9P21-DELETIONS DETECTED BY MATRIX-CGH IN PATIENTS WITH ADULT ACUTE LYMPHOCYTIC LEUKAEMIA INDICATE POOR PROGNOSIS
M.S. Schmid, C. Schwänen, S. Ruf, H. Döhner, S. Wessendorf
University hospital of Ulm, ULM, Germany

Background. The chromosomal band 9p21 harbours 5 different genes with putative tumour suppressor gene (TSG) function: p10, p14ARF, p15INK4b, p16INK4a and the gene for methylthioadenosine phosphorylase (MTAP). Deletions with subsequent inactivation of these TSGs are frequently observed in several malignant tumors including childhood ALL. In this type of childhood leukaemia, 9p21 deletions are associated with poor prognosis. In addition, patients with adult ALL within the German GMALL study are stratified into standard, high risk or very high risk according to underlying chromosomal abnormalities. The karyotype analysis is done by standard cytogenetic procedures such as chromosomal banding in the range of 15·5kb. Small deletions in the range of 1·5kb are not detected by these methods by they might play a role for the prognosis of the affected patients. Aim. We analyzed the 9p21 locus by matrix CGH as well as the expression of the MTAP and p16INK4A genes in three adult ALL patients with an early relapse after initially achieving a complete remission. Methods. Total DNA from three ALL patients (1 T-ALL, 2 c-ALL) was extracted using a commercially available extraction kit. Two patients relapsed either after induction phase II or consolidation II according to the GMALL 07/03 protocol. Patient 3 was initially classified as high risk due to an increased leukocyte count (32.4 G/L) and underwent allrogenic PBSC. She relapsed 8 weeks thereafter. All patients had achieved complete remission after induction phase I. Samples were analysed using a 2·8 kb matrix DNA chip which contained 2800 different clones with 147 clones covering chromosome 9. In addition, the expression of the p16INK4A and MTAP genes was examined by western blot analysis. Results. All three patients had small 9p21 deletions at the time when they relapsed. One patient showed a homozygous deletion and two patients a heterozygous deletion. In one patient (T-ALL), the deletion was already present at diagnosis, but could not be detected by standard cytogenetic Methods. In the other two patients, matrix CGH did not show any abnormalities at diagnosis. No other deletions or chromosomal gains could be identified. In addition, none of the patients expressed the p16INK4A protein, with two patients (T-ALL, 1 c-ALL) where also negative for the MTAP gene. Both genes are located closely (approx. 100 kb) to each other on 9p21. All patients were resistant to several salvage therapies and died within 5 months. Conclusions. Small deletions of chromosomal regions are not detectable by standard cytogenetic Methods. If these regions harbour early childhood leukemia genes they might have a significant impact on the prognosis of the underlying disease as suggested in this study. It is therefore mandatory to include new techniques such as matrix CGH into the diagnostic toolbox, which could change the stratification strategy of risk groups in adult ALL.

0666
EFFICACY AND TOXICITY OF IDA/NOVA-FLAG REGIMEN AS SALVAGE TREATMENT FOR PATIENTS WITH ACUTE LEUKEMIA FIVE-YEAR-EXPERIENCE OF A SINGLE INSTITUTE
Laikon General Hospital, ATHENS, Greece

Background. The observation that Fludarabine administration before Aracantine may lead to larger cytoplasmatic concentrations of the latter, was the origin for the design of Ida/Nova-FLAG regimen. Aim. The present study aims to show that the Ida/Nova-FLAG regimen is a safe and efficacious treatment choice for the patients with early childhood leukemia, refractory or relapsed AL. Methods. During the last five years 44 pts (32 pts<65 year-old and 12 pts≥65 year-old) with AL received in our department the Ida/Nova-FLAG regimen. Their disease was either primary resistant to chemotherapy (17) or relapsed during therapy (21) or within the first year after completion (5). The chemotherapeutic protocol included: Fludara- bine: 25mg/m2 in 1h iv infusion, 4 hours later Aracantine:3 g/m2 (or 1g/m2 for pts≥ 65 years of age) in 3h iv infusion and 1 hour later Idarubicin: 12mg/m2 or Novantrone: 10mg/m2 1/2 h iv infusion. Results. Myelosuppres- sion with febrile neutropenia (55% of episodes with microbiological evidence and the other unspecified-FUO) was the main toxicity of the regimen. Treatment related mortality (TRM) was 22%, 5. The incidence of TRM was 50% among older pts and 20% among pts<65. Six pts died due to infection and in 4 due to hemorrhage. Two pts >65 (16%) and 17 pts< 65 (55%) obtained CR after Ida/Nova-FLAG. Eight pts with primary resistant disease (44,4%), 7 pts who relapsed during therapy (33%) and 4 pts who relapsed shortly after it (80%) were in CR after Ida/Nova-FLAG. The following table shows the response according to type of disease.

Table 1.

<table>
<thead>
<tr>
<th>Disease/number of pts</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>Early Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL (N=15)</td>
<td>8</td>
<td>53.3%</td>
<td>2</td>
<td>13.3%</td>
<td>3</td>
</tr>
<tr>
<td>De Novo AML (N=16)</td>
<td>7</td>
<td>43.7%</td>
<td>2</td>
<td>12.5%</td>
<td>5</td>
</tr>
<tr>
<td>AML from preceeding MDS(N=12)</td>
<td>2</td>
<td>16.6%</td>
<td>1</td>
<td>8.3%</td>
<td>5</td>
</tr>
<tr>
<td>SUM (N=44)</td>
<td>17</td>
<td>38.5%</td>
<td>5</td>
<td>11.4%</td>
<td>13</td>
</tr>
</tbody>
</table>
The median duration of neutrophil (>500/µl) and platelet (>50,000/µl) recovery was 15 days (min: 1 month, max: 72 months). The TTP for pts who achieved CR was 2.5 months. Five pts (11%) achieved allo-BMT after Ida/Nova-FLAG, however 4 experienced relapse within the first trimester after transplantation. 13 pts received third line treatment after Ida/Nova-FLAG (e.g. Mylotarg, L-Asparaginase, Hycamptin-Ara-C, and ESHAP) but only 1 obtained CR and the other did not respond. Conclusion: the state of immunosuppression after Ida/Nova-FLAG for pts with AL presents acceptable toxicity and favorable outcome, even for those with refractory ALL, for whom the data in the literature is sparse. Finally Ida/Nova-FLAG regimen can be a treatment modality for allo-BMT candidates.

**0667**

EFFECT OF INTENSIVE CHEMOTHERAPY ON INNATE IMMUNITY IN CHILDREN WITH ACUTE LEUKEMIA AND NON-HODGKIN LYMPHOMA

M. Jarosz, J. Mysliwiska, A. Balcerska

**Background.** Intensive combination chemotherapy in acute leukemia (AL) and non-Hodgkin lymphoma (NHL) results in a profound systemic immunosuppression. This state, in some patients, may be responsible for recurrent and sometimes life-threatening infections. **Objective.** The aim of this study was to examine the reconstitution of the CD3-CD16+CD56+ NK and the CD3+CD8high+CD57+ immunosuppressive T cells as well as to analyze the neutrophil's and monocyte's phagocytic activity and NK cytotoxic activity in children with acute leukemia and NHL after an intensive chemotherapy. **Patients and Methods.** The study group consisted of 27 children (18 patients with acute lymphoblastic leukemia (ALL) and 9 NHL patients), aged 3 to 16 years, treated in the Department of Pediatrics, Hematology, Oncology and Endocrinology at Medical University of Gdansk. Each patient was examined 2 weeks after cessation of an intensive chemotherapy and thereafter every three months during a year. The study consisted of a medical examination, anamnesis towards infections and laboratory tests. The whole blood count, the lymphocytes subpopulations (NK cells CD3-CD16+CD56+ and the non-specific immunosuppressive T cells CD3+CD8high+CD57+) were analyzed with flow cytometry. NK cytotoxic activity was measured with colorimetric assay based on cytoplasmatic LDH activity released by damaged cells. The investigation of phagocytosis was performed by phagocytosis of FITC labeled opsonized E. coli bacteria by granulocytes and monocytes in whole blood was measured). **Results.** The results of our investigation indicate that: 1. The state of immunosuppression such as leucopenia remained in the patients stable during the observation time; 2. There was noticeable a tendency to a decrease with subsequent rapid increase of cytotoxic NK response with a stable percentage and absolute number of NK cells and the immunosuppressive T cells subset; 3. The phagocytic activity of neutrophils was increased at the beginning of observations and three months thereafter it started to decrease; 4. The phagocytic activity of monocytes remained stable. **Conclusion.** Immune functions were progressively altered during the first 12 months of observation. Only four patients (14,8%) developed severe infections after intensive chemotherapy.

**0668**

RISK-ADAPTED THERAPY FOR ELDERLY PATIENTS WITH ACUTE LYMPHOCYTIC LEUKEMIA

N. Casavilla,1 L. Melillo,1 A. M. Carella,1 G. D’Arena,1 R. De Santis,2 A. P. Falcone,2 M. Dell’Olio,2 M. Nobile,2 E. Merla,2 L. Miglionico,1 M. M. Minervini,1 P. Muerto,1 G. Perla,2 G. Sanpao1,3 F. P. Scalzulli,3 S. Lagodana1

1IRCCS Casa Sollievo della Sofferenza, SAN GIOVANNI ROTONDO, Italy; 2IRCCS CSS Hospital, SAN GIOVANNI ROTONDO, Italy; 3L.N.T. Fondazione G. Pascale, NAPOLI, Italy

**Background.** Acute Lymphoblastic Leukemia is uncommon and scantily curable in patients over 60 years of age because of a greater resistance to chemotherapy, a relative inability of elderly patients to face the toxic effects and complications of therapy and influence of co-morbidities. **Aims.** We review here our experience of 44 consecutive cases of ALL of elderly age collected in the last fifteen years. Median age was 66 years (range 61-83). 12/1L FAB classification 3/6; Median WBC was 1.5x10^9/L (range 1-180); Male/Female ratio was: 26/18. Forty cases (90.9%) belonged to B cell lineage (pre-pre-B 11, common 24, pre-B 5) and 4 (9,1%) to T cell lineage (pre-T stage); CD34 expression was observed in 27/34 cases (79.4%), CD38, CD13 and CD15 surface expression was positive in: 17/35 (48,6%), 14/34 (41,2%) and 5/24 cases (20,8%), respectively; overall, CD13 and CD38 were co-expressed on 9/34 cases (26,5%). Philadelphia chromosome was present in 13 patients (29,5%). **Methods.** Of the 44 reviewed patients, 31 younger patients (median age 65 years, range 61-77, good performance status and without co-morbidity factors), received an intensive treatment such as LAL and others patients (NHL, AML, GIMEMA protocols). In the remaining 13 elderly patients (median age 77 years (range 61-83) and those with severe co-existing cardiac, pulmonary, renal and hepatic disease, a gentle chemotherapy including prednisone and vincristine, 6-mercaptopurine and methotrexate was utilized. **Results.** Six patients (13,9%) of the group treated with curative intent died during the induction phase; 19 patients (61,3%) achieved a CR and at present, 3 patients are alive +10, +46 and +105 months. Out of 13 patients receiving less intensive and supportive treatment, only 4 (30,8%) achieved a short CR: all the patients had an early relapse and died after 4, 5, 6 and 12 months. **Conclusion.** Our data demonstrate that immunophenotypic and karyotypic patterns of these patients differ from those usually observed in children and adults with ALL, therefore confirming the presence of a stem cell disorder and an extremely poor prognosis. In addition, in our experience emerged that to the ‘biologically younger patients’ who can well tolerate an aggressive therapy this approach should not be denied because of it is possible to achieve longer survivals.

**0669**

PROGNOSTIC RELEVANCE OF THE IMMUNOPHENOTYPE IN ADULTS AND CHILDREN WITH T-LINEAGE ACUTE LYMPHOCYTIC LEUKEMIA

N. Casavilla,1 L. Melillo,1 A. M. Carella,1 G. D’Arena,1 M. Dell’Olio,2 R. De Santis,2 A. P. Falcone,2 M. Nobile,2 E. Merla,2 L. Miglionico,1 M. M. Minervini,1 P. Muerto,1 G. Perla,2 G. Sanpao1,3 F. P. Scalzulli,3 S. Lagodana1

1IRCCS Casa Sollievo della Sofferenza, SAN GIOVANNI ROTONDO, Italy; 2IRCCS ‘CSS’ Hospital, SAN GIOVANNI ROTONDO, Italy; 3L.N.T. Fondazione G. Pascale, NAPOLI, Italy

**Background.** T-Lineage Acute Lymphoblastic Leukemia (T-ALL) accounts for 15-20% of newly diagnosed cases of ALL. It is characterized by a male predominance, high WBC count, mediastinal tumors and central nervous system involvement. Historically, T-ALL patients (pts) have a worse prognosis than other ALL patients. **Aims.** In this paper we review our experience on 66 consecutive pts with T-ALL (17 children and 49 adults) diagnosed and treated in our center. Median age of adults and children was 22 (range: 16-75) and 9 (range: 4-15) years, respectively. Male/Female ratio was 47/19 (adults 33/16; children 14/5). **Methods and Results.** Based on their immunophenotypic, all pts were classified in 3 ontogenetic stage-related subtypes: 1. Early T-ALL (immunophenotype: CyCD3+/CD1-/CD1/CD1-; 39 pts (59,1%) belonged to this group. Median WBC was 290x10^9/L (range 1-260); in 14 pts (59,5%) a mediastinal mass was present; CD34 expression was observed in 26/34 cases (76,5%); myeloid antigens (MyAg) (CD13 and/or CD38 and/or CD15 and/or CD65) were co-expressed in 18/35 cases (51,4%). II. Cortical T-ALL (immunophenotype: CD7+/CD1+/CD13-); 20 pts (30,3%) were included. Adult/Childhood ratio was 12/8; Median WBC was 89x10^9/L (range 7-1000); mediastinal tumor was present in 13 pts (65%); CD34 was positive in 4/17 cases (23,5%); and MyAg were co-expressed in 1/6 cases (6,2%). III. Mature T-ALL (immunophenotype: CD7-/CD1+/CD13-): the remaining 7 pts (16,6%) were included. Adult/Childhood ratio was 4/3; Median WBC was 10x10^9/L (range 4-480); mediatinal tumor was present in 4 pts (57,1%); none of them expressed CD34 and MyAg co-expression was only present in one case (14,3%). Therapeutic approaches applied during the twenty years period of the study were those of GIMEMA (for adults) and AIEOP (for children) cooperative groups. Overall, 51 pts (77,3%) achieved Complete Remission: (53 (71,4%) and 16 (94,1%) for adults and children respectively). Co-expressed immunophenotypic subtypes were: 26 (76,2%) early T-ALL, 18 (90%) cortical T-ALL and 6 (58,7%) mature T-ALL pts significantly achieved CR (p=0.035); of these, at present (median follow-up 136 months - range: 5-236), 24 pts are alive in CCR (subtype I: 10 patients (37%); subtype II: 12 pts (66,7%); subtype III: 2 pts (55,3%) p<0.012). **Conclusion.** Our data confirm that T-ALL may be quite heterogeneous in terms of clinical and biological features: a lower incidence of lymphomatous features was observed in the less mature subtypes of T-ALL, in which, in contrast, a higher co-expression of CD34 and MyAg was found. From our experience comes out that
the immunologic classification is the most significant prognostic factor in T-ALL: in fact, in adult as well as in children T-ALL, the cortical subtype showed a better outcome as compared to early and mature subtype.

**0670**

**BONE MINERAL DENSITY IN SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA DURING CHILDHOOD**

A. Gunes Meral, E. Can, B. Baytan, U. Gunay
Uludag university, BURSA, Turkey

Cure rates for children with ALL now approach 80%. Therefore, the late adverse effects of chemotherapy are more frequently observed. These children are especially at high risk of developing low bone mineral density (BMD) predisposing to severe osteoporosis in adulthood. The aim of this study was to evaluate BMD and bone mineral metabolism (BMM) and the influencing factors on them. Method: We analyzed the data of 70 children who achieved complete remission with ALL-BFM protocols. Their median follow up period was 4.3 years. Children were treated according to their leukemia risk specific groups (Standard:16, Median:45, High:9). The groups according to cessation of treatment included within one year, between 1-2 years and longer than 2 years. Their height and weight measurements and percentiles were determined both at the time of diagnosis and when they were included into the study. BMD at post-chemotherapy was also measured in lumbar area with dual X-ray absorptiometry (DEXA), and the results were expressed as age and sex-specific z scores. Serum IGF-1 and 25(OH) D vitamin levels were measured at the time of study and the results were compared with the healthy controls. Using logistic regression test, we compared the association of BMD change with the cessation of treatment, risk groups, the cumulative steroid dose, cranial radiotherapy, passive smoking, duration of television watching and daily calcium intake. Serum IGF-1 and 25(OH) D vitamin levels in each risk group were compared. Logistic regression analyses revealed that the most significant factor influencing BMD was daily calcium intake (OR: 0.997; 95%CI: 0.995-0.999). Results. The mean age of children at the time of diagnosis and study were 5.7±3.4 and 10.6±3.8 years, respectively. Percentiles both for height and weight at diagnosis and postchemotherapy increased non-significantly. The mean BMD and z score were found 0.602±0.15 g/cm² and 0.18), similarly as for high risk ALL.

**0671**

**LONG TERM OUTCOME OF ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: COLLABORATIVE ANALYSIS FROM THREE CENTERS**

A. Czyz,1 J.M. Zaucha,2 L. Gil, B. Nasilowska,3 W. Knopinska-Posluszny, M. Bieniaszewska,2 D. Dytfeld,1 A. Lojko,3 B. Marianska,3 A. Hellman,3 M. Komarnicki1

1University of Medical Sciences, POZNAN, Poland; 2Medical Academy, GDANSK, Poland; 3Institute of Hematology, WARSAW, Poland

The allogeneic and autologous stem cell transplantation (SCT) are accepted treatment options for acute lymphoblastic leukemia (ALL) patients, but criteria for choosing type of transplantation remain controversial. We retrospectively evaluated long-term outcome of SCT in 68 patients with Ph neg-ALL treated between January 1995 and December 2005 from three centers in Poland. There were 39 pts, median age 24 (15-55) years, standard risk- 9 pts (23%), high risk- 30 pts (77%) in alloSCT group and 29 pts, median age 24 (17-50) years, standard risk- 12 pts (41%), high risk- 17 pts (59%) in autoSCT group. High risk patients were defined by at least one of the following criteria: leukocytosis > 30 G/L, age >35 yrs, immunophenotype pre-pre-B, early-T, CR not achieved after 4 weeks of induction. The disease status before SCT was CR1- 36 pts, CR2-6 pts, NR-5 pts in alloSCT group, and CR1-27 pts, CR2-2 pts in autoSCT group. TBI based conditioning regimens were used in 21/39 pts in alloSCT and in 8/29 pts in autoSCT group, whereas other patients were conditioned with chemotherapy: Bu/Cy2 and fludarabine/melphalan in alloSCT group; Bu/CY2, Bu/Cy/VP, BEA and CAV in autoSCT group. CSA and a short course of MTX were given as GVHD prophylaxis after alloSCT from HLA-matched siblings (35 pts) with ATG added in SCT from MUD (4 pts). With a median follow-up of 35 (2-127) months 14/39 pts (36%) died after alloSCT, 3 in first 100 days (relapse-1, VOD-1, infection-1) and 11 after 100 days (relapse-8, GVHD-2, infection-1). With a median follow-up of 41 (7-81) months 13/29 pts (45%) died after autoSCT, 5 in first 100 days (infection-2, bleeding-1, relapse-1) and 8 after 100 days (relapse-5, DFS was 50% (95% CI 32-69) and 52% (95% CI 33-70) at 5 years for alloSCT and autoSCT group respectively estimated with the Kaplan-Meier method. The cumulative incidence of relapse and NRM were 33% (95% CI 19-57) and 14% (95% CI 6-32) respectively for alloSCT group versus .35% (95% CI 35-59) and 15% (95% CI 5-32) for autoSCT group. DFS for standard risk ALL patients treated with alloSCT was 75% (95% CI 45-100) due to no relapse versus 43% (95% CI 15-71) for autoSCT, however the differences did not reach statistical significance (p=0.18), similarly as for high risk ALL pts. Conclusions. There were no statistical significant differences in long-term outcome between alloSCT and autoSCT for patients with Ph-neg ALL. Relapse remains the main cause of treatment failure after both allo- and autoSCT. Our results suggest that novel methods of patients stratification should be studied in further prospective clinical trials to determine which group of patients would benefit the most from each transplant option.
Chronic myeloid leukemia III

0672

CYTOGENETIC AND MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CML IN CHRONIC PHASE ON IMATINIB MESYLATE THERAPY - THE SINGAPORE GENERAL HOSPITAL EXPERIENCE

G.F. How,1 C.T.H. Chuah,1 L.T. Tan,2 A. Amirzargar3
1Singapore General Hospital, SINGAPORE, Singapore; 2Department of Haematology, TEHRAN, Iran

Introduction. Imatinib mesylate (Glivec) has been demonstrated to induce good haematologic and cytogenetic response rates in patients with Philadelphia (Ph)-positive chronic myeloid leukaemia (CML). In addition the development of quantitative PCR technology has enhanced our ability to monitor response and minimal residual disease (MRD) at the molecular level. Aims. Over a 3-year period from 2002 to 2004, 45 patients in chronic phase CML were treated with Glivec 400 mg/day at our institution. Quantitative PCR (polymerase chain reaction) for p210 BCR-ABL transcripts was performed at regular intervals to determine molecular response. Bone marrow studies were also done to determine cytogenetic response. Methods. Real-Time quantitative PCR was performed on the ABI PRISM 7700 Sequence Detection System, following the procedures established by the Europe Against Cancer Program. We defined major molecular response (MMR) as a 3-log reduction in BCR-ABL/ABL ratio from the median baseline of 152%, and complete molecular response (CMR) as BCR-ABL undetectable or a 4-log reduction in BCR-ABL/ABL ratio. Results. The median age of the patients was 45 years (range, 18-76 years) and median follow up was 31 months (range, 11-49 months). Thirty patients (67%) achieved complete cytogenetic remission (CCyR) at 12 months and on further follow-up, another 13 attained CCyR in 18-32 months (median, 24 months). Thus, a total of 43/45 (95.5%) patients were able to achieve CCyR. Among these 43 patients, 27 achieved MMR or CMR in a median of 24 months (range, 6-41 months). Twenty-three patients had subsequent PCR analyses and molecular response was sustained in 10 (43%) patients.

Table 1. Cytogenetic and molecular responses of patient cytogenetic and molecular responses of patients.

<table>
<thead>
<tr>
<th>Total No</th>
<th>No who sustained</th>
<th>No who lost MMR</th>
<th>No who failed to achieve MMR</th>
<th>No who failed to achieve CCyR</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>36</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

One patient lost her CCyR with 5% Ph-positive metaphases detected at 80 months and MMR achieved at 18 months was lost at 27 months. This correlated well with increasing BCR-ABL/ABL ratios from 0.061% (18 months) to 1.071% (32 months). Of the 2 patients who did not achieve CCyR, one remained refractory with less than 0.5 log reduction in BCR-ABL transcript levels after 2.5 years on Glivec. This patient recently had an allogeneic BMT, achieved major cytogenetic response and a 1-log reduction 1 month later. Glivec was then restarted. Further monitoring of cytogenetic and molecular responses is necessary for this patient and the last patient whose latest evaluation was at 18 months.

Conclusions. Overall, 43/45 (95.5%) of patients achieved CCyR in a median of 12 months (range, 0-32 months). This incidence rate appears to be higher than those previously reported (75%-90%). MMR or CMR was achieved in 27 patients (60% of all patients). While CCyR is sustained in most patients, molecular response is sustained in only 43% of patients. We observed that patients with fluctuating levels around 2-2.5 log reduction, remained in CCyR while a trend of persistently rising BCR-ABL transcripts could lead to a loss in CCyR. As data on molecular responses of Glivec-treated CML patients in Asia is limited, we would continue to accrue such patients for molecular monitoring to assess the association of molecular response with prolonged progression-free and overall survival.

0673

KINETICS OF TWO CO-EXISTING MUTATIONS IN THE BCR-ABL KINASE DOMAIN IN FOUR CML PATIENTS

J.S.K. Khorashad, S. Khorashad, C. Marin, M. Goldman, F. Apperley, S. Kaeda
Imperial College, LONDON, United Kingdom

Background. Kinetics of mutant CML clones helps in better understanding their functional role. Aims. Investigation of kinetics of co-existing mutations in CML patients Methods and Results. Pyrosequencing was used to study the kinetics of the mutant Ph+ clones. BCR-ABL transcripts were quantified by Taqman real time PCR. Where applicable, RFLP studies were used for confirmation. Of the Ph-positive CML patients treated with imatinib (IM) at our institution who were screened for KD mutations as described previously, we were able to monitor the kinetics of the mutant clones in 4 patients, each of whom had two distinct KD mutations. Patient no. 1 failed to respond to IM and was found to have a P-loop mutation, Y255F. Treatment was changed to dasatinib, whereupon BCR-ABL transcript levels fell initially by >2 logs and the mutant clone became transiently undetectable. Thereafter transcript numbers increased and quantitative single nucleotide polymorphism (Q-SNP) using pyrosequencing and sequence analysis showed that the sensitive clone consisted almost entirely of mutant cells with both Y255F and T315I mutations. Q-SNP analysis suggested the two mutations were both present in 95% of cells; this was confirmed by restriction enzyme digestion of polymerase chain reaction (PCR) product. Patient no. 2 showed a similar sequence of events. She responded poorly to IM and was found to have F311L mutant clone. She responded briefly to dasatinib with transient reduction in total BCR-ABL transcripts and disappearance of the mutant clone. Thereafter BCR-ABL transcript numbers increased rapidly; the F311L mutation reappeared but a T315I mutation was also detected at the same level, namely 92%, which suggests again that the two mutations co-existed in the same sub-clone (therefore probably in cis). In contrast, patient no. 3 had no significant reduction in total BCR-ABL transcript levels despite treatment with IM. Both Y255F and M351T were detected but Q-SNP data showed that they represented on average 10% and 60% respectively of the total transcript numbers, suggesting involvement of different Ph-positive sub-clones. Similarly, patient no. 4 achieved complete cytogenetic remission with IM; after treatment for 1 year 90% of transcripts had a M351T mutation. After two years on IM he still had the M351T mutation (80%) but a new mutation, H596R, was detected in 46% of transcripts. Thereafter, however, the levels of the two mutations evolved discordantly; for example after 30 months on IM the M351T mutation comprised 45% of transcripts whereas the H596R comprised 20%. These values imply that these two mutations involved distinct sub-clones (therefore in trans) which had different degrees of sensitivity to imatinib. Conclusion. These observations provide further evidence for the sequential acquisition of mutant clones, which may differ in their responsiveness to specific tyrosine kinase inhibitors. It supports the notion that the best method of preventing resistance may be to start treatment with a combination of more than one tyrosine kinase inhibitor.

0674

PROGNOSTIC SIGNIFICANCE OF THE LEVEL OF RESIDUAL DISEASE AFTER 12 MONTHS IMATINIB BASED THERAPY: THE GERMAN CML-STUDY IV

P. Erben1, M.C. Mueller2, L. Sadikaj2, M. Kripp2, A. Amirzargar3, C. Schneider4, T. Lorenz1, T. Schenk1, T. Ernst1, S. Lauber1, J. Kruth1, S. Saußele1, T. Lahaye5, U. Berger6, R. Hehlmann7, A. Hochhaus8
1III. Med. Klinik Mannheim Univ Heidelberg, MANNHEIM, Germany; 2Univ. Heidelberg, MANNHEIM, Germany; 3Univ. Heidelberg, MANNHEIM, Germany

Background. Targeted therapy with imatinib induces high response rates in chronic myeloid leukemia (CML) patients (pts). However, about 4% of pts per year relapse with reappearance of Ph chromosome positive metaphases or loss of hematologic control. Aims. We sought to investigate the relationship between BCR-ABL transcript levels at month 12,
cytogenetic response and relapse-free survival after 2 years of imatinib-based treatment within the German CML-Study IV Methods. Between July 2002 and January 2006, 731 pts were randomized of whom 251 pts were recruited until November 2003 and thereby qualified for a 2-year evaluation. In 189 pts quantitative RT-PCR data at month 12 after start of treatment are available. In parallel pts were monitored by conventional cytogenetic analysis of bone marrow metaphases. A classification of molecular response levels in 4 cohorts was applied: Ratios BCR-ABL/ABL of 0.01%, 0.12%, and 1.4% represent a 4, 3, and 2-log-reduction from a predefined baseline (IRIS definition), respectively. Results. After 12 mo of imatinib-based therapy, ratios <0.01% were achieved in 11 pts (cohort 1, 6%); ratios of 0.01-0.12% in 56 cases (cohort 2, 30%); >0.12-1.4% in 70 pts (cohort 3, 37%); and >1.4% in 52 pts (cohort 4, 27%). The 2-year analysis showed CCR in 7/7 evaluable pts in cohort 1 (100%), 29/29 evaluable pts in cohort 2 (100%), 33/34 evaluable pts (97%) in cohort 3, and 12/22 evaluable pts in cohort 4 (55%, p<0.0001). Ratios BCR-ABL/ABL after 2 years differed significantly between cohorts (cohort 1 0.011%, cohort 2 0.060%, cohort 3 0.39%, cohort 4 6.4%; p<0.0001). Two pts who achieved CCR at month 12 experienced cytogenetic relapse (12 and 32% Ph+ metaphases) at month 24. Their 12 mo BCR-ABL/ABL ratios were 2.5% and 2.2%, respectively, in contrast to 0.11% which represents the median ratio of those pts achieving CCR at month 12 which was ongoing at least until month 24. Taken together pts lacking cytogenetic response (n=15, median 52% Ph+ metaphases) revealed significantly higher BCR-ABL transcript levels after 12 months than those with CCR at 2 years (13.8% vs 0.17%, p<0.0001). Within 2 years of observation 16/251 pts (6%) progressed to blast crisis, of whom two revealed clonal evolution (complex aberrant karyotype, n=2), and another two pts revealed del(9q34) or t(8;9). Tyrosine kinase inhibitors are reported to be effective in tyrosine kinase domain mutation usually detectable by D-HPLC and conventional sequencing 11 mo (M244V) and 1 mo (E555G) before hematologic diagnosis of blast crisis. Conclusions. The assessment of BCR-ABL transcript levels by quantitative RT-PCR at month 12 of imatinib-based therapies shows prognostic significance for 2-year cytogenetic and molecular response. Long term observations will demonstrate its impact on prediction of long term response.

**0675**

**Mutation analysis of the kinase domain of the BCR/ABL fusion gene in chronic myelogenous leukaemia**

Y. Wei,1 R. Hezaveh,2 B. Olsson,1 A. Ricksten,1 L. Palmqvist,1 D. Stockelberg,1 H. Wadenvik1

1Dept of Internal Medicine, Qili Hospital, JINAN, China; 2Sahlgrenska Univ. Hospital, GOTHENBURG, Sweden; 3Internal Med., Sahlgrenska Univ. Hospital, GOTHENBURG, Sweden

Background. Imatinib induces complete cytogenetic remission in a high proportion of CML patients. However, patients in cytogenetic remission usually display residual bcr-abl positive progenitors by RT-PCR. The mechanisms underlying persistence of small numbers of malignant progenitors in imatinib-sensitive patients are unclear. Aims. To gain more information about the biology of bone marrow metastases. A classification of molecular response levels in 4 cohorts was applied: Ratios BCR-ABL/ABL of 0.01%, 0.12%, and 1.4% represent a 4, 3, and 2-log-reduction from a predefined baseline (IRIS definition), respectively. Results. After 12 months imatinib therapy, 6 different mutations were detected in 9 patients who had shown imatinib resistance; 1 out of 31 treated in early-CP, 4 out of 7 treated in late-CP and 3 out of 3 treated in AP. E450G, a mutation locates at the C-terminal of the kinase domain distant to imatinib binding site, was the most frequently detected mutation. However, these mutations do not appear to be relevant for cytogenetic resistance or molecular persistent disease in patients otherwise being imatinib sensitive. Most likely mutant clones that do not expand and cause resistance, can transiently appear during imatinib treatment.

**0676**

**Benefit of imatinib at 600 mg/day for patients with Philadelphia chromosome positive chronic myelogenous leukemia in accelerated phase: A retrospective study of 44 patients**

K. Bouabdallah,1 C. Foucaud, M.P. Fort, G. Mant, F.X. Mahon

Service des Maladies du Sang, PESSAC, France

**Background.** Chronic myelogenous leukemia (CML) is a malignant hematologic disorder with a poor prognosis in advanced stages of the disease. Currently, the only curative approach is based on allogenic stem cell transplantation. Tyrosine kinase inhibitors and in particular Imatinib Mesylate (IM) have provided tremendous and significant improvement in chronic phase of the disease with cytogenetic remissions rates above 75% in numerous studies. The benefit and the dose of IM in accelerated phase remain uncertain. Some few studies have suggested that IM at 600 mg/d could increase the cytogenetic rate with a median survival close to 4 years. We report on a single and retrospective experience with IM 600mg/d in 44 accelerated phase CML patients (pts).

**Patients and Methods.** 44 adult pts (M = 27, F = 17) with accelerated phase CML (IBMTR criteria) have been treated with IM at 600 mg/d. 28 pts had received a previous treatment before IM. The median time between diagnosis and IM treatment is 13.85 months (0.005-189.42). We analyzed the cytogenetic and molecular responses, the overall survival and tried to determine factors possibly linked with survival. Statistical analysis have been performed with Kaplan-Meier method and the comparative curves with the log-rank method. Results. The median age at IM start is 51,18 years (24-80). The median follow-up time is 29,24 months. Sixteen pts died and 23 are still alive. 56 pts (84%) have a complete hematologic response, 27 (61%) a major cytogenetic response (MCyR) from whom 21 (78%) a complete response (CCyR). The probability to be in CCyR is 56% (± 17%) with a median time of 11,6 months (CI 95%: 0,0-32,7). 11 pts have a major molecular response (MMR) with a 3 log decrease of BCR-ABL load and 8 pts reached a complete molecular response (CMR). The median survival is 46,89 months. A CCyR at 3 months, and a MMR seem to be determinant for the survival (respectively p<0.089 and p<0.0007) while anemia and a previous treatment appeared to affect negatively the survival (respectively p=0,0646 and p=0,029). Conclusion. This study emphasizes the benefit of IM at a dose of 600 mg/d in accelerated phase CML pts with substantial cytogenetic and molecular responses. A CCyR at 3 months and an MMR seem to be determinant on the progressive free survival. These results should be taken in consideration in the management of such pts particularly when the question of allogenic stem cell transplantation is raised.

**0677**

**Prame as a secondary target for bcr-abl positive leukemias**

D.D. Carvalho,1 R. Porto-Siqueira,1 J.M.G. Leroy,1 W.O. Fereira,1 M.A. Zanichelli,1 M.D. Colassanti,1 M.A. Zago,1 F.A. Castro,1 V.C. da Silveira,1 M.A. Zago,1 F.A. Castro,1 V.C. da Silveira,1 1BCB-USB, SO PAULO, Brazil; 2Departamento de Clínica Medica-MFMR-USP RIBEIRO PRETO-SP, Brazil; 3Servio Hematologia-Hospital Brigadeiro, SO PAULO, Brazil; 4Hospital Brigadeiro, SO PAULO, Brazil; 5FMF-USP, RIBEIRO PRETO-SP, Brazil; 6DACT-FCFRP-USP, RIBEIRO PRETO-SP, Brazil

**Background.** Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by a clonal expansion of neoplastic hematopoietic stem cell. The neogene Bcr-Abl is a hallmark of CML and it is the neogene Bcr-Abl is a hallmark of CML and it is the
results of the fusion of bcr and c-abl genes. The correlation between the bcr-abl and prame (Preferentially Expressed Antigen in Melanoma) expression has previously been suggested, but this association is still unclear. In this study, our goal was to determine the possible correlation among prame expression, Bcr-Ab1 levels, CML progression, and response to imatinib (Gleevec). Aim and methodology: For this purpose we evaluate prame expression in many cell lines, such as HL-60, HL-60.BcrAbl, HeLa, LeukAbl Bcr-Abl, Jurkat, Jurkat.BcrAbl K562, KBM3, KBM7, KG1a, LAMA-48, SKW-64, SKW.Bcr-Abl, THP1, and THP1.Bcr-Abl (with or without imatinib for 4 hours) and 22 CML patient samples in different phases, and in remission post-imatinib by real-time RT-PCR using taqman assays. Results: We only found a correlation between bcr-abl and prame in HL-60 X HL-60.Bcr-Abl in which prame expression was 48 times higher in HL-60.Bcr-Abl. Moreover, we did not detect any association between imatinib treatment and prame, which indicates that this is probably independent of the Bcr-Abl’s tyrosine kinase activity. On the other hand, a higher prame expression was related to a disease progression, as we found 8-times more prame in accelerated than in chronic phase and 29-times more in blastic than in chronic phase and no prame expression was found in cytogenetic remission post-imatinib. Conclusions: Recently a function of prame was described as a dominant repressor of retinoic acid receptor (RAR) signaling. Signaling through RAR induces proliferation arrest, differentiation, and apoptosis in many cell types. Considering the function and our results, we can suggest that new therapeutic approaches can be developed, aiming to inhibit the function or expression of this gene, for the most delayed phase of the illness, in imatinib-refractory patients.

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0678

GENETIC CHARACTERIZATION OF 203 DE NOVO CHRONIC MYELOID LEUKEMIA PATIENTS IN THE PORTUGUESE POPULATION


CGC, PORTO, Portugal

Background. Philadelphia chromosome (Ph1) is the hallmark of almost all the cases of CML. The vast majority of patients express either the b2a2 (e13a2) or b3a2 (e14a2) BCR-ABL mRNa, characteristic of the p210BCR-ABL fusion protein. A very few patients express the e1a2 mRNa, characteristic of the p190BCR-ABL fusion protein and present in half of the adults who have BCR-ABL positive Acute Lymphoblastic Leukemia (ALL). However, some patients have the protein p280BCR-ABL originated from the e1a2 mRNA, and in some sporadic cases the BCR-ABL transcript is not detectable by standard karyotyping. This work was supported by grant NR/8758-3, Internal Grant Agency of Ministry of Health, Czech Republic.

Table 1. Portuguese patients with the novo CML used in this study.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Average age</th>
<th>Male</th>
<th>Female</th>
<th>Karyotype</th>
<th>Molecular Biology</th>
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<td>100</td>
<td>103</td>
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<td>131</td>
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<td></td>
<td>(49.3%)</td>
<td>(49.3%)</td>
<td>(50.2%)</td>
<td>(50.2%)</td>
<td>(64.5%)</td>
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Results. Ph1 chromosome was found in 96.2% of patients; 3.8% were Ph1 negative BCR-ABL positive. In the Ph1 positive group 6.1% had variants (t(9;22),v(9;22) or t(9;V)) and 12.2% had additional anomalies, while the remaining (77.9%) presented the standard karyotype (46,XX,t(9;22)(q34;q11)) or 46,XY,(9;22)(q34;q11)). 76% of CML patients expressed only BCR-ABL p210 transcripts, 21.2% co-expressed p210 and p190 transcripts, while 2.5% expressed p190 BCR-ABL, p190 (17%) or b2a3 (e13a3) or e6a2, each one with a frequency of 0.56%. Conclusions. Our cytogenetics findings do not differ significantly from those described by other authors, except for the frequency of the Ph1 negative BCR-ABL, positive cases, which is slightly below the one reported. Based on molecular biology studies a discrepancy regarding BCR-ABL expression is shown. According to the literature more than 99% of patients express p210 transcripts, while the remaining express BCR-ABL p190 and other variants, considered rare. In our population the frequency of non BCR-ABL p210 transcripts is higher than the one reported (1.7% for patients expressing p190 and 1.1% for atypical transcripts). Different transcripts may result from alternative splicing between BCR and ABL and within BCR itself. RNA splicing implies the recognition of consensus sequences, including 5’ and 3’ splice sites and a weakly conserved branchpoint in the intron upstream the 3’ splice site. Polymorphisms affecting these sequences could activate cryptic branchpoints that are less efficiently used originating unusual products. Being so, the reported frequency of atypical transcripts in our population might reflect a specific genetic Background. Nevertheless, the complete characterization of BCR-ABL transcripts, namely the uncommon ones, will ascertain correlations with different disease phenotypes and improve the outcome of single patients by individualizing therapeutic strategies.

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References

0679

GENE EXPRESSION PROFILING IN CML PATIENTS RESISTANT TO TREATMENT SPECIFIC PROFILES IN NON-RESPONDERS WITH LOW BCR-ABL TRANSSCRIPT LEVELS

K. Fiser, D. Moucková, E. Otáhalová, J. Moravcová

Inst. of Hematol. and Blood Transf., PRAHA, Czech Republic

CML is characterised by a presence of fusion gene BCR-ABL. The level of BCR-ABL transcript characterises the disease status and BCR-ABL kinetics are an important prognostic factor. However, we found that among patient resistant to therapy there were those whose low BCR-ABL levels did not correlate with the disease status. Moreover, patients with non-correlating BCR-ABL levels had the worst clinical outcome. Our aim was to find gene expression differences underlying this discrepancy. To do this we turn to gene expression profiling using cDNA macroarrays. We analysed 28 samples of patients not responding to treatment. There were samples with BCR-ABL levels corresponding (n=21) and not corresponding (n=7) with the clinical state of disease. Hierarchical clustering (Euclidean distance, Average linkage) was used to cluster simultaneously both samples and genes. Hierarchical clustering showed that out of 28 samples of non-responding CML patients all 7 samples with BCR-ABL level not correlating with the disease status occupied a single cluster, clearly visible on the gene expression matrix. Among gene clusters our focus was kept on genes differentially expressed in non-correlating samples compared to the rest of the non-responders. We found clusters with genes up-regulated in non-correlating samples as well as clusters with genes down-regulated in these samples. Among up-regulated genes there were BAD, CDKN2A, O-6-methylguanin-DNA methyltransferase, Notch4, RhoC and VEGFR1. Clusters of down-regulated genes included e.g. Akt2, MAPK8, cyclins A, G1 and D3 and several caspases. In conclusion, we have found a group of CML patients not responding to the treatment whose BCR-ABL transcript levels were not correlating with the clinical disease status. This group was characterised to have clearly different gene expression profiles to the other non-responding patients. The genes differentially expressed in these samples are candidates for further investigations on mechanisms of both therapy resistance and possible lose of BCR-ABL dependency in CML. The BCR-ABL independency in these patients was further supported by our preliminary data on Western blot analyses and other kinase inhibitor experiments.

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0680

THE EXPRESSION OF PROTO-ONCOGENES IN THE COURSE OF CHRONIC MYELOID LEUKEMIA

A. Vidovic, G. Jankovic, M. Colovic, D. Tomin, M. Perunicic, I. Djunic, D. Antic, J. Bila, M. Bakrac, O. Markovic, V. Cemenick, D. Boskovic

Institute of Hematology, BELGRADE, Serbia and Montenegro; Clinical Center ‘Bezansjka Kosa’, BELGRADE, Serbia and Montenegro

Background. The chronic phase (CP) of chronic myelogenous leukemia (CML) is characterised by the presence of chimeric BCR/ABL gene and a proliferate growth of mature polymorphonuclears. The accelerated
We studied 85 patients (pts.) with the median age of 50 years. Among them, 71 pts. were in CP, 25 in an AP, and 31 in the BC. The temporal expression (percentage positivity per 1000 analysed cells) of c-kit, c-myc, H-Ras, cyclin A1, p53, bcl-2 and VEGF proto-oncogene proteins over the course of CML was studied using the immunohistochemical technique which utilizes relevant monoclonal antibodies. It was correlated with the laboratory findings (Hb, WBC and platelet counts, and the percentage of blasts) and clinical parameters (organomegaly, duration of CP, AP, and BC of disease progression. Results. The level of c-kit expression differed significantly in time with the largest values observed in the BC (x2, p=0.025). The level of anti-apoptotic protein bcl-2 increased significantly with the progression of CML (x2, p=0.005). Conversely, the expression of c-myc was highest in CP (x2, p=0.003). The expression of VEGF protein was most pronounced in an AP (ANOVA, p=0.033). There was no significant difference in the level of expression of H-Ras, cyclin A1 and p53 over the course of CML. The level of VEGF expression correlated inversely with degree of organomegaly (Pearson, r=-0.400, p=0.011). The c-kit expression correlated directly with the extent of bone marrow fibrosis (Spearman, r=0.407, p=0.000). High expression of VEGF correlated with a longer duration of CP (log rank, p=0.003) and with a longer overall survival (log rank, p=0.042). Conclusion. The significance of changes in oncoprotein expression, estimated by a histochemical approach over the course of CML, is of great importance in understanding the progression of the disease and a new approach to their therapy. The details of the temporally-related changes in oncprotein expression in leukemic cells require the study at the molecular level.

**0681**

**P190 BCR-ABL CHRONIC MYELOID LEUKEMIA PARTLY RESEMBLING CHRONIC MYELOMONOCYTIC LEUKEMIA IN A YOUNG PATIENT TREATED WITH IMATINIB**

J. Diamond,1 H. Aliazi,2 R. Domingues,3 M. Gomes Silva,4 M.J. Frade,4 A. Parreira4

1Portuguese Institute of Oncology - IPO, LISBON, Portugal; 2Hematology Lab, Hematology Dept, IPO, Lisbon, Portugal; 3Hematology Lab, IPO, Lisbon, Portugal

Background. In Ph-positive CML, the breakpoint on the BCR gene nearly always occurs within the major breakpoint cluster region (M-bcr), and the BCR-ABL fusion gene encodes a protein of 210 kDa molecular weight (P210). In most Ph-positive ALL, by contrast, the breakpoint on chromosome 22 occurs within the first intron of the BCR gene, or minor bcr (m-bcr). In these cases, the first BCR exon is fused to ABL exon 2 (e1a2 junction) and a BCR-ABL protein of 190 kDa is formed (P190). This form of CML was reported as having some unusual clinical and haematological features, partly resembling chronic myelomonocytic leukemia (Melo et al., 1994). Here, we describe a 24-year-old female patient, diagnosed in July 2005 with leukaemia, when she volunteered as a blood donor, and was diagnosed as chronic phase CML. Methods and Results. She was asymptomatic, with only splenomegaly detected on physical examination. The peripheral blood examination showed a WBC count of 29.7×10⁹/L, basophilia (4%), monocytosis (5%) and a platelet count of 713×10⁹/L. No pseudo-Pelger-Huet hypolobulation or peripheral blood myeloblasts were detected. Bone marrow cytogenetic analysis at diagnosis showed a karyotype 46,XX (t(9;22)(q34;q11)) in 20 metaphases. Molecular studies detected the presence of an e1a2 transcript. FISH analysis confirmed the m-bcr as the sole type of BCR-ABL rearrangement in bone marrow cells. She was put on hydroxyurea (HU) 2 g daily, with a partial haematological response; 3 weeks later she was started on α-IFN 3 MU/day. Treatment with imatinib was initiated in October at a dose of 400 mg a day and after one month the dose was increased to 600 mg daily. The patient achieved complete haematological response and remained clinically well. After 5 months of imatinib therapy, the abnormal clone persisted and C-bcr-PCR quantification showed a 50% BCR-ABL ratio. Despite remaining in complete haematological remission, the abnormal clone persists 4 months after initiation of imatinib therapy. Twenty-one cases of CML with a breakpoint in the m-bcr, resulting in P190 type BCR-ABL have been reported, so far, and only 17 of them in detail. This is to our knowledge, the first P190 CML case reported in a very young patient, in contrast to those previously described whose age ranged from 32 to 83 years (median 53.5). It remains to be seen whether the long term response to imatinib in this type of CML will compare to that observed in classical P210 cases or will resemble more the poorer response achieved in Ph-positive ALL.

**0682**

**ANGIOGENIC ACTIVATORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: EFFECT OF TREATMENT WITH IMATINIB MESYLATE**

L. Smolej,1 J. Voglova,1 C. Andryss2

1Charles Univ.Hospital and Medical School, HRADEC KRALOVE, Czech Republic; 2Charles Univ. Hospital, HRADEC KRALOVE, Czech Republic

Background. Angiogenesis is nowadays considered an important factor in the evolution of various haematological malignancies including chronic myeloid leukaemia (CML). Several studies have recently reported elevated levels of angiogenic activators such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in CML patients. However, there have been only few data on the influence of imatinib mesylate (IM) treatment on the levels of angiogenic cytokines in CML. Aims. To analyze peripheral blood levels of angiogenic activators in patients with newly diagnosed CML and during imatinib treatment. Methods. We measured plasma concentrations of VEGF, bFGF and soluble endoglin (sCD105) using sandwich enzyme-linked immunosorbent assay (ELISA) in 16 patients with chronic-phase CML and 80 healthy blood donors; furthermore, repeated samples during the therapy with (IM) were analyzed. Results. We found a statistically significant increase in VEGF (mean ± SD [standard deviation], 491.0 ± 365.3 vs. 64.2 ± 69.5 pg/ml, 95% CI [confidence interval] of mean, 296.4-685.7 vs. 51.0-77.5 pg/ml, p<0.0001) and sCD105 (mean ± SD, 7.0 ± 1.9 vs. 4.5 ± 1.51 pg/ml, 95% CI [confidence interval] of mean, 5.80-11.3 vs. 4.20-4.93 ng/mL, p=0.0001) but not bFGF (p=0.606) in comparison to the control group. VEGF levels significantly decreased in 7 patients who achieved hematological remission (6 complete remissions, 1 partial remission) during therapy with IM (mean ± SD, 679.6 ± 431.5 vs. 152.7 ± 63.3 pg/ml, 95% CI, 280.6-1076.8 vs. 74.1-191.8 pg/ml, p=0.015). There was no significant change in bFGF or sCD105 (p=0.950 and 0.125 respectively). Conclusions. We found significantly elevated VEGF and sCD105 levels in CML patients. In addition, successful treatment with IM resulted in significant decrease of VEGF. These data lend further support to the importance of angiogenesis in pathophysiology of CML. Further studies incorporating larger number of patients are needed to confirm our findings. Supported by research project MZO 00179906 from Ministry of Health of Czech Republic.

**0683**

**THE E19A2 BCR-ABL BREAKPOINT: MORE FREQUENT THAN OTHER ATYPIICAL BCR-ABL VARIANTS IN CHRONIC MYELOGENOUS LEUKEMIA?**

I. Iacobucci,1 G. Martellini,1 G. Rosti,2 F. Castagnetti,2 M. Amabile,1 A. Poerio,2 S. Soverini,2 S. Colarossi,2 A. Gnanì,2 S. Armadori,2 A. Abruzzese,2 D. Cillonì,2 G. Saglio,1 M. Baccarani,1 F. Pane1

1University of Bologna, BOLOGNA, Italy; 2Inst. of Hematology Sergnoli, BOLOGNA, Italy; 3University Tor Vergata, ROME, Italy; 4Hematology, University of Turin, TURIN, Italy; 5Ceinge Biotecnologie Avanzate, UNIVERSITY FEDERICO II, NAPLES, Italy

In the vast majority of patients diagnosed as having chronic myelogenous leukaemia (CML) and t(9;22), the breakpoint on chromosome 22 occurs in the major region of the BCR gene (M-BCR); this translocation usually results in a hybrid BCR-ABL mRNA with a b2a2 and/or b3a2 junction, which encodes a p210 fusion protein. However, the E19A2 BCR-ABL breakpoint was initially described in a 45-year-old male hemoglobin was 14.7 g/L, white blood cell count 7.1×10⁹/L, neutrophils 64%, lymphocytes 8%, monocytes 2%, eosinophils 0.9%, basophils 0.4%, metamyelocytes 30%, myelocytes 20% and platelet count 9.9×10¹⁰/L. In a 30-year-old female hemoglobin was 9.4 g/L, white blood cell count 10.8×10⁹/L. In all 7 patients cytogenetic analysis of 20 bone marrow...
Despite the continuously increasing control of CML, the treatment may become more complex than ever. The cytogenetic and molecular responses, respectively. Quantitative RT-PCR techniques (qRT-PCR) were used to measure BCR-ABL and WT1 transcript levels, and conventional karyotyping was used for measuring the cytogenetic response. Good cytogenetic responders were defined as patients having obtained a major cytogenetic response (MCgR), i.e. < 36% Ph-positive metaphases, within 1 year of treatment. Results. At diagnosis the seven CML patients with a suboptimal cytogenetic response were found to have significantly higher WT1 transcript levels compared to those 18 patients with a good cytogenetic response (MCgR) (p=0.02). This difference was seen both when peripheral blood and bone marrow samples were used as templates. No relationship was seen between BCR-ABL transcript levels at diagnosis and the cytogenetic response to imatinib therapy obtained during the first year of treatment. Conclusion. A high WT1 gene expression level at diagnosis might identify those CML patients that will have a suboptimal cytogenetic response to imatinib therapy. It appears warranted to study this hypothesis in a larger patient material.

**0684**

**GENE EXPRESSION PROFILE OF PATIENTS INNATELY RESISTANT TO IMATINIB MESYLATE**

A. Santoro,1 G. Cammarata,1 M. La Rosa,1 D. Turri,1 S. Tringali,1 A. Marfia,1 C. Aguelli,1 V. Ruzzo,1 R. Giustolisi,1 S. Mirto1
1Cervello Hospital, PALERMO, Italy; 2Cattedra Ematologia Università Cattana, CATANIA, Italy

**Background.** Imatinib (IM) a specific ABL tyrosine kinase inhibitor has been reported to have a significant clinical effect on chronic myeloid leukemia (CML). Some patients treated with IM acquire resistance probably due to selective pressure on cells that carry amplified copies of the BCR-ABL oncogene or point mutations in the ABL affecting the binding site of IM. In other cases resistance appears to exist prior to drug exposure. Such innate resistance is poorly understood, some evidences suggest that development of alternative pathway, may confer BCR-ABL independent survival to CML cells. Comparative genome expression studies have long been known to provide important insight into biological process such as proliferation, differentiation, apoptosis and transformation. Only few gene expression profiling-based studies of CML and IM treatment have so far published. Moreover, only three studies have been performed on patient's samples, resulting in heterogeneous conclusions. Aims. To investigate about the molecular events involved in innate IM resistance in CML we compared the expression profile of a set of 380 genes on resistant patients versus responder patients. We chosen 380 genes involved in process like apoptosis, cell adhesion, cell proliferation, signal transduction, chromosome/DNA dynamics. Methods. A set of 15 patients (3 female, 10 male, median age 50) with CML were selected from several diagnosed at Division of Hematology of the Cervello Hospital of Palermo. Patients were defined a responder to IM if they achieved reduction of BCR-ABL transcript greater than 3 log within 6 months, while resistant those with less than 1 log of reduction after 6-12 months of treatment. We use the TaqMan Microfluidic Card (Applera). This technology is a method for real-time RT-PCR that can simultaneously assays the RNA expression levels of up to 380 genes on a single card. RT-PCR data were quantified using the SDS 2.1 software and normalized using the GAPDH as endogenous control. Results. After the analysis of seven responder and six no responder samples we detected differential expression of 18 genes that correlate with the imatinib resistant phenotype. The resistant cells over express (1.9 fold /7.5 increase) genes of different categories: signal transduction (SOX1,PEA15,STAT5B), apoptosis (BCL2, BAX), genes involved in cell adhesion (SELL, ITGB7), genes related to cell cycle progression (CCND2, CDK4) and transcription factors genes (ETS2, SMAD1, KLF7). Conclusions. In the pathogenesis of CML the expression of BCR-ABL activates many signaling cascades. It has been recently demonstrated that IM treatment increase BCL6 expression through the inhibition of PI3K/AKT pathway; BCL6 replace STAT5 at STAT5/BCL6 site in CCND2 promoter repressing CCND2 expression and arresting the cell cycle progression. In this study we identified several genes implicated in cellular process that are involved in the PI3-K /Stat5 integrated mechanism, in particular we noted an over-expression in IM resistant patients of BCL6, CCND2 and CDK4 suggesting that an activation of these pathways may represent a novel mechanism for the persistence of BCR-ABL-positive cells in IM-treated patients.

**Funding:** this work was supported by progetto regionale AIRC coordinated by Prof R Giustolisi

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**0685**

**PROGNOSTIC SIGNIFICANCE OF WT1 GENE EXPRESSION IN CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH IMATINIB**

M.H. Hardling,1 Y. Wei,1 K. Ekeland-Sjoberg,1 D. Stockelberg,1 A. Ricksten,1 H. Wadenvik2
1Hematology Section, GOTEBOG, Sweden; 2Department of Clinical Chemistry, GOTEBOG, Sweden

**Background.** Despite the availability of imatinib (Glivec), Novartis, NJ, USA) as the accepted standard approach to the treatment of newly diagnosed patients with CML, the overall management of the disease has become more complex than ever. The cytogenetic and molecular response to imatinib in patients in first chronic phase overides the current therapeutically relevant risk factors, i.e. Sokal score. New baseline biological markers predicting the response to imatinib therapy would thus be valuable to identify patients in whom imatinib will fail, so that timely adjustments can be made to the overall therapeutic strategy. Aims. The objective of this study was to evaluate if the WT1 (Wilms tumor gene 1) gene expression at diagnosis bears any prognostic information, i.e. if it relates to the cytogenetic response obtained during the first year of imatinib therapy in chronic phase CML patients. Methods. Peripheral blood (PB) and bone marrow (BM) samples were obtained in 25 newly diagnosed chronic phase CML patients, before commencing imatinib treatment, for analysis of WT1 gene expression. In addition, BM and PB were sampled at 3-month intervals to determine the cytogenetic and molecular response, respectively. Quantitative RT-PCR techniques (qRT-PCR) were used to measure BCR-ABL and WT1 transcript levels, and conventional karyotyping was used for measuring the cytogenetic response. Good cytogenetic responders were defined as patients having obtained a major cytogenetic response (MCgR), i.e. < 36% Ph-positive metaphases, within 1 year of treatment. Results. At diagnosis the seven CML patients with a suboptimal cytogenetic response were found to have significantly higher WT1 transcript levels compared to those 18 patients with a good cytogenetic response (MCgR) (p=0.02). This difference was seen both when peripheral blood and bone marrow samples were used as templates. No relationship was seen between BCR-ABL transcript levels at diagnosis and the cytogenetic response to imatinib therapy obtained during the first year of treatment. Conclusion. A high WT1 gene expression level at diagnosis might identify those CML patients that will have a suboptimal cytogenetic response to imatinib therapy. It appears warranted to study this hypothesis in a larger patient material.
G1, 3 in G2 and 1 in G3) of these 13 pts, molecular response was confirmed in two or more consecutive tests. In addition, all live evaluable pts (G4) who were in complete karyotypic response at the time of the beginning of STI-INF combination, obtained a molecular response. This was confirmed in three or more consecutive tests over a period ranging from 15 to 23 months in three pts and in non-consecutive tests in the remaining two. From a minimum of 1 to a maximum of 10 tests (median value 4) were performed in the 18 molecular responsive patients including the first negative RT-PCR assay, in the calculation. The results obtained by RT-PCR were concordant with those obtained by RQ-PCR.

Conclusions. It is not possible to achieve any firm conclusion regarding the effect of STI-INF combination on molecular response because of the small sample size of treated patients. However, our findings suggest an additive effect of STI and INF in Ph'- clone control as indicated by the improvement of the quality of remission in long lasting karyotypic, but not molecular responsive patients when this combination therapy was utilized.

**0687**

PROLONGED MOLECULAR REMISSION IN A LATE CHRONIC PHASE CML PATIENT AFTER DISCONTINUATION OF IMATINIB TREATMENT


S. Luigi Hospital, ORBASSANO, TORINO, Italy

Background. Treatment with imatinib results in complete cytogenetic response (CCR) in the majority of patients with Ph-positive CML in chronic phase. However, in spite of a rapid decline in BCR/ABL positive cells during treatment with a standard dose of 400 mg/day, imatinib fails to eliminate all residual disease. It has been observed that a higher dosages of 800 mg/day may induce a more rapid CCR, and the role of hghedose as well as the association of imatinib with interferon or ARA-C are under investigation in large trials, but it is not yet clear whether these more aggressive modalities of treatment will eventually lead to increased rate of response in a more prolonged follow-up. Major molecular remission (MMR), as defined by a ≤3-log reduction in leukemic cells, relates with good prognosis and low risk of disease progression. However, complete disappearance of the hybrid transcript, when sensitive methods such as nested RT-PCR and RQ-PCR are used, is very rare. When patients discontinue imatinib treatment because of side effects, levels of BCR/ABL rise rapidly indicating the reexpansion of the leuemic clone (Michor F et al., Nature 435:1268, 2005). Aim of the study. To evaluate the clinical, cytogenetic and molecular features of a CML patient who obtained a complete molecular remission confirmed over a 30-month period during a standard dose imatinib treatment and persisting 22 months after imatinib discontinuation. Methods and Results. Standard cytogenetics, FISH analysis, nested RT-PCR and quantitative PCR with TaqMan technology were used for the follow-up of the patient. He was a 58-year-old man diagnosed as having Ph-positive CML, low Sokal risk, in 1995. He underwent an allogeneic donor bone marrow transplantation. He was retreated with interferon and subcutaneous low-dose ARA-C and went into CCR at month 18; however this was subsequently lost and a minor clone with associated trisomy 8 was observed. The patient continued IFN + ARA-C until September 2000, when BM cytogenetics showed 80% normal cells, 15% Ph+ cells and 5% Ph- cells with +8. He was started on imatinib, 400 mg/day, in October 2000. A CCR was obtained at month 3 and it was confirmed in all following controls. RT-PCR and RQ-PCR became negative for the presence of BCR/ABL transcript at month 12 and this data was consistently confirmed in eight controls done every 6 months on BM and twelve PB samples, done every 3 months. In May 2004, after 48 months of imatinib treatment, therapy was stopped because of side effects. The patient remains in complete hematologic, cytogenetic and molecular remission at March 2006. Conclusions. Complete molecular remission as defined by the absence of any detectable BCR/ABL transcript is usually observed only in patients who underwent a allogeneic bone marrow transplantation and is uncommon in imatinib treated cases. However, the data reported here indicate that a small proportion of CML patients could achieve eradication of the disease with standard imatinib treatment. It remains to be established if the previous therapy with interferon or other immunological mechanisms may contribute to this phenomenon.

**0688**

CHRONIC MYELOID LEUKAEMIA AFTER TREATMENT WITH 131-I FOR THYROID NEOPLASMS: TWO NEW CASES AND REVIEW OF THE LITERATURE


Hospital Universitario de Canarias, LA LAGUNA - TENERIFE, Spain

Background. Chronic myeloid leukaemia (CML) is a clonal disorder arising from a somatically mutated pluripotent stem cell. It is a process of unknown aetiology, although there is a higher incidence between populations exposed to irradiation: Japanese atomic bomb survivors, patients with thyroid carcinoma, and women treated with orthovoltage cauterisation irradiated for ankylosing spondylitis, among others. CML after treatment with 131-I for thyroid carcinoma is a rare condition. We report two new cases of CML associated with 131-I treatment for thyroid carcinoma, and a review of the literature. Case Report 1. A 48-year-old female was diagnosed in February 1993 of chronic lymphocytic leukaemia (CLL). She had a 2-year history of euthyroid multinodular goiter, and two years later she developed a papillary thyroid carcinoma. The thyroid gland was partially removed, and the patient began radiation therapy receiving a cumulative 131-I dose of 525 mCi, for ablation of thyroid remnants. Nine years later she developed leukocytosis and thrombocytosis, and Philadelphia (Ph) chromosome-positive CML was diagnosed. She received imatinib as treatment, and at the present time she has only a clonal lymphocytosis in blood and bone marrow. This case is a rare combination of C. L. L. and C. M. L. 131-I-related, and it is possible the unique known in the literature. Case Report 2. A 41-year-old man presented in November 1986 with a short history of leukopenia, leukocytosis, and thrombocytosis. Bone marrow was obtained and stromal cells bearing this gene are positively selected by virtue of a growth advantage in vitro. There is a delay of several years between the initial mutational event and the development of clinical symptoms that lead to the diagnosis of CML. It was calculated that the elapsed time from occurrence of a single cell containing the Ph chromosome to a leukemic burden of 100,000 cells/μL was 6.5 years. A literature review disclosed only 10 cases similar to ours. The earliest cases of CML were diagnosed 4 to 5 years after the exposition to radiation. Although there is no evidence to prove whether the development of CML after thyroid carcinoma represents a treatment-induced complication, patients treated with 131-I may need a long-term blood count follow-up to investigate the appearance of myeloproliferative disorders such as CML.

**0689**

FOLLOW-UP OF CML PATIENTS WITH CLONAL CYTOGENETIC ABNORMALITIES IN PH NEGATIVE CELLS DURING TREATMENT WITH IMATINIB

A.C. Oliveira, 1 M. Arnan, 1 A. Alonzo, 2 B. Espinet, 3 C. García, 3 C. Boqué 3 1 Institut Català d’Oncologia, BARCELONA, Spain; 2 Hospital Universitari Bellvitge, BARCELONA, Spain; 3 Hospital del Mar, BARCELONA, Spain

Background. Clonal cytogenetic abnormalities in Philadelphia (Ph) negative cells of patients with Chronic Myeloid Leukaemia (CML) treated with imatinib have been reported in the past years. The aberrations most frequently described are trisomy 8, monosomy 7 and deletion 20q. For many years it was believed that these cells did not have any clinical relevance, but it is now evident that the presence of such cells during treatment with imatinib may predict a worse outcome and shorter progression free survival. This is probably due to the fact that the Ph-negative clones are自媒体 mutated by the treatment with imatinib. The role of the Ph-negative clones during treatment with imatinib is still not known. In most cases the Ph-negative clones disappear during treatment with imatinib. However, the data regarding bone marrow findings and clinical outcome are presented. Methods. The three patients were studied by conventional cytogenetics (CC) and by FISH with the following probes: CEFS, 5q1 (EGR-1), 7q31 (D7S486), 20q12 (D20S108) of VYS15. All the patients had received imatinib for at least 9 months when the abnormal clone was first detected. Bone marrow was examined for morphologic dysplasia at the moment of clonal detection as well as at the moment of this study. Clinical and cytogenetic data are summarized in Table 1. Results. Patient 1 had myelodysplasia when the abnormal clone was first detected as well as at present. During a 26 months follow up, imatinib dosis had to be decreased because of progressive cytopenias resulting in the lost of complete cytogenetic response (CCR). Patient 2 also had bone marrow dysplasia when cytogenetic anomalies were found. CCR only lasted six months. At present, she is...
in blastic phase 29 months later despite of imatinib, although never having received more than 300mg because of cytopenias. Patient 3 showed no morphologic signs of dysplasia at any moment. During a 24 months follow up he also required decreases in imatinib dosis because of cytopenias. Conclusions. 1. Abnormal clones were always detected in Ph negative cells. 2. The three studied patients presented cytopenias that limited increases on imatinib dosis, independently of bone marrow morphological features. 3. The correct control of the disease in these patients might be difficult because suboptimal dosis of imatinib certainly contribute to treatment failure.

### Table 1

<table>
<thead>
<tr>
<th>Case/Gender/Age</th>
<th>Cytotype (first analysis)</th>
<th>FISH (first analysis)</th>
<th>Cytotype (at present)</th>
<th>Months of follow-up/Comments</th>
</tr>
</thead>
</table>

1. Abnormal clones were always detected in Ph negative clone was detected and at the moment of this study.

### Results

#### Aims
- The frequent extranodal site was lung (55%), 51% pts had pleural effusion and 28% - pericardial effusion. 52 pts (82%) received CHOP as a first-line treatment, whereas other 19 pts were treated with other regimens (9 RCHOP and 10 MACOP-B). Medialistinal radiation therapy (RT) at dose 30-36 Gy was given to 24 (34%) pts.
- Among 52 pts who received CHOP, 13 (25%) achieved complete response (CR), 11 (21%) partial response (PR), 7 (13%) responders pts had early relapse. All pts, who relapsed/failed the initial treatment, underwent to salvage CT, 10 not responding pts had progressive disease and died. Projected 3-years RFS and OS were 37% and 52% respectively. RCHOP and MACOP-B were highly effective regimens in all 19 pts (11 CR and 9 PR), but short follow-up and few number of pts were not indicative for the superiority of these regimens over the CHOP. Conclusions. Our data confirm that most patients with PMLBCL presented cytopenias that limited increases on imatinib dosis, independently of bone marrow morphological features. The three studied patients presented cytopenias that limited increases on imatinib dosis, independently of bone marrow morphological features.

### Methods
- The frequent extranodal site was lung (55%), 51% pts had pleural effusion and 28% - pericardial effusion. 52 pts (82%) received CHOP as a first-line treatment, whereas other 19 pts were treated with other regimens (9 RCHOP and 10 MACOP-B). Medialistinal radiation therapy (RT) at dose 30-36 Gy was given to 24 (34%) pts.
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ed active disease in 16 of 30 patients (53%). Sensitivity in gastric MALT (7/17, 41.1%) was lower compared to non-gastric MALT (9/18, 69%). The CT findings obtained from the fused PET/CT data allowed differentiation between physiological and pathological FDG uptake, especially in the stomach. PET/CT detected active disease in 7/7 (100%) patients with advanced disease (stage III-IV) but was positive in only 9/25 (36.1%) with early stage disease (I-II). The incidence of gastric FDG uptake was higher in patients presenting with gastric ulcer than in subjects with minimal or no macroscopic findings on gastroscopy. Of the 30 patients in the study cohort, nine had a follow up PET/CT after therapy. Of these, 3 had biopsy proven relapse during follow-up. PET/CT detected relapse in 3 patients (including one patient who had negative PET on diagnosis). Conclusions: We report the initial results of a PET/CT imaging in MALT lymphoma patients. Our data suggest that PET/CT is a useful tool for both initial staging of disease and for follow-up after therapy. The anatomic data obtained from the CT part of the study allows for better interpretation of the corresponding scintigraphic abnormality detected on PET, mainly since MALT appears to involve organs which may be associated with physiologic 18F-FDG uptake.

0692

BENIGN STRICTURES FOLLOWING TREATMENT FOR PRIMARY GASTRO-INTESTINAL NON-HODGKINS LYMPHOMA: CASE SERIES

J. Kerr,¹ M. Turner,¹ M. Ashton-Key,¹ G.M. Mead,² P.W. Johnson²
¹Cancer Research UK; SOUTHAMPTON, United Kingdom; ²Southampton General Hospital, SOUTHAMPTON, United Kingdom

Background. We report a novel complication, benign intestinal strictures, developing after treatment for gastrointestinal lymphoma (GINHL). Between 25 and 35% of non-Hodgkin’s lymphomas arise at extranodal sites, and around half of all extranodal lymphoma is in the gastrointestinal tract, the stomach being the commonest site. Treatment for GINHL carries a risk of internal haemorrhage, and perforation of an abdominal viscus, although studies have found that these complications are relatively rare, with incidence rates of around 0-2%. Methods. This is a retrospective case series. Five patients in whom a stricture complicated therapy for lymphoma were identified from the centre records over a six year period. Information was gathered from clinical records, radiology and stored histology samples. Available histology was reviewed by an independent histopathologist and x rays were reviewed by an independent radiologist.

Figure 1. Duodenal stricture with replaced muscularis propria.

Results. Three patients were male and two female. The median age was 55 (range 34-75). Three patients had localised diffuse large B-cell lymphoma of the small intestine (all in the duodenum or jejunum). One patient had post-transplant lymphoproliferative disorder affecting the small bowel and another follicular lymphoma involving the gastro-duodenal junction. In 3 the stricture developed de novo after chemotherapy and in the other 2 this occurred during remission at 10 months and two years from the end of therapy, respectively. One case was treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) (having previously received rituximab at presenta-
The development of a Non-Hodgkin’s lymphoma (NHL) is one of the most serious complications in patients with autoimmune diseases. Mucosa associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma (DLBCL) are the most common subtypes in these patients and chemotherapy is the therapy of choice in this setting. The combination of cyclophosphamide, doxorubicin, vincristine, prednisolone and rituximab (R-CHOP) seems to be the most effective regimen for lymphoma cell eradication at the moment. On the other hand, B lymphocytes are not only the key target in NHL but play also an integral part in the pathogenesis of autoimmunity. In keeping with these findings, one might hypothesize that immuno-chemotherapy administered to treat lymphoma might also diminish (or even eradicate) auto-reactive cell clones and might therefore improve the underlying autoimmune condition. Aims. As patients with B-cell lymphomas suffering from an underlying autoimmune condition undergoing therapy with R-CHOP offer the unique possibility of monitoring effects of therapy on various rheumatologic parameters, we have evaluated serologic autoimmune markers and the clinical outcome of patients with autoimmune diseases who received lymphoma treatment with R-CHOP during the course of their disease. Patients and methods. We have retrospectively analysed 13 patients with Non-Hodgkin’s lymphoma who concurrently suffered from autoimmune diseases and were treated with the R-CHOP regimen (Table 1).

### Table 1. Patient characteristics.

<table>
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<tr>
<th>No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Disease</th>
<th>Prior Duration (months)</th>
<th>Prior Treatment</th>
<th>RF</th>
<th>CR</th>
<th>C3c</th>
<th>C4</th>
<th>RD</th>
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</table>

No: number; M: male; F: female; MALT: mucosa associated lymphoid tissue lymphoma; DLBCL: diffuse large B-cell lymphoma; RD: rheumatic disease; RF: rheumatoid factor; CR: complete remission; C3c: complement C3c; C4: complement C4; MTX: Methotrexate.

At every visit, patients were asked for the presence of joint pain, intake of corticosteroids, disease modifying anti-rheumatic drugs (DMARDs), biologic anti-rheumatic drugs (BSAIDs), as well as quality of life. The rheumatoid factor (RF), antinuclear antibodies (ANA) and the complement factors C3c and C4 were measured immediately before institution of chemotherapy and then at regular intervals during the course of chemotherapy. Lymphoma response to treatment was classified according to the International Working Group recommendations. Results. The median levels of RF were 89 IU/ml (IQR: 12-960) before and 65.5 IU/ml (IQR: 20.25-577.75) after therapy (p=0.046). The median levels of ANA were 240 (IQR: 70-1600) before and 40 (40-60) after therapy (p=0.068). 10 (77%) patients showed clinical improvement of their autoimmune symptoms during the course of chemotherapy. Two (15%) patients reported no difference with regard to their autoimmune disease and one (7%) patient who suffered from rheumatoid arthritis experienced a worsening of her symptoms during therapy with R-CHOP. The autoimmune related symptoms recurred after a median time of 7 weeks (IQR: 6-8) in 7 patients. One patient who had suffered from vasculitis before initiation of had a durable remission after completion of R-CHOP now ongoing for 8 months. In terms of lymphoma-response, 11 patients achieved a complete- and 2 a partial remission. Summary/Conclusions. This analysis suggests that R-CHOP given for lymphoma treatment is also effective for therapy of concurrent rheumatoid diseases. Both rheumatoid parameters as well as clinical symptoms showed a significant decrease during treatment with this immunotherapy. However, patients treated with R-CHOP should be of limited duration. With regard to the lymphoma, R-CHOP displayed an excellent efficacy which seems comparable, or even better, to patients with NHL without autoimmune diseases.
0696
Efficacy and Safety of DepoCyte (Liposomal Cytarabine) in Patients with Central Nervous System Involvement from Non-Hodgkins Lymphoma: A Report on 23 Patients Treated in Spain
A. Garcia-Marco,1 C. Pérez,2 B. Navarro,1 L. Palomera,3 E. Sanz,4 FJ. Capote,2 A. Fernández de Sevilla,5 E. Sánchez,4 A. Cantalapiedra,6 R. Pérez7, C. Catanzariti,8 M. Medina-Villanueva,9 F.R. García,10 Y. Martín11; A. Alvarez1; T. García1; C. Panizo1
1Hospital Universitario Puerta de Hierro, MADRID, Spain; 2Hospital Clinico Universitario Lozano Blesa, Zaragoza, Spain; 3Puerta del Mar, CADIZ, Spain; 4Institut Català d’Onkologia Duanes i Reina, BARCELONA, Spain; 5Hospital Universitari Vall d’Hebron, BARCELONA, Spain; 6Rio Ortega, VALLADOLID, Spain; 7Gregorio Marañón, MADRID, Spain; 8Meixoeiro, VIGO, Spain; 9Clínica Universitaria San Carlos, MADRID, Spain; 10Complejo Hospitalario de Pontevedra, PONTEVEDRA, Spain; 11Principe de Asturias, ALCALA DE HENARES, MADRID, Spain; 12Del Mar, BARCELONA, Spain; 13Hospital Universitar Arnau de Vilanova, LLEIDA, Spain; 14Clínica Universitaria de Navarra, PAMPLONA, Spain

Background. Lymphomatous meningitis (LM) occurs in ~5% of patients with diffuse large B-cell lymphoma (DLBCL), and more frequently in patients with Burkitt’s lymphoma (BL) and lymphoblastic lymphoma (LL). The treatment of LM involves multiple (2-3) intrathecal injections of cytarabine or methotrexate, which increases the burden on patients, carers and medical providers. As very few long-term survivors have been reported in any patient series, the major goals of therapy are relief from neurological symptoms, prevention of neurological progression, and preservation of quality of life. DepoCyte® is a sustained-release formulation of cytarabine for intrathecal injection, which maintains therapeutic concentrations in the cerebrospinal fluid (CSF) for 2 weeks. Treatment with DepoCyte® does not require an Ommaya reservoir, and offers the advantage of less frequent injections and potentially greater efficacy than conventional treatment. Methods. We report here on a series of 23 patients (median age 43 years, range 21-74; 13 male) with LM (9 relapsed) from 17 Spanish hospitals who were treated with intrathecal DepoCyte® (mean 3.5 injections, range 1-9) from March 2004 to December 2005. Concurrent dexamethasone was given as prophylaxis for arachnoiditis. Results. Cytological responses (clearance of lymphoma cells from the CSF) were seen in 8 of 12 patients with DLBCL, 3 of 3 with BL, 1 of 1 with LL; 1 of 2 with mucosa-associating lymphoid tissue (MALT) lymphoma; 2 of 2 with follicular lymphoma (FL); 1 of 1 with central nervous system lymphoma (PCNSL); and 1 of 2 with T-cell non-Hodgkin’s lymphoma (NHL). Neurological responses were seen in 8 of 12 patients with DLBCL (6 complete remissions [CR], 2 stable disease [SD]); 2 of 3 with BL (1 CR, 1 partial remission [PR]); 1 of 1 with LL (PR); 1 of 2 with MALT lymphoma (CR); 2 of 2 with FL (2 CR); 1 of 1 with PCNSL (SD); and 2 of 2 with T-cell NHL (1 PR, 1 SD). The overall response rate was 74% for both cytological response and neurological response (45.5% CR, 13% PR, 17.5% SD). Neurological progression occurred in 53% of patients, including 9 of 12 patients with DLBCL, 2 of 3 with BL, 1 of 1 with LL after 28-90 months (2 alive); 1 with LL after 30 months (died); 2 of 2 with MALT NHL after 8 and 95 months (died); 2 of 2 with FL after 150 and 300 months (both alive); 1 of 1 with PCNSL (died); and 1 of 2 with T-cell NHL after 20 months (alive); 48% of patients remained alive at the last report. DepoCyte® showed good tolerability. Fifty-two per cent (12/23) of patients experienced no side effects, and the most common side effects associated with DepoCyte® injection were headache (n = 8), vomiting (n = 4) and nausea (n = 5), with one occurrence each of fever and neurological deficits. Conclusions. This series demonstrates the feasibility, efficacy, safety and tolerability of DepoCyte® in the treatment of LM involving different histological subtypes of NHL. A substantial number of patients had a cytological response (74%) or a neurological response (74%), and 48% of patients were still alive at last report. Two-weekly (or monthly) DepoCyte® injections are much more convenient than the conventional alternatives. We consider that DepoCyte® may be the agent of choice for LM.

0697
Lack of Humoral Response to Acute EBV Infection May Identify Patients with Fulminant EBV-Associated NK/T-Cell Lymphoproliferative Disorder
C.L. Tan,1 L.P. Koh,2 B.C. Koh,3 S.M. Loh,4 T.H. Chuah,5 Y.T. Goh6
Singapore General Hospital, Singapore

Background. EBV-associated NK/T-cell lymphoproliferative disorder is a rare and distinct clinical entity. In most instances, it is refractory to conventional treatment and confers a poor prognosis. We report the clinicopathologic features of 7 patients with EBV-associated NK/T-cell lymphoproliferative disorder treated between 2002 and 2005 in a single institution. Aims. We compare the presenting features and treatment outcomes of these patients. In doing so, we hope to identify trends that may help optimize the diagnosis and management of this rare and fatal disease. Methods. The investigation is a retrospective study. Patients with the diagnosis of interest were identified from our lymphoma registry and clinical information of the cases obtained from pathological reports and clinicians. Results. All patients were of Asian origins (6 Chinese and 1 Malay). Other than a patient aged 66 years old with a history of recent transplant and was on immunosupression, the other 6 patients were aged between 32 to 40 years with no significant past medical history. All patients had a preceding history of acute upper respiratory tract infection prior to their dramatic presentation. They demonstrated the haemophagocytic syndrome with severe systemic symptoms including high fever, acute pancytopenia, mixed cholestatic/hepatitic transaminis and coagulopathy. Five patients had maculopapular rashes. Other than the finding of mild hepatoesplenomegaly, no bulky diseases or significant lymphadenopathy were found on CT scans of all patients. LDH and β2 microglobulin were invariably elevated. Diagnoses of EBV associated lymphoproliferative disorder were made based on detection of in-situ hybridization for EBV-encoded early small RNA (EBER) on bone marrow trephine, skin and liver biopsies. Three patients demonstrated the classic NK/T-cell lymphoma, nasal type phenotype (CD3-, CD4-, CD8-, CD56+), with clinical manifestation of aggressive NK-cell leukaemia at the outset, with no prior history of nasal disease. Four had peripheral T cell lymphoma phenotype with one showing TCR t-rearrangement. PCR for EBV DNA performed in 3 patients showed high viral loads. Despite features of acute EBV infection, EBV capsid Ag IgM was surprising negative in all patients, while IgG was positive in all. Median survival was 55 days from time of presentation and the causes of mortality included liver failure, neutropenic sepsis and severe bleeding. Four patients received chemotherapy. Two had CHOP regime upfront and two had immunosuppression with etoposide, prednisolone and cyclosporin prior to full dose chemotherapy. Although mortality was uniform, those who received immunosuppression first had a longer survival. Summary/Conclusions. This disorder should always be suspected in EBV-positive patients with haemophagocytic syndrome. Its epidemiological predisposition may be accounted for by a higher prevalence of EBV infection and carrier status in the Asian population. Important pitfalls in diagnosis is the lack of serological evidence of acute EBV infection. This lack of humoral response may be the predisposing factor for clonal T-cells proliferation post-EBV infection. Reasons for this immune defect in a predominantly young and seemingly well adult population remains elusive. Immunosuppression may have a role in controlling the cytokine storm before chemotherapy is started.

0698
Dose-Dense CHOP (CHOP-14) Plus Rituximab for Newly Diagnosed Aggressive B Cell Lymphoma: A Prospective Multicentric Study. Grupo para el Estudio de Hemopatias Malignas-Galicia
E. Lavilla1, M. Perez Encinas,2 E. Romero,3 A. Simiele,4 E. Gomez Torreiro,5 M.J. Plaza,6 J. Arias7
1Hospital Xeral, LUGO, Spain; 2Hospital Clinico Universitario, SANTIAGO DE COMPOSTELA, Spain; 3H. Arxitecute Marde, FERROL, Spain; 4C. Povea, VIGO, Spain; 5H. Comarcal, MONFORTE, Spain

Background. Standard front line therapy for aggressive B cell lymphoma is CHOP every 21 days associated with rituximab; CHOP every 14 days is CHOP-14. CHOP-14 has also shown an improvement in response and survival. Methods. We report the results of a randomized phase III trial of 90 pts and clinicians. Results. All patients were of Asian origins (6 Chinese and 1 Malay). Other than a patient aged 66 years old with a history of recent transplant and was on immunosupression, the other 6 patients were aged between 32 to 40 years with no significant past medical history. All patients had a preceding history of acute upper respiratory tract infection prior to their dramatic presentation. They demonstrated the haemophagocytic syndrome with severe systemic symptoms including high fever, acute pancytopenia, mixed cholestatic/hepatitic transaminis and coagulopathy. Five patients had maculopapular rashes. Other than the finding of mild hepatoesplenomegaly, no bulky diseases or significant lymphadenopathy were found on CT scans of all patients. LDH and β2 microglobulin were invariably elevated. Diagnoses of EBV associated lymphoproliferative disorder were made based on detection of in-situ hybridization for EBV-encoded early small RNA (EBER) on bone marrow trephine, skin and liver biopsies. Three patients demonstrated the classic NK/T-cell lymphoma, nasal type phenotype (CD3-, CD4-, CD8-, CD56+), with clinical manifestation of aggressive NK-cell leukaemia at the outset, with no prior history of nasal disease. Four had peripheral T cell lymphoma phenotype with one showing TCR t-rearrangement. PCR for EBV DNA performed in 3 patients showed high viral loads. Despite features of acute EBV infection, EBV capsid Ag IgM was surprising negative in all patients, while IgG was positive in all. Median survival was 55 days from time of presentation and the causes of mortality included liver failure, neutropenic sepsis and severe bleeding. Four patients received chemotherapy. Two had CHOP regime upfront and two had immunosuppression with etoposide, prednisolone and cyclosporin prior to full dose chemotherapy. Although mortality was uniform, those who received immunosuppression first had a longer survival. Summary/Conclusions. This disorder should always be suspected in EBV-positive patients with haemophagocytic syndrome. Its epidemiological predisposition may be accounted for by a higher prevalence of EBV infection and carrier status in the Asian population. Important pitfalls in diagnosis is the lack of serological evidence of acute EBV infection. This lack of humoral response may be the predisposing factor for clonal T-cells proliferation post-EBV infection. Reasons for this immune defect in a predominantly young and seemingly well adult population remains elusive. Immunosuppression may have a role in controlling the cytokine storm before chemotherapy is started.
RITUXIMAB AND ESHAP PLUS G-CSF AS AN EFFECTIVE PERIPHERAL BLOOD PROGENITOR CELL MOBILIZATION REGIMEN IN PRETREATED B-CELL NON-HODGKIN'S LYMPHOMA: A PRELIMINARY REPORT OF COMPARISON WITH ESHAP PLUS G-CSF

C. Suh, S. Kim, Y.H. Cho
Asan Medical Center, SEOUL, South-Korea

Background. The ESHAP is reported as excellent mobilization chemotherapy in patients with relapsed and poor-risk aggressive non-Hodgkin's lymphoma (NHL). Rituimub was added to ESHAP (R-ESHAP) has been tried as salvage therapy for relapsed and poor-risk B-cell NHL. Mobilizing stem cells following R-ESHAP should decrease time to autologous stem cell transplantation (ASCT) by making separate mobilizing chemotherapy unnecessary, while controlling a patient's lymphoma. Aims. The aim of this study was to prospectively evaluate the efficacy of mobilization by R-ESHAP plus G-CSF regimen in relapsed or poor-risk B-cell NHL. Methods. Twenty B-cell NHL patients were enrolled. R-ESHAP plus G-CSF (Neutrogin®, Choongwae Pharma Corp., Seoul, Korea) was used to mobilize peripheral blood progenitor cells. The results were compared with those of 24 patients with NHL whose mobilizing chemotherapy was ESHAP. Results. The R-ESHAP and ESHAP groups were well balanced for age, sex distribution, prior chemotherapy cycles, number of chemotherapy regimens, and radiotherapy to the axial skeleton. Total duration of G-CSF administration was not different between the two groups. The median number of total CD34+ cells harvested per patient was 10.59×10^6/kg (range, 4.88-52.55×10^6/kg) in the R-ESHAP group and 15.34×10^6/kg (range, 0.04-48.0×10^6/kg) in the ESHAP group (p=0.42). The median number of CD34+ cells collected per apheresis was 4.50×10^6/kg (range, 0.30-21.60×10^6/kg) in the R-ESHAP group and 4.40×10^6/kg (range, 0.01-26.50×10^6/kg) in the ESHAP group (p=0.71). Adequate collection (total harvested CD34+ cells > 2×10^6/kg) was achieved in all 20 patients from R-ESHAP group and 22 of 24 (92%) patients from ESHAP group (p=0.19). Optimal collection (total harvested CD34+ cells > 5×10^6/kg) was attained in 85% (19/22) of patients in the R-ESHAP group and 92% (22/24) of patients in the ESHAP group (p=0.67). Kaplan-Meier product limit estimate and log-rank test revealed that the apheresis days to adequate and optimal CD34+ cell collection were not statistically different between the two groups. Thirteen patients in the R-ESHAP group and 19 patients in the ESHAP group underwent ASCT and there were no differences in days to neutrophil engraftment and platelet engraftment. Summary/Conclusions. These preliminary results indicate that R-ESHAP plus Neutrogin® is an excellent mobilization regimen in patients with relapsed and poor-risk B-cell NHL.

0700

THE OCCURRENCE OF CNS RELAPSES IN HIGH-RISK AGGRESSIVE LYMPHOMA PATIENTS TREATED WITH INTENSIFIED INDUCTION AND HIGH-DOSE CONSOLIDATION PROTOCOLS OF THE CZECzech LYMPHOMA STUDY GROUP

Masaryk University Hospital, BRNO, Czech Republic; First dept. of internal medicine, VFN, PRAHA, Czech Republic; Faculty Hospital, HRADEC KRALOVE, Czech Republic; Hematology Hospital Kralovsky Vinohrady, PRAHA, Czech Republic; Faculty Hospital Motel, PRAHA, Czech Republic; Hospital CESKE BUDJOVICE, Czech Republic; CLSG Data Center, PRAHA, Czech Republic

Background. CNS relapse of systemic NHL is usually fatal and identification of risk group and effective prophylaxis are controversial issues. Aims. to analyse our cohort of high risk NHL patients in terms of CNS relapses and identify risk factors for CNS relapse. Patients and Methods. We analysed a cohort of 135 patients younger than 65 years with high-risk (age adjusted IPI 2,3) aggressive lymphomas (73% DLBCL, 15% mediastinal DLBCL, 5% peripheral T-cell, 2% mantle cell, 1% anaplastic large cell, 4% others with no Burkitt and no lymphoblastic lymphoma). Patients were prospectively treated with intensified induction and high-dose consolidation protocols designed by CLSG in the period 1998 - 2004. Treatment protocols. Protocol 1: induction 3-4 courses of high-dose CHOP (cyclophosphamide 5 g/m2, doxorubicin 75 mg/m2, vincristine 1 mg, predison 300 mg/m2 + G-CSF every three weeks (21 pts). Protocol 2: 3 courses of standard CHOP-21 followed by 3 courses of ESHAP (26 pts). Protocol 3: 3 courses of high-dose CHOP + 3 courses of ESHAP (29 pts). Protocol 4: same as protocol 3 with addition of rituximab to each cycle of chemo (59 pts). PBPCs were mobilized after 2nd or 3rd high-dose CHOP in protocol 1 and after 1st or 2nd ESHAP in protocol 2,3,4. Patients in complete or partial remission after all types of induction treatment were consolidated with BEAM and ASCT. If radiotherapy was administered to initial bulk or to residual mass after chemo. CNS involvement at diagnosis was an exclusion criterion. Intrathecal CNS prophylaxis consisted of 15 mg methotrexate + 40 mg ara-C and it was recommended but not mandatory part of the protocols. Intrathecal prophylaxis received 50% of patients with median number of 3 cycles for patient (range 1-8). Results. The median age of the whole cohort was 46 years, male/female ratio 76/59. 58% had IPI 2 and 42% IPI 3. We observed 9 CNS relapses (7%), 7 on therapy, one 1 month after completion of the therapy and one late relapse following 15 months after diagnosis. Original histologies in CNS relapsing patients were DLBCL 6x, 1x mediastinal DLBCL, 1x PTL, 1x FL. 5 of these patients received CNS prophylaxis (all 5 pts received i.t. MTX 15 mg+ara-C 40 mg, median 5 cycles). Median time from study entry to CNS recurrence was 5 month (range 2-15). Median survival after diagnosis of CNS relapse was 12 months (0.1-16). Non-CNS relapses were 9 due to progression, one after 16 month in next CR due to pneumonia. Evaluated risk factors for CNS progression were IPI, clinical stage, B symptoms, performance status, LDH level, intrathecal prophylaxis and type of treatment protocol. None of these risk factors were significantly predictive for CNS relapse. Summary/Conclusions. CNS progression/relapse in this cohort of high risk lymphoma patients is relatively low, but outcome of all patients is fatal. Our patients did not benefit from intracranial prophylaxis. More precise detection of patient at risk for CNS involvement and detection of occult disease at diagnosis are needed to differentiate the treatment protocols with appropriate CNS prophylaxis for these patients.

0701

BURKITT AND NON-BURKITT TYPES OF CHILDHOOD B-CELL LYMPHOMAS ( B- NHL) - COMPARISON OF TREATMENT RESULTS. A REPORT OF POLISH PAEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP (PPLSSG)

Wroclaw Medical University,Poland; WROCŁAW, Poland; Warsaw Medical University, WARSAW, Poland; Zakrzew Medical University, ZABRZE, Poland; Cracow Medical University, CRACOW, Poland; GDANSK, Poland; Bialystok Medical University, BIALYSTOK, Poland; Lublin Medical University, LUBLIN, Poland; Poznan Medical University, POZNAN, Poland
The efficacy of the LMB-89 protocol for children with NHL-B has been investigated. The patients (pts) were treated in 10 onc hematological centers of PFLLSG between 1993 and 2006. A total number of 149 children with NHL-B were included into analysis: 105 (70%) of them with of B-NHL Burkitt (I gr.) and 44 (30%) with B-NHL non t Burkitt (II gr.) histopathological types (17 - Burkitt-like, 6 Large B-cell, 4 immunoblastic, 11 polymorphic). Median observation time was 56 months. Methods. The diagnosis was based on histomorphological investigation and supplemented with immunophenotyping. The S. Murphy staging system was used for prognostic stratification. Treatment intensity was adapted to 3 risk groups (A, B, C), according to LMB-89 protocol. Results. In both groups majority of children presented on admission advanced stage II and IV disease. 95% and 15% for I gr. and 45% and 13% for II gr., respectively). Eighty-two (82%) pts were classified to B risk group. Complete remission (CR) was achieved in 94 (90%) pts with Burkitt and 42 (96%) pts with non-Burkitt types: 16 (94%) - Burkitt-like, 6 (100%) - Large B-cell, 6 (100%) 1 centroblastic, 5 (75%) immunoblastic, 11 (100%) 1 others. There were 13 (9%) non-responders: 11 in I gr. and 2 in II gr. 8 early deaths were observed: 7 in I gr. (4 advanced tumour in diagnosis, 1 acute renal failure+peritonitis, 1 St.aureus sepsis+varicella, 1 multiorgan failure+ myelosuppression) and 1 in II gr. (advanced tumour in diagnosis). 11 relapses were observed: 7 in I gr and 4 in II gr. (1 Burkitt like, 1 centroblastic, 2 others). 8 pts died after RC: 5 in I gr. due to disease relapse, 3 in II gr. (2 relapse, 1 toxicity related death (lungs failure)). Probability of EFS was 0.86 for all pts (in previous study 0.75). The EFS of I + II, III and IV stages were: 0.93, 0.87 and 0.71 respectively (p=0.02). The EFS for B-NHL Burkitt was 0.83 (in previous study 0.81) and for non-Burkitt 0.87. Conclusions. The treatment with children with B-NHL, especially with Burkitt type has improved in comparison to previously reported observations. Higher EFS and overall survival of B-NHL, including Burkitt type, could be achieved thanks to quick diagnosis after first tumour clinical symptoms and an improvement of intensive supportive care (adequate blood product substitution, regular infection supervision, prophylaxis, amelioration of MTX therapy monitoring) for therapy toxicity elimination (compared with previous study). The worst results are observed in children with bone marrow involvement and in those with large tumor burden at diagnosis in two examined pts groups.

0702

HAEMATOLOGICAL AND EXTRHAEMATOLOGICAL MALIGNANCIES AFTER AUTOLOGOUS TRANSPLANTATION FOR LYMPHOPROLIFERATIVE DISEASES


Istituto di ematologia UCSC-Roma, ROMA, ITALY

Background. Secondary myelodysplastic syndrome (sMDS), acute myeloid leukemia (aAML) and severe aplastic anemia (SAA) are severe complications of high-dose chemotherapy followed by autologous bone marrow (BMT) or peripheral blood stem cell transplantation (PBSCT) for lymphoproliferative diseases (LPD). These complications are associated with very poor prognosis. Aims. We evaluated the incidence of secondary malignancies after autologous PBSCT for non-Hodgkin's lymphoma (NHL), Hodgkin's disease (HD), multiple myeloma (MM) or chronic lymphocytic leukemia (CLL). Methods. We studied 142 patients (pts) affected by LPD (79 NHL, 31 MM, 26 HD, 6 CLL) undergone from 1988 to 2004 to autologous transplant. At PBSCT all patients showed absence of karyotypic abnormalities. We considered a minimum of 12 months of follow up after PBSCT without receiving chemo-radiotherapy in order to assess sMDS without interference of conditioning regimen. The evaluation of sAML and secondary severe aplasia was done starting from transplant without any delay. The patients withdrew from the study for progression disease (PD) requiring treatment or patient's death. The evaluation, including morphological studies of bone marrow aspiration and peripheral blood's smears, cytogenetic and FISH analysis, was performed after 12 months from PBSCT and at least every 12 months during the follow up. Eighty-one pts were male, 61 were female with median age of 44 years (range 17-63). At PBSCT 69 pts were in complete remission (CR), 64 pts in partial remission (PR) and 9 pts in PD. Before transplant, after front-line therapy, 53 pts underwent second line chemotherapy, 26 third line or more, 24 pts second line plus radiotherapy, 7 pts second line plus rituximab, 7 pts bone marrow conditioning with radiotherapy and 17 pts second line plus first PBSCT. Results. Median follow up was 48 months from PBSCT (range 12-182) for a total of 376 morphologic evaluation, 256 cytogenetic analyses and 46 FISH analysis. We observed one case of sAML with deletion of chromosome 7 occurred 10 months after PBSCT in pt affected by heavily pre-treated NHL (5 lines chemotherapy); he died 2 months later in progression disease and two cases of MDS. The first patient with CLL developed sMDS (refractory cytopenia with multilineage dysplasia) with del (20) detected by FISH and cytogenetic analysis 15 months after PBSCT, he is alive 28 months after sMDS without therapy. The second patient affected by heavily pre-treated LN (Second line chemotherapy plus rituximab) developed sAML-II with detected by FISH 16 months after PBSCT. Three pts developed secondary solid malignancies (lung, pancreas, colon) at median time of 32 months (range23-126). Summary/Conclusion. sMDS/sAML are very late complications after allogeneic transplant, while recent studies observed an increased incidence after autologous PBSCT for LPD. This evidence suggests that their development is resulted from accumulating multiple factors in the recipient cells caused by prior exposure to subablative chemotherapy. A stringent and complete follow-up is requested in order to rapidly detect these complications offering to the pts a chance of treatment.

0703

THE COMPARISON OF PERIPHERAL T-CELL LYMPHOMA WITH DIFFUSE LARGE B-CELL LYMPHOMA WHEN TREATED WITH SAME FIRST LINE CHOP CHEMOTHERAPY


‘Ulsan University Hospital, ULSAN, South-Korea; ‘Asan Medical Center, SEOUL, South-Korea; ‘Pusan Bank Hospital, PUSAN, South-Korea

Background. The prognosis of peripheral T-cell lymphoma, unspecified (PTCLU) is usually regarded poor when compared with that of diffuse large b-cell lymphoma (DLBL). Previously published papers, however, gave no consistent data regarding the differences between two lymphomas and enrolled many various cell types as well as different treatments resulting in obscure observation for a certain lymphoma type. Aims. We wanted to compare the clinical features and prognosis of T-cell lymphoma with those of aggressive b-cell lymphoma, especially between PTCLU and DLBL when same first-line chemotherapy was applied. Methods. Patients with PTCLU and DLBL were selected when their first-line chemotherapies were CHOP. Clinical data were collected through retrospective review of medical records. Progression-free survival (PFS) and overall survival (OS) were calculated from the first day of CHOP chemotherapy.

Figure 1. PFS and OS.
an, both not reached; \( p=0.019 \); Figure 1B). Summary/Conclusions. PTCLu showed more frequent relapse rate and poor PFS after CHOP chemotherapy, in spite of similar response rate to first-line CHOP chemotherapy compared with DLBCL. Responses and relapse rate to second-line chemotherapy were similar and OS was not different in both groups.

0704
RITUXIMAB-CHOP AND RADIOTHERAPY FOR PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: AN UPDATE
T.P. Vassilakopoulos,1 M.K. Angelopoulou,1 Z. Galani,1 S. Sachanas,1 A. Katsigiannis,1 E. Vrakidou,1 N. Constantinnou,1 M.N. Dimopoulou,1 S.I. Kliakis,1 E. Michali,1 E.M. Dimitriou,1 M.P. Siakantaris,1 P. Panayiotidis,1 P. Roussou,1 G.A. Pangalis1
1University of Athens, ATHENS, Greece; 2Hygeia Hospital, ATHENS, Greece; 3Theagton Hospital, THESSALONIKI, Greece

Background. MACOP-B or even chemotherapy (CT) with consolidation high dose therapy with autologous stem cell support (HDT-ASCT), have been considered superior to CHOP in PMBCL. However, in the absence of randomized trials, there is no established optimal treatment for these patients. Recent data have shown that R-CHOP is superior to CHOP in patients with diffuse LBC, so that it is rapidly becoming the new standard of care for this subtype of aggressive lymphoma. 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 R-CHOP in the particular case of PMBCL, which usually affects young patients, is not well established yet. Aims. The evaluation of the efficacy of R-CHOP±RT in PMBCL and the comparison of this approach with CHOP±RT, administered to historical controls. Patients and Methods. Between 1994 and 2005, 62 patients with PMBCL were treated in 4 participating centers. R-CHOP displaced CHOP in the treatment of PMBCL at a given timepoint in each center. Thus 23 consecutive patients who received R-CHOP, were compared to 39 consecutive historical controls, who had been treated with CHOP prior to that point. Results. The median age of the patients was 32 years (17-65) and 42/62 (68%) were females. Age-adjusted IPI was 22 in 39% and 46% of patients who received R-CHOP and CHOP respectively (\( p=0.61 \)). All individual IPI parameters as well as B-symptoms were also balanced between the two groups. The complete response (CR) rate was 100% for R-CHOP±RT vs 64% for CHOP±RT (\( p=0.001 \)). All relapses after CHOP occurred within 22 months from diagnosis. No relapse has been recorded after R-CHOP, while a single patient with CR but persistent PET abnormality underwent stem cell transplantation and was considered as failure. The 3-year failure free survival (FFS) was 95±4% vs 51±3% for patients who received R-CHOP±RT vs CHOP±RT (\( p=0.001 \)). Within the subgroup of patients with LLI risk IPI the corresponding 3-year FFS rates were 100% vs 57±11% (\( p=0.008 \)), while they were 39±10% vs 44±12% (\( p=0.04 \)) among patients with H/H risk IPI. The 3-year event free survival (EFS) for all patients was 91±6% vs 51±8% (\( p=0.005 \)). The 4-year overall survival was 96±4% vs 66±3% (\( p=0.02 \)), while the 4-year lymphoma specific survival was 100% vs 66±8% (\( p=0.008 \)). Conclusions. R-CHOP and RT provided impressive results with only one failure and lymphoma-related deaths among 23 patients. In comparison to CHOP-treated historical controls, highly significant differences in favor of R-CHOP were recorded in terms of CR, FFS, EFS, and LSS rates. Overall survival was also improved. Based on these results we continue to treat PMBCL patients with R-CHOP and RT. The need for more aggressive strategies, such as MACOP-B or ASCT, is therefore questionable. Whether RT is needed after R-CHOP, especially when post-chemotherapy PET-scan is available, should be investigated.

0705
TREATMENT STRATIFICATION ACCORDING TO EARLY RESPONSE TO MEGA-CHOP, BASED ON CT AND GALIUM 67 SCAN WITH OR WITHOUT IFE SALVAGE THERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENT WITH POOR PROGNOSIS AGGRESSIVE LYMPHOMA
E. Conde,1 R. Arranz,1 J. Rodriguez,1 C. Grande,1 M. Gandarillas,1 J.L. Bello,1 C. Albo,1 M.L. Gutierrez,1 S. Garzón,1 M.T. Hernández,1 J.A. García Vela,1 P. Fernández,1 M.L. Amigo,1 D. Caballero1
1Hospital Valdecilla, SANTANDER, Spain; 2-Hematología, Hospital Valdecilla, SANTANDER, Spain; 3Hospital La princesa, MADRID, Spain; 4Hospital Sou Dureta, PALMA DE MALLORCA, Spain; 5Hospital 12 de Octubre, MADRID, Spain; 6Hospital Xeral, SANTIAGO DE COMPOSTELA, Spain; 7Hospital Xeral, VIGO, Spain; 8Hospital Sierrallana, TORRELAVEGA, Spain; 9Hospital de Jerez de la Frontera, JEREZ DE LA FRONTERA, Spain; 10Hospital Universitario, TENERIFE, Spain; 11Hospital de Getafe, GETAFE, Spain; 12Hospital General, ALICANTE, Spain; 13Hospital San Pedro de Alcántara, CACERES, Spain

Background. Patients with IPI > 2, large cell lymphoma have a poor outcome with long term survival lower than 50%. Evaluation of response only with CT scan shows often residual masses which can be tumoral or fibrotic. Gallium 67S discriminate better these two situations and therefore can be helpful to decide further strategies. Aims. To assess the efficacy of PBSCT patients with poor prognosis aggressive NHL according to previous early response to Mega-CHOP evaluated with CT & Ga67S. Patients and Methods. Inclusion criteria were: G67S positive large cell B cell lymphoma with IPI score > 2 or IPI > 2 with high b2 microglobulin or peripheral T cell lymphoma (PTCL), except ALK+ anaplastic lymphoma regardless of IPI. Patients were evaluated after 3 cycles of Mega-CHOP. Those in CR (CT scan, Ga67S negative) or CR (CT scan positive, Ga67S negative) received a 4rd Mega-CHOP followed by BEAM and PBSCT. Those with positive Ga67S received IFE or ESHAP (x2) regimen followed by BEAM and PBSCT. Patients with refractory disease (RD) were dropped from the study. Since 2001, 112 patients have been registered and 87 have finished the treatment. Median age was 52 years (20-67 years) and 49% were males. Seventy one (72%) had a DLBL, 8% a grade 3 FL and, 24 (21%) PTCL. Sixty two (88%) had IPI > 2, and 12% IPI 1. Doses were for Mega-CHOP: Cyt 1.5 g/m2 and 40 mg/m2 and VCR 2 mg on day 1 and Pred 60 mg/m2 on days 1-5 on a 21 day treatment schedule and for IFE: fosfamide 10 g/m2 and VP16 900 mg/m2 (days 1-3) with Mesna. Results. After 3 Mega-CHOP, 47 patients (42%) were considered on CR or uCR due to a negative Ga67S, 46(41%) were on PR and 18 (16%) were refractory. One patients were early deaths. After IFE 18(16%) achieved CR, 19 (41%) PR and 9 (20%) progressed. Overall, 87 patients received PBSCT and are valuable for response. Thirty one patients (28%) died, 23 (21%) due to lymphoma and 5 (7%) due to toxicity. With 36 months of median follow-up (8 to 69 months), 81 patients are alive, 67 (60%), disease free. Five-year overall survival according to clinical response and Ga67S the 5 initial Mega-CHOP were 70% (CR, PR and Ga67S neg.), 67% (CR, PR Ga67S pos.) and 37% (failure Ga67S pos.), respectively (\( p=0.0004 \)). In the univariate analysis, the significant variables associated with outcome were clinical response combined with positive/negative Ga67S after the initial therapy (\( p=0.0004 \)) and disease status at ASCT (\( p=0.01 \)). Conclusion. Our preliminary results suggest that early salvage therapy can overcome the poor outcome of patients with bad prognosis aggressive lymphoma. Moreover, this early evaluation could identify patients with poor prognosis who only need a short treatment (4-Mega-CHOP+PBSCT).
**0706**

**PRELIMINARY RESULTS FROM A PHASE II STUDY OF LENALIDOMIDE MONOTHERAPY IN RELAPSED/REFRACTORY AGGRESSIVE NON-HODGKIN Lymphoma**

H. Wiernik,1 I.S. Lossos,2 G. Justice,1 J.M. Tuscano,1 J.B. Zeldis,1 K. Takeshita,1 D. Petronigoro,1 T. Habermann3 1New York Medical College, BRONX, NY, United States of America; 2University of Miami, MIAMI, FL, United States of America; 3Pacific Coast Hematology/Oncology, FOUNTAIN VALLEY, CA, United States of America; 4University of California, SACRAMENTO, CA, United States of America; 5Celgene Corporation, SUMMIT, NJ, United States of America; 6Mayo Clinic College of Medicine, ROCHESTER, MN, United States of America

**Background.** Lenalidomide (Revlimid®) is an immunomodulatory drug of the IMiD class, recently approved in the US for myelodysplastic syndrome associated with a deletion 5q[31] cytogenetic abnormality that also has activity in multiple myeloma, chronic lymphocytic leukemia and cutaneous T-cell lymphoma. Thalidomide, a less potent IMiD, has activity in non-Hodgkin’s lymphoma as both monotherapy and in combination with rituximab. Aim: To assess the safety and efficacy of lenalidomide as first-line treatment in patients with aggressive NHL.

**Methods.** Subjects with relapsed/refractory aggressive NHL following > 1 prior treatment regimen with measurable disease are eligible. Subjects receive 25 mg lenalidomide orally once daily on Days 1-21 every 28 days and continue therapy for 52 weeks as tolerated or until disease progression. Response and progression are evaluated using the IWLLC methodology. Results. 19 subjects of a planned 40 were enrolled of which eight subjects are currently evaluable for tumor response and safety. The median age of the evaluable subjects is 66 (45-80) and 5 are female. Histology is diffuse large cell lymphoma (n=7) and follicular center lymphoma grade 3 (n=1). Median time from diagnosis to lenalidomide monotherapy is 2.3 (1-6) years and median number of prior treatment regimens per subject is 3 (1-6). Median duration of follow-up is 3.5 (1-5) months. Three of the eight subjects exhibited a PR with decreases in their tumor burden of 95%, 79% and 52%. Two subjects had stable disease and three, disease progression. Grade 3 or 4 hematological adverse events (neutropenia, thrombocytopenia, anemia) occurred in five subjects including one febrile neutropenia and one of the five also exhibited Grade 3 sub-acute autoimmune hemolysis and Grade 4 general malaise. Conclusion. Preliminary data for lenalidomide monotherapy in relapsed/refractory aggressive NHL are encouraging.

**0707**

**RITUXIMAB SIGNIFICANTLY IMPROVES THE OUTCOME OF YOUNG POOR RISK PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA - ON BEHALF OF CZECH LYMPHOMA STUDY GROUP**

M. Trneny,1 D. Belada,1 J. Vasova,1 R. Pytlík,1 T. Kozák,1 A. Sykorova,1 K. Kubackova,1 J. Fírnos,1 I. Bolomska,1 M. Hamouzova1 1Charles Univ Gen Hospital, PRAHA, Czech Republic; 2Univ Hospital, HRADEC KRALOVE, Czech Republic; 3Charles Univ Gen Hospital, PRAHA, Czech Republic; 4Hospital, GESKE BUDJOVICE, Czech Republic; 5Masaryk Mem. Hosp, USTNI LABEM, Czech Republic, 6Charles Univ Hosp, PRAHA, Czech Republic

**Background.** There is a robust evidence of significant patients outcome improvement by adding rituximab (R) to chemotherapy (CHT) in patients (pts) with DLBCL who are older (Coiffier, 2002) or younger than 60y registered in CLSG registry since Jan 1999 till Dec 2006. An analysis that was carried out to evaluate the efficacy of R-CHT vs CHT in younger DLBCL patients who are upstaged 27/105 pts (26%) and for 17% of pts the upstaging modified primary treatment (0 → 1, 1 → 2, 2 → 3). The age of patients in the CHT group was 61% vs 64% in the R-CHT group. The only difference between groups was in the number of pts exposed to HDT with ASCT (38.5% vs 60%, p=0.01). The median follow up in CHT group was 4.6 years vs 2.4 in R-CHT group. The 3 years probability of overall survival - OS - and event free survival EFS (time from dg to progression/relapse or death, whatever occurred earlier, in all pts) were considered as primary endpoints. Epiinfo and GraphPad programs were used for analysis (ANOVA, Wilcoxon test and log rank tests were used). Results. The probability of EFS and OS in the whole group was 52% and 61% resp. The probability of EFS in CHT vs R-CHT was 40.1% vs 71.4% (p<0.0001) and of OS 31.9% vs 67.7% (p<0.0001). The comparison of subgroup of pts who all were treated with HDT as part of the induction according to R administration (CHT vs R-CHT) reveals the significant differences for EFS: 55.5% vs 88.3% (p=0.005) as well as for OS: 61.4% vs 91.4% (p=0.01). There were also significant difference in EFS between CHT and R-CHT in the subset of pts who had primary HDT were analyzed: EFS: 32.9% vs 50.9% (p=0.02) and OS: 45.0% vs 67.7% (p<0.01). There was no found no difference between intermediate-high and high subgroups. Conclusion. This retrospective analysis suggests: Young pts with DLBCL and poor risk IPI have significantly better outcome if they are treated with rituximab containing chemotherapy. Moreover the R-CHT significantly improves the outcome of patients who are designated to HDT with ASCT in comparison of pts who are treated with CHT without R followed by HDT with ASCT. Supported by grant IGA MZ CR: NR 8231-3

**0708**

**WHAT IS THE SIGNIFICANCE OF FDG-PET/CT SCAN AT DIAGNOSIS OF NON HODGKIN LYMPHOMAS?**

R. Sancetta1, M. Greggiani,2 E. Dei Rossi,3 E. Cracco,2 P. Pregno,3 U. Vitolo,1 L. Ragucci,1 E. Merli,1 T. Chiessi2 1Ospedale Civile Umberto I, VENEZIA-MESTRE, Italy; 2Ospedale Civile Umberto I, VENEZIA-MESTRE, Italy; 3Az. Ospedaliero S. Giovanni Battista, MOLINETTE - TORINO, Italy; 4DAG - Università di Firenze, FIRENZE, Italy; 5Arcispedale S. Maria Nuova, REGGIO EMILIA, Italy

**Background.** Correct staging is important for the appropriate treatment in lymphoma patients. Most cancers, including lymphomas, metabolize glucose at a higher rate than normal tissue so FDG-PET/CT scan is a useful tool in the evaluation of patients with lymphoma. Many authors in these last years have shown the importance of FDG-PET/CT analysis at diagnosis of lymphomas and the differences according to histologic subtypes. Aim: The III (Italian Lymphoma Intergroup) evaluated: 1) the role of FDG-PET/CT versus CT scanning in staging the non-Hodgkin lymphoma, 2) the significance of FDG-PET/CT according to histologic subtypes, 3) the ability of FDG-PET/CT in showing extranodal localizations. Methods. We have retrospectively analysed at diagnosis 105 patients (58 male, 52 female) with both FDG-PET/CT and conventional CT scanning. The histologic subtypes were diffuse large cell lymphoma (DLCL) and follicular lymphoma (FL); 37 pts (35%), marginal zone lymphoma (MZL) 7 pts (6%), mantle cell lymphoma (MCL) 4 pts (4%), Burkitt and Burkitt-like lymphoma (BL) 3 pts (3%), primary mediastinal B-cell lymphoma 2 pts (2%), other lymphomas (small lymphocytic, peripheral T-cell, extranodal) 3 pts (3%). The PET/CT evaluation (GE, Discovery, LS) were performed 60 min. after the i.v. injection of 18F-FDG (5.5 MBq/kg) with a whole-body acquisition with a field of view extending from the head to the upper part of the thighs. All patients fasted for at least 8 hours prior to FDG injection and had a glucose level < 120 mg/dL. Results. We have evaluated nodal (18) and extranodal (12) stations. Considering all cases, the agreement between FDG-PET/CT and CT scanning was 89% in nodal stations and 95% in extranodal ones, while discordance was 9% (7% toward PET/CT and 2% toward CT), and 5% (4% toward PET/CT and 1% toward CT) respectively. The percentage was similar in all the different histologic subtypes. The extranodal localizations in which there were more discordant results (20%) were spleen (7 pts), brain (1 pt) and bone (1 pt). FDG-PET/CT upstaged 27/105 pts (26%) and for 17% of pts the upstaging modified therapy (0 → III-IV in 4 pts (4%), I → III-IV in 3 pts (3%), II → III-IV in 10 pts (10%). The FDG-PET/CT downstaged only 9/105 pts (9%): II → I in 1 pt (1%), III-IV → II in 5 pts (5%), I → 0 pts (3%). Conclusions. FDG-PET/CT and CT scanning are concordant, for nodal and extranodal localizations, in staging of Non-Hodgkin lymphomas. FDG-PET/CT shows more nodal localizations (7%) and extranodal localizations (4%) than CT scanning. There isn’t a substantial difference in concordance between FDG-PET/CT and CT scanning according to the various histologic subtypes. It is important to use FDG baseline for early and late evaluation during and after therapy. FDG-PET/CT is essential for staging lymphomas also as exclusive method.
Non-Hodgkin’s Lymphoma - Clinical IV

0710
INTERLEUKIN-8 AND INTERLEUKIN-10 LEVELS IN DIFFUSE LARGE B-CELL LYMPHOMA: CORRELATION WITH INTERNATIONAL PROGNOSTIC INDEX AND OUTCOME
A. Duletic-Nacicovic,1 S. Dvornic,1 I. Host,1 J. Kalcic,1 B. Krajnovic,1 D. Petranovic,1 M. Sever-Preblic,1 T. Valkovic,1 N. Jonjic,2 K. Lucin,2 S. Stifter2
1Clinical Hospital Centre Rijeka, RijeKA, Croatia; 2Department of Pathology, RijeKA, Croatia

Background. Cytokines play important roles in the pathogenesis of lymphomas. The secretion of cytokines can provide growth advantages for tumor cells in either an autocrine or a paracrine fashion. An elevated serum level of cytokines can contribute to the clinical and histopathologic alterations associated with malignant lymphomas. The aim of this study was to determine the relations between serum levels of interleukin-8 (IL-8) and interleukin-10 (IL-10) and outcome in diffuse large B-cell lymphoma (DLBCL). Methods. Serum levels of IL-8 and IL-10 were measured using a sensitive enzyme-linked immunosorbent assay in the pretreatment frozen serum from 46 patients with diffuse large B-cell lymphoma, and from 30 healthy control subjects. The median follow-up duration was 49 months (33–68 months). Cytokine levels were correlated with clinical features and survival. Results. Serum IL-8 levels were higher in NHL patients (median undetectable; range: undetectable to 22.2 pg/mL) than in control subjects (median undetectable; range: undetectable to 0 pg/mL) (p<0.001). Serum IL-10 levels were higher in NHL patients (median 4.9 pg/mL; range: undetectable to 299.2 pg/mL) than in control (median undetectable; range: undetectable to 22.2 pg/mL) (p<0.001). In 34.8% of patients two cytokines were elevated in parallel. Patients with DLBCL were divided in high- and low-risk group according to International Prognostic Index (IP). In the high-risk group, serum levels of IL-8 (range: undetectable-126; median 20.3 pg/mL) were also higher than serum levels of IL-8 (range: undetectable-101.9; median undetectable) in the low-risk group (p<0.015). In addition, IL-10 in high-risk group were found to be higher (range: undetectable-299.2; median 30.7 pg/mL) than serum levels of IL-10 in the low-risk group (range: undetectable-58.6; median undetectable) (p<0.001). Superior response to therapy (complete remission) was achieved in patients with lower serum levels of IL-8 (p=0.011) and IL-10 (p<0.001). Patients who were effectively treated had a significant reduction in cytokine levels (p<0.05). Patients with elevation of serum IL-10 had poor median and 5-year survival (p=0.007), while IL-8 levels did not affect overall survival (OS). Using univariate analysis, overall survival in all patients was affected by presence of systemic symptoms, Ann Arbor stage, performance status score, elevated levels of beta-2 microglobulin, pretreatment serum levels of cytokines IL-8 and IL-10 and the number of cytokines increased. Conclusions. Serum assay of IL-8 and IL-10 before the treatment in patients with diffuse large B-cell lymphoma may help us to have some perception about the possible prognosis and to decide on therapeutic approaches for individual patients.

0711
ANTI-LEUKEMIC AND ANTI-ANGIOGENESIS EFFICACY OF ARSENIC TRIOXIDE IN NEW CASES OF ACUTE PROMYELOCYTIC LEUKEMIA
Z. Sanaat
Tabriz University of Medical Science, Tabriz, Iran

Arsenic trioxide is now considered the standard agent in treatment of refractory cases of acute promyeocytic leukemia (APL). This drug is also shown to have anti-angiogenesis effect against APL cells in vitro. This study evaluated clinical efficacy and anti-angiogenesis effect of arsenic trioxide in 17 new cases of APL. Arsenic trioxide was given in a dosage of 0.15 mg kg-1 and remission rate, survival rate, toxicities and effect on vascular density of bone marrow was studied. The bone marrow vascular density was examined using immunohistochemistry for von Willebrand Factor (vWF) and CD31 markers. Bone marrow vascular density was reduced as identified by anti-vWF immunohistochemical staining. Mean before treatment = 201.6 mm² ± 20.4 (SEM), mean after treatment = 109.4 ± 17.2 (SEM), p<0.001 and anti-CD31 immunostaining (mean before treatment = 199.17 mm² ± 21.5 (SEM), mean after treatment = 99.5 mm² ± 22.1 (SEM), p<0.05). Treatment efficacy results showed
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100% complete remission rate after median of 30 days and 72% survival rate after median 60 days of follow-up. Main toxicities included hyperleukocytosis, hepatic toxicity and APL differentiation syndrome. The results imply that arsenic trioxide is an effective anti-leukemia and anti-angiogenesis agent in new cases of APL.

0712 SALVAGE TREATMENT WITH HIGH-DOSE THERAPY AND PBSCT IN HIGH GRADE NHL - FROM THE GISL (GRUPPO ITALIANO STUDIO LINFOMI)

P. Maza, M.R. Specchia,* M. Dell’Olio,* L. Marcheselli, L. Annino

Our study demonstrated that nineteen patients - 17 R-IEV and 2 R-MINE - presented with a purging agent.

B. Anaclerico, 1

p=0.056. The OS of relapsed patients is 26 months median, 21 and > 84 months for CT and HDS, respectively. The 3 years survival is 38 and > 70%, for CT and HDS, respectively (p=0.029). The analysis for therapy efficacy done show a better significance of survival according to two arms of treatment (p=0.025). Conclusions. Our study demonstrated that HDS in relapsed patients with HG-NHL exerts a better outcome than CT, whether the same result could be obtained in refractory patients should be further investigated by prospective studies even if a positive trend could be already disclosed in this subset of patients.

0713 THE EFFICACY OF RITUXIMAB PLUS IFOSFAMIDE SECOND LINE APPROACH IN RELAPSED/REFRACTORY NON HODGKIN LYMPHOMA

V. Bongarzoni,* A. Chierichini,* P. Anticoli Borza,* B. Anaclerico,* M. Bartolini,* S. Fenu,* M. Cedrone,* M. Vittori,* M. Persiani,* B. Ronci,* M. Cantonetti,* M.C. Petti,* L. Annino*

Our experience, if limited to a small series of patients, shows that the addition of Rituximab to second line chemotherapy: a) increases the rate of CR; b) improve progression free survival with respect to those in partial response. CR rate after median 860 days of follow-up. Main toxicities included hyperleukocytosis, hepatic toxicity and APL differentiation syndrome. The results imply that arsenic trioxide is an effective anti-leukemia and anti-angiogenesis agent in new cases of APL.
patients rarely showed an intrasinusoidal BM infiltration pattern or a stroma component, both reported features of this type of lymphoma. As mentioned by other authors, CD5-positive SMZL cases seem to be more common that previously thought.

0715
IS DOUBLE-BALLOON ENDOSCOPY USEFUL AND NECESSARY FOR THE EVALUATION OF SMALL INTESTINE INVASION IN PATIENTS WITH NHL?
T.K. Kato
Gifu University School of Medicine, Gifu-CITY, Japan

Background and Aims. It is difficult to diagnose correctly the invasion of non-Hodgkin lymphoma (NHL) in small intestine. Recently, double-balloon endoscopy (DBE) (Fujinon Co. Ltd., Tokyo) has been produced and spread as a new and easy-to-use method of endoscopy for whole small intestine. In this study, we studied the usefulness of DBE in patients with NHL for diagnosis of invasion in small intestine. Patients and Methods. From February 2005 until January 2006, DBE was underwent in twelve patients with NHL. Six patients were systemic NHL, five patients were gastric NHL, and one patient was rectal NHL. They were seven males and five females, with an average age of 63.6 years (range: 48 to 78). The pathological findings were 7 diffuse large B cell lymphoma (DLBCL), 3 follicular cell lymphoma (FCL), 1 Maltoma, 1 mantle cell lymphoma, and 1 IPSID. DBE was basically twice done in every case both from mouth and anus on different day as possible. All patients were also underwent biopsy at DBE. Results. DBE was safely underwent in all 12 patients. Characteristic endoscopic findings of small intestine were revealed in six patients with NHL. However, in only 4 patients, biopsy specimen showed positive. In the rest two patients, there was no pathological finding of NHL, which was considered due to chemotherapy at the previous hospitals. Both of two FCL cases had endoscopic and pathological findings in the small intestine. They were diagnosed intestinal perforation because of chemotherapy at the previous hospitals. If they were noticed that their NHL was invasive in small intestine, we were able to speculate their small intestine might be perforated after chemotherapy. Only 2 gastrointestinal NHL patients had small intestinal lesion. On the other hand, 3 systemic NHL patients had also invasion in small intestine. Especially, IPSID patient was diagnosed with only DBE. Aspiration pneumonia was happened in one patient. Other severe complication was not found. Conclusions. DBE must be available for diagnosis of NHL invasion in small intestine. DBE must be selected before chemotherapy for NHL.

0716
NODAL VS. PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMAS: A COMPARISON OF PRESENTING FEATURES, RESPONSE TO TREATMENT AND OUTCOME
D. Mihou, 1 P. Konstantinidou, 1 Fr. Patakouta, 2 I. Tomaskova, 2 Z. Kral, 2 D. Salek, 2 F. Jedlicka, 2 L. Smardova, 2 J. Mayer 2
1 Theagenion Cancer Center, THESSALONIKI, Greece; 2 Masaryk University Hospital, BRNO, Czech Republic

Background. Diffuse large B-cell lymphomas (DLBCL) represent the commonest subtype of non-Hodgkin’s lymphomas (NHL) in Western countries, comprising 30% of the total. Marked heterogeneity in aspects of morphology, immunophenotype and genetics is their main characteristic. Approximately 80% of them are of primary extranodal origin. It has already been proposed that primary extranodal DLBCL could be regarded as two distinct clinical entities, since molecular differences between them suggest a different genetic origin. Aim. To assess the main clinical presenting features, response to treatment and outcome of a large number of patients with DLBCL according to the primary site of origin, nodal or extranodal. Methods. Between 1976 and 2005, 598 consecutive patients with DLBCL were treated in our department. CHOP and CHOP-like regimens were administered to a total of 353 (68.8%) patients, 60 (17%) of which received additionally radiotherapy, 74 (21%) rituximab and 35 (9.9%) both radiotherapy and rituximab. Patients were divided into 2 groups (DLBCL1, 2 follicular cell lymphomas (FCL)) patients with DLBCL of primary nodal origin and group B, that consisted of 210 (52.8%) patients with DLBCL of primary extranodal origin. Patients’ characteristics (gender, age, stage, IPI, presence of B symptoms, bulky disease and bone marrow infiltration), the kind of treatment (chemotherapy, radiotherapy, rituximab, radiotherapy) and response rates were compared between the two groups. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Results. Group B patients presented with early stage disease (I-II, no bulky disease), low IPI (0-1), no B symptoms, and no bone marrow infiltration with a significantly higher frequency than group A patients (83.8% vs. 45.7%, 62.8% vs. 39.9%, 20% vs. 34.6%, 2.4% vs. 12.2% respectively, p<0.003). Patient distribution according to the kind of treatment administered, was not different between the two groups (p>0.05). Median follow-up time for groups A and B was 55 (1-425) and 56 (1-426) months respectively (p>0.05). On an intention-to-treat basis, complete response rates were similar between groups A and B (81.9% vs. 84.8% respectively, p>0.05). Actuarial 5-year DFS rate was significantly higher in group B compared to group A (80% vs. 68.3% respectively, p=0.006). Actuarial 5-year OS and FFS rates were not significantly different between groups A and B (71.3% vs. 70.8% and 55.2% vs. 61.4% respectively, p>0.05). In the study, patients with DBLCL of primary extranodal origin demonstrated similar and consistent presenting clinical features and a higher DFS rate than patients with nodal DLBCL. Nevertheless, OS and FFS rates did not seem to be affected by the primary site of origin.

0717
THE LATE CARDIOTOXICITY OF DOXORUBICIN CONTAINING REGIMENS IN THE TREATMENT OF MALIGNANT LYMPHOMAS
I. Vasova, 1 L. Ebl, 1 M. Navratil, 1 I. Tomaskova, 2 Z. Kral, 2 D. Salek, 2 F. Jedlicka, 2 L. Smardova, 2 J. Mayer 2
1 Masaryk University Hospital, BRNO, Czech Republic; 2 University Hospital, BRNO, Czech Republic

Background. Chronic cardiotoxicity of doxorubicin occurs later than one year after completion of the chemotherapy and it represents a serious late treatment related complication. Aims. to determine the cumulative late cardiac and also anemia with the development and the treatment. Methods. 96 patients with Hodgkin’s and non-hodgkin’s lymphoma treated in the period 1995 -2000 at our department were consecutively included in the prospective study. Male/female ratio 47/49, median age 41 (23-79), median follow-up 6 years (5-10). The maximum cumulative dose of doxorubicin (CD DOX) used in the treatment protocols was 377-147 (median 300, 50-880) mg/m². 32 (33%) of the patients received another treatment after primary regimen for high risk disease at the time of diagnosis or for later relapse. Patients were examined by rest echocardiography before initial treatment, after its completion and at the minimum of 5 years follow-up in the survivors. Dynamic stress echocardiography and cardiology exercise test were performed during control examination. Decline of left ventricular ejection fraction (LVEF) below 50%, progression of decline of LVEF >10% compared to baseline value and drop-off of peak oxygen consumption pVO2<20 mL/kg/min were considered as pathological. Doppler parameters of left ventricular diastolic function and index of global left ventricular function (myocardial performance index, MPI) were evaluated too. Results. Clinical signs of cardiotoxicity were observed in 4% of pts, subclinical cardiotoxicity in 31%. Impairment of diastolic function was present in 38% pts and a pathological value of MPI in 31% pts. A stress increment of EF was 15-4% (median 12; 5-25). Decreased value of pVO2 was find out in 15% patients. Decrease of LVEF significantly correlates with duration of follow-up after treatment. The risk factors for late cardiac toxicity detected in multivariate analysis were CD DOX > 800 mg/m², pre-existence of cardiovascular diseases and age >60 years (OR=0.05, age p<0.01; concomitant cardiovascular disease p<0.01, r=0.57 and p=0.02 for whole model). Additional treatment following the initial treatment is associated with higher risk only for finding of diastolic dysfunction (OR=2.37, p<0.05), but not for drop of LVEF. Reduced cardiopulmonary performance was diagnosed only in 15% of survivors and is significantly affected by age and diastolic impairment. Summary/Conclusions. Our data demonstrate, that cardiac function should be long-term monitored at least by means of rest echocardiography in patients after anticyclic containing regimens.

0718
PROGNOSTIC SIGNIFICANCE OF GAMMA-DELTA T CELL RECEPTOR EXPRESSION IN BONE MARROW LYMPHOCYTE POPULATION OF LYMPHOMA MALIGNUM PATIENTS
S.E. Sowinska, U.Z.L. Usnarska-Zubkiewicz, K.K. Kuliczkowski
Wrocław Medical University, Poland, WROCŁAW, Poland

Background. Gamma-delta T lymphocytes (γδT) appear to posses intrinsic cytolytic antitumour activity in different carcinomas, sarcomas, myeloma and leukemia. Activated γδ T cells express antigen CD25+
(late activator) and CD69+ (early activator) on their surface. Aim 1 determine a mean percentage (%) of γδ T cells in bone marrow of untreated NHL patients, 2) γδ T cells% comparison in bone marrow and peripheral blood of NHL pts, 3) verify the impact of γδ T cells presence in bone marrow on the NHL clinical outcomes. Material and Methods. 18 newly patients (pts) with NHL diagnosis, admitted to Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Wroclaw Medical University between 2002-2005 were included into analysis. The S. Murphy staging system was used for prognostic stratification (pts in III or IV stages). Samples of bone marrow and blood were taken at the time of diagnosis. γδ T cells were estimated by flow-cytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies (MoAbs: Ab-anti γδTCR-FITC (Becton-Dickinson), Ab-anti CD14-PE/CD-45-FITC [Leukogate], CD3-PE and CD25-PE,CD69-PE for identification activated γδ T cells. Results. In 18 of NHL patients (pts) γδ T mean% in bone marrow was 4,38±3,9 and was significantly (p=0,04) higher than in peripheral blood: 3,04±1,88. Similarly γδ T CD25+ and γδ T CD69+ in bone marrow: 0,29±0,22 and 1,56±3,26, were significantly (p=0,006 and p=0,01) higher than in blood: 0,1±0,1 and 0,64±1,21. Two positive correlations were found: between γδ T CD25+ and γδ T CD69+ cells percentage in bone marrow and blood: r=0,53, p=0,02 and r=0,54, p=0,04. After 4 cytostatic cycles of 8 pts received disease regression; complete, partial remission or stabilization (group R) and 10 of 18 pts had lymphoma progression (group P). Despite statistical significance a favorable trend was observed, that bone marrow γδ T cells activated γδ TCDB25+ and γδ TCD69+ mean% in group R: 5,6±5,52; 0,36±0,53 and 2,31±3,49 were higher than in group P: 3,4±1,64; 0,23±0,23 and 0,96±0,99, respectively. Overall survival time (OS) in group R of 12 patients was lower than in group: 1,9±2,2 months, 16,8±5,0 was higher than in group P: 4,2±2,9 months, 13,3±8,6. Two positive correlations between bone marrow γδ T CD25+ and γδ T CD69+ percentage and OS were found, r=0,54, p=0,04 and 0,53, p=0,05 respectively. Conclusions. In NHL, γδ T lymphocytes activation by tumour antigens occurs first in bone marrow than in peripheral blood. As higher γδ T CD25+ and γδ T CD69+ cells mean percentage in bone marrow than in blood were observed. Moreover, higher bone marrow γδ T CD25+ and γδ T CD69+ lymphocytes percentage at the time of diagnosis can be used as a good prognostic marker in NHL patients.

0719 EXPRESSION OF A FEW T CELL ANTIGENS AND EARLY T ORIGIN OF T-ALL/LBL PREDICT FOR POOR SURVIVAL OF ADULT PATIENTS TREATED ON ALL PROTOCOLS

Background. Immunophenotype subtypes of T-lineage ALL (early, thymic, and mature) are conventionally used for risk stratification and selection of post-induction therapy. It is less clear if the same risk factors apply to T-ALL and T-lymphoblastic lymphoma (T-LBL) treated with the ALL strategy. Aim To assess the prognostic value of immunophenotype patterns and clinical features of adult pts with precursor T-cell leukemia or lymphoma (T-ALL/LBL) treated with the ALL protocol. Methods. From 1997 to 2008 35 adult patients with T-ALL/LBL were treated according to the GMALL (German Multicenter Study Group for Adult ALL) 05/93 protocol (D. Hoelzer et al., Blood 2002; 99:4579). Immunophenotype was determined by flow cytometry of cells from the lymph node, mediastinal tumor or bone marrow. Subtypes definitions: early - cCD3+ (cytoplasmic), thymic (cortical) CD1a+, mature sCD3+ and CD1a-. Assessment of the pan-T-cell CD antigens (pT-CD) expression included: CD1a, CD2, sCD3, CD4, CD5, CD7, CD8. Survival rate was calculated by Kaplan-Meier method and compared using logrank test. Prognostic factors were analyzed by Cox's model. Results. Patient characteristics: males - 26 (74%), age < 35 - 30 (86%), median WBC - 12 G/L, ALL (25% BM+)- 14 (40%), LBL - 21 (60%), mediastinal mass - 51 (86.6%), primary CNS - 4 (11%). Immunophenotype: early - 16 (45.7%), cortical - 17 (48.6%), mature - 2 (5.7%), 17 pts (46.8%) expressed CD1a1 antigen. Differential expression of pan-T-antigens: 0-3 antigens - 35.3%, 4-5 antigens - 26.5%, and 6-7 antigens - 38% of pts. The median follow up for surviving patients was 45 months. 5-year overall survival (OS) for all 35 pts was 42.5%, for T-ALL - 45%, and for T-ALL - 34.2% (p=0.99). Complete remission (CR) rate was 83% (29/35). Disease free survival for 29 CR pts was 60.3%, for T-LBL - 58.8% and for T-ALL - 63.6% (p=0.59). Patients with cortical or mature subtype had better sYOS than those with early subtype: 56% vs 15% (p=0.056). sYOS advantage of CD1a+ pts vs. CD1a- pts (64% vs. 39%) was not significant (p=0.075). Expression of 2 4 pT-CD markers vs. < 4 antigens correlated with better survival p=0.015, sYOS for 0-3 pT-CD - 0%, 4-5 pT-CD - 50%, and for 6-7 pT-CD - 60%. On the multivariate Cox's analysis of clinical and immunophenotypic features only female sex was predictive of poor survival (HR 6.54; p=0.002). Female sex and age > 35 correlated with progressive disease (p=0.007, p=0.046). Conclusion. Adult pts with precursor T-cell leukemia or lymphoma treated with the GMALL 05/93 protocol have similar outcome. Early T phenotype is a poor risk factor in both leukemic and non-leukemic patients. Expression of 3 or less panT antigens is predictive of dismal outcome.

Figure 1. Number of pant/cd marker number of pant/cd markers.

0720 RITUXIMAB-CHOP EVERY 14 DAYS IN NAIVE PATIENTS WITH DIFFUSE B-LARGE CELL LYMPHOMA
Lozano Blesa University Hospital, ZARAGOZA, Spain; Miguel Servet University Hospital, ZARAGOZA, Spain; Obispo Polanco Hospital, TERUEL, Spain; Reina Sofia Hospital, TUDELA (NAVARRA), Spain; Virgen del Camino Hospital, PAMPLONA, Spain; San Jorge Hospital, HUESCA, Spain; Barbaso Hospital, BARBASTRO (HUESCA), Spain

Introduction. Some studies have shown that patients with aggressive lymphoma may benefit from dose intensified schedules as CHOP-14. The addition of rituximab (R) improves response rate and survival. The support with G-CSF in dose intensification regimes may provide a good complementation of courses and advantage compared with schedules standard-dose as R-CHOP: Purpose: To evaluate the efficacy of R-CHOP-14 in naive patients with diffuse B- large cell lymphoma (DLBCL) (REAL classification). Design: observational, prospective and multicentric trial in a consecutive and previously untreated patients diagnosed of DLBCL CD20+. Exclusion criteria: HIV positivity, other malignancies and CNS involvement. Patients and Methods. Since June 2005 to January 2006, 51 patients were included in an R-CHOP regimen administered every 14 days (8 courses). At baseline assessment: clinical and physical exam, blood counts, serum and urine biochemistry, albumin, β2-microglobulin and LDL level, body scan, bone marrow biopsy. Patients were classified according ECOG, clinical stage and PI. All patients receiving prophylaxis with haematopoietic factors. Re-staging studies were performed every 4 cycles. Responses were classified as complete remission (CR), partial remission (PR), and non response (NR). Statistical analysis: Overall survival (OS), relapsed free survival (RFS). Survival analysis was performed using Kaplan-Meier and Cox regression. Results. Mean age 54 (20-78), 66.6%. ECOG 0(20), 1(21), 2(5), 3(3). B symptoms 55%. 8 symptoms 55%. IPI score 0(3), 1(21), 2(13), 4(3), 5(1), stage I(3), II(9), III(12), IV(27). Bulky disease 12 patients, only extranodal location 4, with extranodal location 31, haemoglobin<10 g/dL 12, albumin<3 g/dL 15, high LDH 34, high β2-microglobulin 30. After 4 cycle: 47 valuable patients (92.1%); response: 43(91.4%), 12 CR (25.5%), 31 PR (65.9%), 4 NR. After 8 cycle: 34 valuable patients (66.6%), 32 CR (94.1%), 1 PR, 1 NR. 5 patients have relapsed (5.9%) and 12 died (23.8%) (progression 6, infection 5 > 70 years 4). Adverse events 187 episodes: myelotoxicity 72% (grade 3-4 neutropenia and thrombocytopenia were observed in
80% and 16% respectively), infection 12%, gastrointestinal (5%), others (10%) OS was 3 months and mean PFS 40 months. Conclusions. A high response rate to R-CHOP 14 in adults naïve DBLC patients (94.1%) was observed in this study with acceptable toxicity. No differences in response were observed according to age groups but higher myelotoxicity and adverse events was present in older than seventy

0721
INFLUENCE OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR UPON TUMOR PROGRESSION IN PRIMARY SMALL INTESTINE LYMPHOMA

O. Kamabeda, N. Gaydukova
Kiev Medical Postgraduate Academy, KIEV, Ukraine

The aim of this study was attempted to clarify the relationship between the serum vascular endothelial growth factor (VEGF) and clinicopathological characteristics in patients with primary small intestine lymphoma. Materials and Methods. The 30 patients with primary small intestine lymphoma ranged age was from 34-72 years (mean, 56 years) and included 21 men and 9 women. All cases satisfied the criteria for primary gastrointestinal lymphoma. All histologic materials were obtained by endoscopic biopsy, surgery. Immunophenotyping were assessed by monoclonal antibodies. After dividing the cases into either B-cell or T-cell phenotypes, B-cell lymphomas were classified according to the Revised European-American Classification of Lymphoid Neoplasms. The stage of tumor was classified according to modification of Musshoff et al. of the Ann Arbor staging system. Serums were assayed for VEGF quantitative sandwich ELISA. The minimum detectable level of VEGF was 9 pg ml-1. Analysis of differences in VEGF levels between two groups of various prognostic factors was performed with Mann-Whitney U test. The posttreatment survival probability of the patients was calculated by Kaplan-Meier method in all 30 patients and compared with the log-rank test. Results. The serum VEGF levels were significantly higher in patients with colorectal and/or gastric involvement than those who did not (715±17 pg ml-1 vs. 314±2 pg ml-1, p<0.001), in patients with diffuse infiltration under macroscopy than those who did not (789±0.15 pg ml-1 vs. 404_0.15 pg ml-1, p<0.001), in patients with high grade histology than those who did not (767±19 pg ml-1 vs. 367±0.06 pg ml-1, p<0.001) and in patients with perforation than those who did not (779±2.0 pg ml-1 vs. 589±1.14 pg ml-1, p<0.001). Those patients with MALT type tumors, less advanced stage of disease, B-cell phenotype had significantly lower serum VEGF levels. The high serum VEGF levels were significantly associated with poor survival. Conclusion. The high serum VEGF levels (>575 pg ml-1) appears to have poor prognosis among patients with primary small intestine lymphoma. Our study may provide a basis for the better evaluation of biological characteristics and a new therapeutic strategy.

0722
D-PACE REGIMEN: AN EFFECTIVE CHEMOTHERAPY REGIMEN TO CYTOREDUCT REFRACTORY AND EXTENSIVELY PRETREATED LYMPHOMAS BEFORE ALLOGENIC STEM CELL TRANSPLANTATION

Istituto Nazionale per lo Studio e la Cu, MILAN, Italy

Background. Lymphoma patients who relapse after several lines of chemotherapy or after autologous stem cell transplantation (SCT) have a very poor prognosis with a long-term survival <15%; currently there is no standard salvage chemotherapy regimen for this subset of patients. Aims. The objective of the study was to assess the efficacy of DT-PACE, originally used in multiple myeloma as salvage chemotherapy regimen in patients affected by relapsed lymphoma. This regimen has advantage of the continuous infusion (CI) principle; we chose the D-PACE regimen (without Thalidomide), in which doxorubicin is administered as a continuous infusion over 72 h, in order to: 1) cytoreduce the disease in patients eligible for an allogenic SCT 2) overcome the MDR-1-mediated resistance in tumor cells by continuous exposure to low drug concentrations 3) reduce the risk of cardiotoxicity in patients that were heavily pretreated with CHOP-like regimens. Methods. D-PACE regimen consisting of Dexametazone 40 mg days 1-4 i.v, Cisplatin 10 mg/ms CI days 1-4 i.v, Dxorubicin 10 mg/ms CI days 1-4 i.v, Cyclophosphamide 400 mg/ms CI days 1-4 i.v, Etoposide 40 mg/ms CI days 1-4 i.v. In responding patients the regimen was planned for four cycles. Between June 2001 and July 2005 40 patients affected by relapsed or refractory lymphoma entered the study: 20 patients with Non-Hodgkin Lymphoma (NHL) (16 aggressive and 4 indolent), and 20 patients with Hodgkin’s disease (HD).

The International prognosis index (IPI) value was 0-1 in 18 cases (39%), and 2-3 in 31 cases (61%), respectively. All patients with 0-1 IPI had a bulky disease, stage IV or less than complete response after the initial chemotherapy regimen. ASCt was performed as part of the first-line therapy in poor risk patients (n=39, 79.6%) or at the time of relapse (n=10). Conditioning regimen consisted of BEAM (n=45), of melphalan respectively (n=2) and total body irradiation associated with chemotherapy (n=4). In order to evaluate the response, [18F]FDG-PET was performed systematically before and 3 months after ASCt. The median follow-up of living patients is 12 months [8-48 months].
Results.

- - : [18F]FDG-PET negative before and after ASCT
+ - : [18F]FDG-PET negative before and positive after ASCT
+ + : [18F]FDG-PET positive before and after ASCT

ζ : [18F]FDG-PET positive before and negative after ASCT

Conclusion. This study allows demonstrating that: i) a negative [18F]FDG-PET before ASCT has a good prognosis value; ii) as expressed, a positive [18F]FDG-PET before and after ASCT is associated with a poor outcome; iii) interestingly, patients with positive status before ASCT and negative status after ASCT have a prognosis similar as those negative before and after ASCT (under reserve because of the low number of patients and the short follow-up).

0724

LONG TERM SURVIVAL DATA OF PEDIATRIC NHL PATIENTS


Aghia Sophia Children’s Hospital, ATHENS, Greece

Background. Evaluating NHL treatment result data can provide insight and useful information to guide our future approach and possibly improve the care of our children. Aims. The characteristics of the patients with NHL, treated in our Department over the last 15 years are analyzed. The results are summarized in total and by the different time course of presentation and treatment schedule. Methods. From 1990 to 2005, 47 children (10 girls) were diagnosed with NHL. Mean age at diagnosis was 8,40 years (range, 0,33 to 14,5). During the 1st, 2nd and 3rd decade 14 (5), 17 (3) and 16 (4) patients (girls) were diagnosed, respectively. Based on pathology, B-NHL, T-NHL and Ki-1 (+) NHL was diagnosed in 31, 11 and 5 patients, respectively. Most common presenting sites were the mediastinum (15), the neck area (12) and the abdomen (8). For all patients, stage I, II, III, IV was found in 3, 14, 25 and 7 patients, respectively. Treatment varied through the last 15 years. The approach of the BFM protocol was applied since 1995 (BFM-NHL 90 and from 1997 the BFM-NHL 95 protocol). Irradiation was given to 5/47 patient (with B-NHL 2/5 and with T-NHL 3/5) and autologous SCT to 4 patients, all with B-NHL (1 with CNS disease, 1 with residual disease at the end of treatment and 2 at relapse). Results. Thirty eight (85) patients are alive: 35, 2 and 1 in 1st, 2nd and 3rd remission, respectively. Nine (9) patients in total have have succumbed (2 died soon after admission from other hospitals due to acute stage complications and 5 patients died during the 1st decade of our retrospective study (with T- histology and extensive disease). EFS (55 of 47 patients) is 74,4% and OS (58 of 47 patients) is 80,9%, for a median follow-up time of 6,1 years (range, 0,01 to 14,7) for all patient. For the 34 patients treated with the BFM-95 protocol since 1997, EFS and OS is 79,4% and 88,2%, respectively, for a median follow-up time of 4,8 years. Conclusions. Overall and events free survival and outcome of our patients with NHL treated during the last 15 years is standing high. Due to continuous improvement of the supportive care and understanding of the protocol philosophy while by implementing the BFM NHL treatment approach for our patients the mentioned high standing outcomes have been documented. There has been limited use of irradiation and stem cell transplantation.

0725

PREVALENCE OF HEPATITIS B IN PATIENTS WITH Hodgkin AND NON-Hodgkin’S LYMPHOMAS

P. Konstantinidou, E. Verrou, E. Georgiou, C. Chatziaggelidou, C. Zervas, N. Constantinou

Theagenion Cancer Hospital, THESSALONIKI, Greece

Introduction. High prevalence of hepatitis B infection has been observed in patients with lymphomas in previous studies, but it is still not clear whether there is an association between malignant lymphomas and hepatitis B virus (HBV). Aim. The aim of this study was to investigate the incidence of hepatitis B amongst patients with Hodgkin and non-Hodgkin lymphoma. Patients and Results. We retrospectively studied 1191 patients with lymphoma who were admitted to our hospital unit from January 1980 to December 2005. They consisted of 404 cases of Hodgkin lymphoma (HL) and 787 cases of non-Hodgkin lymphoma (NHL). Patients were tested for hepatitis B antigen (HBAg) during their first admission to the hospital. Nine out of 404 patients with HL (2.25%) and 46 out of 787 patients with NHL (5.84%) had positive HBAG. The rate of hepatitis B infection in patients with HL and NHL was higher than the general Greek population (0.9%). When compared statistically by the x2 test the prevalence in patients with HL was not significantly higher than normal persons (p=0.085), while the prevalence in patients with NHL was significantly higher than the general population (p<0.001). HBV infection is known to cause immune disorders and clonal expansion of B lymphocytes, probably contributing to lymphomagenesis. In addition the immunodeficiency that preexists and leads to chronic hepatitis B may also be a predisposing factor for the development of a malignant lymphoma. It is not known whether patients with hepatitis B have the same response rates to treatment and survival rates with the rest of the patients. Larger series of patients are needed to investigate it. Conclusions. We observed high rate of HBAG in patients with lymphomas especially NHL. Hepatitis B virus may play a role in lymphomagenesis. Further studies are required to clarify the association between HBV infection and malignant lymphomas.

0726

SAFETY AND EFFICACY OF RITUXIMAB COMBINED WITH CHEMOTHERAPY FOR LYMPHOMA DURING PREGNANCY

J. Rey, N. Bouayed, V. Roger, J.A. Gastaut, R. Bouabdallah, D. Coso

'Institut Paoli-Calmettes, MARSEILLE, France; 'Hôpital de la conception, MARSEILLE, France

Background. Management of non-Hodgkin lymphoma during pregnancy remains a difficult challenge for both patients and doctors. Treatment may allow to obtain a complete response for the mother without side effects for the fetus. Few rituximab have been published on the safety of rituximab during pregnancy. Rituximab is a chimeric IgG1 antibody, which can cross the placenta and interact with fetal B cells. Aims. We report the case of a woman with a diffuse large B cell lymphoma during pregnancy who was treated with rituximab. Methods. A 28 year old woman was diagnosed with CD20+ diffuse large B cell lymphoma in her 18 week of pregnancy. Staging show a stage II with mediastinal bulky. After careful consideration and patient informed consent, the patient was treated with a combination of rituximab and chemotherapy with standard CHOP: rituximab 375 mg/m2 D1, cyclophosphamide 750 mg/m2 D1, doxorubicin 50 mg/m2 D1, vincristine 2 mg D1, and oral prednisone 100 mg D1 to D5 given in 3-week cycles. There were no infusions reactions. During treatment, the intrauterine development was closely monitored. She received four cycles of treatment before delivery. She delivered in the 33 week of pregnancy and in very good partial remission, a 2000 g healthy child via caesarean section. Two weeks after caesarean, she was treated with two another cycles of chemotherapy with rituximab. Tep scan concluded to a complete remission. The child is now 10 month old and has a completely normal growth. After a follow up of 10 months, our patient is still in complete remission. Results. Little is know about the safety and efficacy of rituximab during pregnancy. To our knowledge, there are three cases treated with rituximab during the first (one patient) or second (two patients) trimester of pregnancy. Rituximab seems safe and without significant consequences for the fetus. Although B cells were extremely low at birth and during first weeks in the child, no infectious complications have been reported. Conclusions. Combination of rituximab with chemotherapy is safe and might be a valuable treatment option for pregnant women with CD20+ lymphoma. Controlled studies are necessary to confirm this data.
FALSE POSITIVE PET FINDINGS IN NHL PATIENTS AFTER CHEMOTHERAPY RELATED TO MACROPHAGE-RICH LESIONS

M. Gomes, C. Pinto, P. Figueiredo, A.M. Leite
IPO Coimbra, COIMBRA, Portugal

PET (Positron Emission Tomography) imaging uses the glucose analogue 18F-FDG as a tracer, and is an excellent method to detect small focal sites of high metabolic activity, which are frequently indicative of tumors. However, 18F-FDG uptake is not tumor specific. Various forms of inflammatory lesions and healing tissues, that have a high concentration of inflammatory cells (neutrophils, activated macrophages), also take up 18F-FDG, and are a major cause of false positive Results. 18F-FDG PET represents a major advance in both staging and restaging of Non Hodgkin Lymphoma (NHL), however the sensitivity in the setting of restaging is lower, and is associated with a significant number of false positive findings. Here we describe two cases, that illustrate the caution needed in the interpretation of PET scans in the restaging context of NHL patients. Case 1. A 57 year old male, with a solitary nodule with 10 cm of diameter, localized to the segment IV of the liver, histologically compatible with Diffuse Large B-Cell NHL (stage IE A). After treatment with R-CHOP, we observed in CT scan, a 60% reduction of the hepatic lesion dimensions. The PET/CT showed increased 18F-FDG uptake in the segment IV of the liver, compatible with the persistence of NHL. A left hepatectomy and resection of the segment IV was performed. The histological examination of the surgical specimen showed a large nodular area of tissue necrosis, surrounded by a fibrosis capsule and numerous activated macrophages, but no signs of persistent disease. One year after surgery, the patient is in complete remission. Case 2. A 53 year old male, with Diffuse Large B-Cell NHL, stage II A, bulky (infra-diaphragmatic), treated with R-CHOP. After treatment, a persistent thickening of the mesenteric fat was seen in the CT scan. The PET/CT showed increased 18F-FDG uptake in multiple abdominal confluent masses, in the pre-aortic and mesenteric regions, compatible with the persistence of NHL. A laparotomy with multiple biopsies was performed. The histological examination of the surgical specimen showed large areas of fat necrosis, and no evidence of NHL. In conclusion, 18F-FDG PET is an essential tool in the management of NHL patients. However, in case of positive 18F-FDG-PET findings, histological confirmation is required, in order to exclude post treatment benign inflammatory lesions, and to avoid unnecessary treatment approaches.

DIVERSE NICHES WITHIN MULTIPLE MYELOMA BONE MARROW SAMPLES AFFECT PLASMA CELL ENUMERATION AND FCM PROFILE

N.L. Nadav,1, B.Z. Katz,1, S. Baron,2, L. Yossipov,2 A. Polliack,1 V. Deutsch,1, B. Geiger,1 E. Naparstek2
1Weizmann Institute of Science, REHOVOT, Israel; 2Tel-Aviv Sourasky Medical Center, TEL AVIV, Israel; 3Hadassah University Hospital, JERUSALEM, Israel

Background. The diagnosis of MM is based on the combination of clinical and laboratory criteria including bone marrow (BM) morphological assessment (percentage of plasma cells counted), often combined with flow cytometry (FCM). Aims. In this study we compared the bone marrow plasmacytosis by microscopic examination of BM aspirates, to the FCM results in samples obtained from MM patients. We also tested whether the noted discrepancy between these two methods applies only to MM, or represents a trend in other hematopoietic malignancies as well. Methods. The number of plasma cells in BM aspirates from 41 MM patients were analyzed simultaneously by morphological evaluation and FCM using the following panel of antibodies: CD38, CD117, CD138, and IgG isotype controls. Each sample was assessed independently by two qualified laboratory specialists and/or hemato-pathologist. Seven BM samples from patients with acute myeloid leukemia (AML) were compared in a similar manner. Results. In MM it was evident that FCM underestimated the number of BM plasma cells by an average of 60%, compared with conventional morphological evaluation. On the other hand in AML there was a good correlation between the morphological and FCM assessments of the blast cell population, indicating that the discrepancy observed in the MM BM samples may be related to unique characteristics of the malignant plasma cells. This discrepancy may results partially due to the fact that bone marrow aspirates contain cells associated with the lipid-enriched spicules, while flow cytometry analysis is performed on the bone marrow fluid which is depleted of these fat tissue -adhesive plasma cells. When disrupted spicules from MM BM samples were isolated (by repeated passages through 21g needle), a 40% increase in the plasma cell percentage was noted, compared with the fluid of the same BM samples. In order to determine the FCM profile of the cells in these two fractions, we isolated BM derived spicules from aspirates of MM patients, and either sheared them mechanically with repeated passages through a 21g needle, or treated them with a cocktail of three extracellular matrix (ECM) degrading enzymes (heparinase I, chondroitinase ABC and hyaluronidase), followed by mechanical shearing. Only a combination of these two methods (shearing and ECM degrading enzymes) released the highly adhesive plasma cells from the spicules. The released myeloma cells displayed a different FCM profile and in particular had a higher level of CD138 expression. Summary. We have shown a major discrepancy between the percentage of MM cells obtained by routine BM morphology and flow cytometry counts. It is possible that this discrepancy is partially attributable to the two distinct microenvironmental components occupied by MM cells in the BM sample - the lipid spicules, and the fluid phase. MM cells located in the different niches of the BM also differ in their FCM profile. This study indicates that multiple myeloma patients contain heterogeneous populations of malignant plasma cells. These sub-populations may play distinct roles in the different biological and clinical manifestations of the disease.

COMBINATION OF BORTEZOMIB, MELPHALAN, PREDNSIONE AND THALIDOMIDE IN ADVANCED MYELOMA: A PHASE II CLINICAL TRIAL

A. Palumbo,1 M.T. Ambrosini,1 G. Benevolo,1 N. Pescosta,2 V. Callea,1 C. Cangialosi,1 T. Caravita,1 F. Morabito,1 F. Pregno,1 F. Gay,1 I. Avonto,1 C. Rus,1 P. Falco,1 S. Brighen1 M. Boccadoro1
1Divisione di Ematologia Univ. Torino, TORINO, Italy; 2Italian Myeloma Network, CINEWS, Italy

Background. Bortezomib (Velcade™) and Thalidomide are effective for the treatment of refractory multiple myeloma (MM), in vitro studies showed that Bortezomib can restore sensitivity to Melphalan-resistant MM cell lines [Clin. Cancer Res 2003; 9:1136-1144]. In newly diagnosed patients (pts), the addition of Thalidomide to the standard oral Melphalan/Prednison combination significantly increased response rate and event free survival [Cancer 2005;104:1425-53]. Aims. A phase II trial was
initiated to evaluate the efficacy and the safety of the combination therapy of Velcade™, Melphalan, Prednison, Thalidomide (VMPT) in advanced myeloma. Methods: Oral Melphalan was administered at 6 mg/m² on days 1-5, oral Prednison at 60 mg/m² on days 1-5 and Thalidomide at 100 mg/day continuously. Velcade™ was administered by IV bolus on days 1, 4, 15, 22 at three dose levels: in the first cohort (10 pts) at 1.0 mg/m²; in the second cohort (10 pts) at 1.3 mg/m² and in the third cohort (10 pts) at 1.6 mg/m². Each course was repeated every 35 days for a total of 6 courses. Dose Limiting Toxicity (DLT) was defined as the occurrence of any grade 3-4 non hematological toxicities, a grade 4 neutropenia > a week, or any grade 4 hematological toxicity except neutropenia. Results: Thirty pts with relapsed or refractory myeloma were enrolled, median age 66 years (range 58-79), 67% IgG, 17% IgA, 17% Binic Jones. The median β2 microglobulin was 3.4 mg/L (range 0.4-11.8). Fourteen pts received V-MPT as second line therapy, 16 as third line. Twenty pts received prior autologous transplant, 10 conventional chemotherapy and 9 thalidomide-based regimens. After a median of 5 courses, 20 pts (66.7%) achieved an objective response (complete response 16.7% and partial response 50%). Furthermore, 2 pts (6.7%) achieved a minimal response and 3 (10%) s; le disease. Five pts (16.7%) were refractory to treatment and experienced progressive disease. In the first cohort, 3 DLT were observed (grade 3 pneumonia, grade 3 febrile neutropenia and grade 3 vasculitis); in the second cohort, 5 DLT were observed (grade 3 Herpes Zoster infections, grade 4 thrombocytopenia and grade 4 anemia); in the third cohort 5 DLT were observed (grade 4 thrombocytopenia, grade 3 fatigue, sensory neuropathy grade 5, grade 3 Candida esophagitis). The most common grade 1-2 toxicities were: infections, fatigue, peripheral neuropathy and constipation. After introduction of prophylaxis with acyclovir, no new HSV reactivations was observed. Among the 8 pts with baseline peripheral grade 1 neuropathy before VMPT treatment, 5 worsened (one grade 3). Treatment-related neuropathy developed de novo in 4 pts (one grade 3). Conclusions. Initial results showed that VMPT is a promising regimen for advanced myeloma.

0730
SERUM CONCENTRATIONS OF DICKKOPF-1 PROTEIN ARE INCREASED IN PATIENTS WITH MULTIPLE MYELOMA AND REDUCED AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

M. Politou,1 D.J. Heath,2 A. Rahemtulla,2 R. Szydlo,3 A. Anagnostopoulou,2 M.A. Dimopoulos,1 P.L. Croucher,3 E. Terpos1 1Faculty of Medicine Imperial College, LONDON, United Kingdom; University of Sheffield Medical School, SHEFFIELD, United Kingdom; University of Athens Medical School, ATHENS, Greece

Background. Dickkopf-1 (DKK-1) protein, a soluble inhibitor of Wnt signalling, has been implicated in the pathogenesis of myeloma bone disease. DKK-1 protein was detected in plasma cells isolated from myeloma patients with bone lesions but not in normal plasma cells or in plasma cells isolated from MM patients with no lytic disease. However, it is unclear whether serum DKK-1 concentrations are elevated and whether this is related to other markers of bone and/or tumour development in myeloma (MM). Aims. The aim of the study was to evaluate, for the first time, circulating serum DKK-1 concentrations in MM patients at diagnosis, before and after autologous stem cell transplantation (ASCT) and in patients with monoclonal gammopathy of undetermined significance (MGUS) and examine possible correlations with clinical data. Methods. We studied 52 patients with MM at diagnosis, 18 MM patients pre- and post-ASCT, 18 patients with MGUS, and 22 healthy controls of similar age and gender. Evidence of bone involvement was documented using plain radiography. A series of serum bone remodelling indices: i) bone formation markers [NTX and TRACP-5b], ii) bone formation markers [bone-alkaline phosphatase and osteocalcin], and iii) osteoclast function, as assessed by bone formation markers. These results, if confirmed in a larger series of patients, could provide the basis for developing drugs that block DKK-1, thus restoring osteoblast function, and countering the increased osteoclastogenesis observed in MM.

0731
DEVELOPMENT OF A NOD-SCID HU ANIMAL MODEL TO INVESTIGATE WALDENSTRUMS MACROGLOBULINEMIA* W. Nakayama,1,2 E. Emmanouilides,3 S. Sotiropoulos,4 J. Jobing,5 M. Bestor,6 E. Terpos1 1Aristotle University of Thessaloniki, THESSALONIKI, Greece; 3University of Sheffield Medical School, SHEFFIELD, United Kingdom; 2University of Athens Medical School, ATHENS, Greece

Waldenstrom’s macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by predilection for bone marrow involvement and secretion of IgM paraprotein. The purpose of this study is to establish an animal model mimicking closely the disease in humans. We implanted into NOD-SCID mice human cancellous bone obtained from adults undergoing total hip arthroplasty or hemiarthroplasty. Cancellous bone was harvested in compact cores from the femoral head and was implanted in the hindlimb muscles of ten NOD-SCID mice. Mice were used when between 6 and 8 weeks of age (25-30 grams). The size of the bone implant was between 16 and 22 mm³. Eight to twelve weeks after the bone implantation, 3-5×10⁶ WM cells freshly harvested from a WM patient were injected i.m. very close to the bone implant into 4 mice, and 1×10⁶ cells from the same WM patient were injected i.v. into the tail vein of 2 mice bearing human bone implants. Also, two freshly harvested bone marrow (BM) core biopsies from a patient with active WM were implanted as described. All animals had a human bone fragment from non WM individuals in the opposite hindlimb. Tumor progression was determined by monitoring human immunoglobulin M (IgM) levels in murine plasma. Immunohistopathologic evaluation was performed on the human bone grafts, and murine tissue including the femurs, and tibia, the brain, liver, spleen, lung and kidney. One out of four mice injected i.m. into the bone fragment vicinity with WM cells showed elevated levels of human IgM indicative of the development of the disease. One out of two i.v. injected mice had elevated IgM one month following the injection of the WM cells. Both mice implanted with the bone marrow core biopsies showed a declining level of IgM directly after the implantation of the biopsy, but 3 months following the implantation IgM started increasing and reached levels above baseline. Histopathologic analy-
s was performed using antihuman reagents for expression of CD20 and IgM. Positive cells for both CD20 and IgM were found in BM core biopsies from the WM patients and the bone marrow graft opposite to the injected/implanted site. The stain was present in the cytoplasm and/or the surface of the positive cells. Murine tissue needs further histopathologic evaluation. Mice may need to be followed for more extended periods of time to fully assess the pattern of WM growth in this model. In conclusion, this SCID-hu-WM model may efficiently resemble the human disease. It differs from the recently created WM model by Tassone et al. (2005), since the utilization of adult bone compared to fetal, along with the implantation of WM bone biopsy, allows us to study the biology of the malignant cells in their native BM microenvironment.

This study is supported by a grant from the International Waldenström Macroglobulinemia Foundation (IWMF) to A.S.T. and C.E.E.

**0732**

**COMBINATION OF BORTEZOMIB AND DEXAMETHASONE FOR PATIENTS WITH AL AMYLOIDOSIS**

E. A. Kastritis,1 A. Anagnostopoulos,1 G. Bozas,1 S. Toumanidis,1 S. Mellou,1 J. Nanas,1 M. A. Dimopoulos2

1University of Athens, ATHENS, Greece; 2Henry Duyan Hospital, ATHENS, Greece

Background. Primary systemic amyloidosis (AL) is a clonal plasma cell dyscrasia and is characterized by widespread deposition of abnormal amyloid fibrils derived from abnormal light chains, leading to multisystem organ failure. Aggressive treatment of AL amyloidosis with high-dose melphalan and autologous stem cell transplantation (HDM-ASCT) is the current treatment of choice for selected patients, while the recombination of melphalan and dexamethasone is used for patients who are not eligible for HDT. Bortezomib is a proteasome inhibitor with proven activity in relapsed/refractory Multiple Myeloma, alone or in combination with dexamethasone. Aims. To evaluate the activity and feasibility of the combination of Bortezomib and Dexamethasone (BD) in patients with primary systemic amyloidosis. Methods. We treated consecutive patients with histologically proven, symptomatic AL amyloidosis who had measurable disease, defined as a serum M-spike >0.5 g/dL or urine M-spike >200 mg/24 hours or involved immunoglobulin free light chain (FLC) ≥100 mg/L and an abnormal FLC ratio. None of the patients had a history of multiple myeloma. Patients were treated with the combination of Bortezomib 1.5 mg/m² on days 1, 4, 8 and 11, and Dexamethasone 40 mg on days 1 to 4, every 21 days, for 4-6 cycles. Dose modifications were made based on toxicity. For the assessment of hematologic and organ response we followed the recommendations of the 10th International Symposium on Amyloid and Amyloidosis (Gertz et al., Am J Hematol (2005) 79: 319-328).

**Table 1. Patient characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Male/Female</td>
<td>4/3</td>
</tr>
<tr>
<td>Age (median/range)</td>
<td>61 (45-79)</td>
</tr>
<tr>
<td>Light chain type k/λ</td>
<td>436 (41-694)</td>
</tr>
<tr>
<td>Bone marrow Plasma cells (median/range)</td>
<td>20% (15-30%)</td>
</tr>
<tr>
<td>Number of Major organs involved</td>
<td>2/2/3</td>
</tr>
<tr>
<td>Heart involvement (number of patients)</td>
<td>5/4/2</td>
</tr>
<tr>
<td>Ejection fraction (median/range)</td>
<td>74% (47-84)</td>
</tr>
<tr>
<td>U rate urea mm (median/range)</td>
<td>17 (10-20)</td>
</tr>
<tr>
<td>NYHA class &gt;1</td>
<td>4</td>
</tr>
<tr>
<td>BNP &gt;120 pg/mL</td>
<td>5</td>
</tr>
<tr>
<td>Troponin &gt;0.05 ng/mL</td>
<td>0</td>
</tr>
<tr>
<td>J2Microglobulin &gt;2.7 mg/dL</td>
<td>4</td>
</tr>
<tr>
<td>Creatinine &gt;1.7 mg/dL</td>
<td>2</td>
</tr>
<tr>
<td>Creatinine clearance &lt; 50 ml/min</td>
<td>2</td>
</tr>
<tr>
<td>Albumin &lt;3.5 g/dL</td>
<td>5</td>
</tr>
<tr>
<td>Urine protein (mg/24 hrs) (median/range)</td>
<td>2800 (1500-4950)</td>
</tr>
<tr>
<td>Alk. Phosphatase &gt;1.5 ULN</td>
<td>0</td>
</tr>
</tbody>
</table>

Results. Over the last 6 months, 7 patients have started treatment with BD. Their characteristics are shown in table 1. Three had at least one prior therapy, one with HDM-ASCT followed by melphalan/prednisone, one with VAD and one patient with melphalan and dexamethasone. Four patients are not eligible for upfront HDM-ASCT received BD as primary treatment. Among 6 evaluable patients so far, two had a complete hematological response (CR) and 3 had partial hematologic response (PR). Hematologic response was achieved 3 to 12 weeks (median 5 weeks) after the initiation of treatment. One patient who achieved CR to BD was subsequently treated with HDM-ASCT. It is too early to evaluate organ response. Toxicity was manageable; 5 patients had grade 1 or grade 2 events. One had grade 1 fatigue, 4 had grade 1 edema, two patients had grade 1 diarrhea and two had grade 1 constipation. Dose reduction was needed only in one patient due to fatigue. Five of one toxicities was neutropenic fever. Grade 3 lymphopenia and grade 1 thrombocytopenia were the main toxicities.

Conclusions. The combination of BD is feasible for patients with AL amyloidosis. Patients achieve a rapid hematologic response with manageable toxicity but additional follow up is needed to assess organ response. Further investigation is needed to explore this combination in the treatment of AL patients either in relapsed patients or in the frontline treatment of patients not eligible for HDM-ASCT.

**0733**

**LENALIDAMIDE (REVLIMID), IN COMBINATION WITH CYCLOPHOSPHAMIDE AND DEXAMETHASONE IS AN EFFECTIVE REGIMEN FOR HEAVILY PRE-TREATED MYELOMA PATIENTS**

E.E. Davies,1 G.J. Morgan,1 S.A. Schey,2 P. Wu,1 M. Srikant,1 K.J. Phekoo,1 M. Jenness,1 A. Field Smith,1 S. Dines,1 A. Qureshi,1 G. Ravindranathan,1 R. Saso1

1Royal Marsden Hospital, LONDON, United Kingdom; 2Kings College Hospital, LONDON, United Kingdom

Background. Lenalidomide (Revlimid) is an oral immunomodulatory drug that has been shown to be effective for the treatment of relapsed/refractory myeloma, and in vitro laboratory studies suggest that its action in one patient due to fatigue. Four of one toxicities was neutropenic fever. Grade 3 lymphopenia and grade 1 thrombocytopenia were the main toxicities.

Conclusions. The combination of BD is feasible for patients with AL amyloidosis. Patients achieve a rapid hematologic response with manageable toxicity but additional follow up is needed to assess organ response. Further investigation is needed to explore this combination in the treatment of AL patients either in relapsed patients or in the frontline treatment of patients not eligible for HDM-ASCT.

Results. Over the last 6 months, 7 patients have started treatment with BD. Their characteristics are shown in table 1. Three had at least one prior therapy, one with HDM-ASCT followed by melphalan/prednisone, one with VAD and one patient with melphalan and dexamethasone. Four patients are not eligible for upfront HDM-ASCT received BD as primary treatment. Among 6 evaluable patients so far, two had a complete hematological response (CR) and 3 had partial hematologic response (PR). Hematologic response was achieved 3 to 12 weeks (median 5 weeks) after the initiation of treatment. One patient who achieved CR to BD was subsequently treated with HDM-ASCT. It is too early to evaluate organ response. Toxicity was manageable; 5 patients had grade 1 or grade 2 events. One had grade 1 fatigue, 4 had grade 1 edema, two patients had grade 1 diarrhea and two had grade 1 constipation. Dose reduction was needed only in one patient due to fatigue. Five of one toxicities was neutropenic fever. Grade 3 lymphopenia and grade 1 thrombocytopenia were the main toxicities.

Conclusions. The combination of BD is feasible for patients with AL amyloidosis. Patients achieve a rapid hematologic response with manageable toxicity but additional follow up is needed to assess organ response. Further investigation is needed to explore this combination in the treatment of AL patients either in relapsed patients or in the frontline treatment of patients not eligible for HDM-ASCT.

**0734**

**ACQUIRED ACTIVATED PROTEIN C RESISTANCE , MULTIPLE MYELOMA AND THROMBOSIS DURING THE INDUCTION PHASE OF TREATMENT**

V. Jimenez, V. Domínguez

Inmmnz, MEXICO CITY, Mexico

Background. Thrombosis is increasingly recognized as a common complication in patients with malignancy. Despite the common finding of VTE in patients with cancer, significance of coagulation test abnormalities predicting for deep venous thrombosis (DVT) still remain to be proven, although recently has been reported the impact of diagnosing acquired activated protein C resistance (APC) on DVT development in myeloma patients. Thalidomide has been used to treat refractory MM, ...
and an increased risk of thrombosis has been reported when it is employed in combination with other chemotherapeutic agents. Aims. The purpose of this study was to examine the association between chemotherapy, thalidomide and APC-R with DVT development in a cohort of newly diagnosed MM patients. Methods. One hundred and twenty newly diagnosed multiple myeloma patients were evaluated. Methods. We designed a descriptive, retrospective, longitudinal and observational study. Patients diagnosed with deep vein thrombosis and MM were evaluated. Coagulation tests were performed in the last 60 patients including acquired activated protein C resistance, Leiden factor V, factor VIII, serum S and C protein. Thrombosis was documented using standard criteria and diagnostic imaging.

Time to thrombosis was defined as the period of time between multiple myeloma diagnosis and the thrombotic event. Statistical analyses were performed using SPSS version 10.0. Fisher’s Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. Cohen’s Mantel Haenszel methods were used to evaluate the association between APC resistance and DVT occurrence while controlling for thalidomide exposure. A p value 0.05 was considered as statistically significant. Results. The frequency of response (CR, VGPR/NCR, PR) in the group of thalidomide and dexamethasone was 80% (CR, 22.8% VGPR/NCR 20% and PR, 37.2%) being higher than VAD, NCR, PR (35.5%) in stage III, with corresponding median OS 76 (95% CI: 66-86), 40 (95% CI: 35-45) and 23 (95% CI: 19-27) months. According to WSS, 177 (57.4%) patients belonged to stage I, 126 (26.8%) to stage II and 167 (35.5%) to stage III, with median OS 64 (95% CI: 54-74), 43 (95% CI: 38-48) and 23 (95% CI: 19-27) months respectively. Statistically significant difference in survival was detected between all stages in both staging systems (p<0.001). There were 42 patients with B2M 3.5 mg/L and alb 3.5 g/dL, who when analyzed separately, had a median OS of 40 (95% CI: 32-48) months, having no statistically significant difference with stage II patients of either staging system (p>0.05). Conclusions. Both ISS and WSS achieved a homogeneous distribution of patients among the three stages and demonstrated a high discriminatory efficacy. Stage I patients according to WSS had a lower OS compared to stage I patients in ISS. This is due to the inclusion of all patients with B2M 3.5 mg/L in stage I, irrespective of their alb level, while in facts, patients with B2M 3.5 mg/L belong to stage II. So, alb cannot be excluded from the ISS model, since it is absolutely necessary in order to identify true low-risk patients.

**0736**

Molecular characterization of a panel of multiple myeloma cell lines: a model for an integrative genomics approach


*Fondazione IRCCS Ospedale Policlinico, MILANO, Italy; Istituto di Patologia Molecolare, ONCOLOGIA ORALI, BELLINZONA, Switzerland; Dip Prog Chim Ing, Università degli Studi, PADOVA, Italy*

Background. The availability of Human Myeloma cell lines (HMCLs) has significantly contributed to elucidate the molecular and biological aspects of Multiple Myeloma (MM), such as the identification of the most recurrent IGH translocations and the complex network of cytokines affecting plasma cell growth and angiogenesis. Recently, genes targeted by the chromosomal translocations, as well as the activity of novel candidate specific therapeutic agents, have been investigated in HMCLs. However, it is well known that the establishment in culture perses and the continuous passages in culture confer to the HMCLs a progres-sion independence from the biological factors as well as the presence of multiple genetic lesions. Aims. The purpose of the present study was to characterize a panel of 23 HMCLs using a genomic integrative approach combining Fluorescence in situ hybridization (FISH) and both gene expression and genome-wide profiling. Aims. The most recurrent IGH translocations were determined by FISH and RT-PCR in 23 HMCLs. Gene expression profiling (GEP) of the 23 HMCLs has been generated using Affymetrix HG-U133A high-density oligonucleotide arrays. Expression data has been analyzed with unsupervised (two-dimensional hierarchical clustering) and supervised (SAM, Significant Analysis of Microarrays) Methods. Genome wide profiling data for 17 HMCLs has been generated on high-density SNP arrays and independently analyzed to investigate copy number alterations. Results. In the studied panel of 23 HMCLs, 8 lines displayed the t(4;14) translocation, 4 the t(11;14), 5 the t(14;16), 2 the t(6;14), 1 the t(14;20) and 13 the t(8;14), with the complex network of genetic lesions. Aims. Gene expression profiling data showed that only t(4;14) HMCLs could be grouped in a clearly distinguishable cluster. A subset of 6 HMCLs, 4 of which without any known IGH translocations, showed the overexpression of the members of the GAG tumor antigen, previously described as associated to unfavourable tumor progression in MM patients. Interestingly, the GEP analysis revealed that MFAP expression is not strictly related to the presence of the t(14;16) since its expression was found in cell lines negative for the translocation. In the group of HMCLs overexpressing MAF or MFAP, the specific deregulation of the known MAF target genes, including CCND2 and ITGB7,
was observed. Finally, our data show that all HMCLs are characterized by a complex chromosome abnormality, the most prominent being the gain of chromosome arm 1q and the loss of chromosome arms 1p, 13q and 17p. Conclusions. In the present study, we extend the characterization of most of the known HMCLs, making it possible a more accurate selection as appropriate model of MM for in vitro experiments and provide insights into the characterization of novel potential genetic lesion in primary tumors.

0737
MONOCLONAL GAMMOPATHY: NATURAL HISTORY STUDIED WITH A RETROSPECTIVE APPROACH
H. Steingrimsdottir,1* V. Haraldsdottir,2 V. Guinasson,1 I. Olafsson,1 H.M. Ogmduttir1
1Landskóp University Hospital, REYKJAVIK, Iceland; 2The Icelandic Heart Association, KOPAVOGUR, Iceland; 3University of Iceland, REYKJAVIK, Iceland

Background. Monoclonal gammopathy of undetermined significance (MGUS) indicates the presence of monoclonal immunoglobulin in serum without evidence of multiple myeloma (MM); Waldenström macroglobulinemia (WM), amyloidosis or other malignant lymphoproliferative disease. The prevalence of MGUS and the probability of progression to malignant lymphoproliferative disease vary between studies reflecting different populations studied and sometimes referral bias. The probability of progression from MGUS to malignant plasma cell disease is reported to be 12%, 25%, 30%, at 10, 20, 30 years, respectively, in the largest series so far (Kyle et al. 2002). The size of the initial paraprotein and the non IgG type were the strongest predictors of progression. Other smaller studies have supported these findings. Although it is known that a significant proportion of cases with MGUS will progress to malignant disease, sometimes after a long benign phase, it has never been investigated in how many cases MM or WM have been preceded by MGUS. Aim. The objective of this study was to examine the natural history of monoclonal gammopathy using a retrospective approach, with a long observation period, in an effort to estimate the proportion of MGUS cases that progress to a prodromal MM phase. Methods. Patient information was obtained from the Icelandic Cancer Registry for all MM and WM cases in Iceland since 1955 (1991 for WM) and compared with the Icelandic Heart Association’s (IHA) biobank registry. Frozen serum samples were collected from MM and WM cases. These samples were collected between 1967 and 1999 by IHA as part of the population-based Reykjavik Study in a nonselected manner. Protein electrophoresis (PE) and immunofixation (IF) was performed on all samples from the cases and two controls for each case, matched for age, gender and sampling time. Results. Paraprotein was found with PE in 26% of the samples from cases (n= 21, MM=20, WM=1) and 1.3% from controls. With IF paraprotein was found in 46% of the samples from cases (n=38, MM=32, WM=5) and 2.6% from controls. The time in years from sample collection to diagnosis was 10.14 (mean), 9 (median, range: 1-23.5) and 14.33 (mean), 13.5 (median, range: 8-31.4) in cases with detected paraprotein in the sample and those with no paraprotein detected, respectively. All cases diagnosed with MM or WM in the same year as the sample was collected were excluded from this analysis. The type of paraprotein detected was IgA in 33.4% of cases. IgG in 57% and IgM in 8.5%. Conclusion. This study indicates that MGUS precedes MM and WM in nearly half of the cases when analyzed with IF but only a quarter could be detected with PE. MGUS prevalence in the control subjects was in concordance with large population-based studies. The prevalence of IgA paraprotein in the MM cases with a prodromal MGUS phase was much higher than commonly reported in MGUS, reflecting the findings of other large studies that IgA MGUS has the highest risk of progression to malignant disease.

0738
PHASE I STUDY OF BORTEZOMIB AND 153SM-LEXIDRONAM COMBINATION FOR REFRAC TORY AND RELAPSED MULTIPLE MYELOMA
H.S. Yeh,1* R. Swift,2 D. Ferretti,3 R. Mapes,4 W. Goekeler,4 J. Berenson1
1Inst. for Myeloma & Bone Cancer Research, WEST HOLLYWOOD, CALIFORNIA, USA; 2Institute for Myeloma & Bone Cancer Res, WEST HOLLYWOOD, USA; 3Ontotheonapeutics, WEST HOLLYWOOD, USA; 4Cytogen, Inc., PRINCETON, USA

Background. Multiple myeloma (MM) is a highly radiosensitive B-cell malignancy and radiation therapy is an effective treatment for these patients. Recent preclinical studies have demonstrated that the bone-seeking radionuclide, Samarium Sm.153 lexidronam (Sam) in combination with the proteasome inhibitor, bortezomib (Velcade [Vel]), can synergistically inhibit proliferation of myeloma cell lines in vitro and reduce MM growth in mice bearing murine MM without significant myelotoxicity. These results provide the basis for a new targeted therapeutic approach for refractory and relapsed MM patients that involve combining Vel with Sam to improve the anti-MM effects of these agents without increasing their toxicity. Aim. The primary objective of this dose escalation Phase I study is to determine safety and tolerability as well as the response rate as determined by the Blade criteria of Vel + Sam treatment for patients with relapsed or refractory MM. Methods. MM patients who had failed more than 2 prior treatments will be enrolled on the Phase I dose-escalation trial which involves six cohorts with three patients each. Previous treatment with Vel is allowed. Dose escalations in parallel arms are as attached. A complete treatment cycle is 8 weeks. Vel is given on days 1, 4, 8 and 11 followed by a 45-day rest period. Sam is administered only on day 3. The cycle is repeated on Day 57 if disease is stable or improved and platelets and neutrophils recover to better than or equal to Grade 1 toxicity (may be delayed for up to four weeks). Dose limiting toxicity (DLT) is defined as cycle 1 Grade 4 hematologic or Grade ≥3 non-hematologic toxicity.

Table 1. Outline of patient cohort.

<table>
<thead>
<tr>
<th></th>
<th>Arm 1</th>
<th>Arm 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sam</td>
<td>Vel</td>
<td>Sam</td>
</tr>
<tr>
<td>Cohort 1</td>
<td>0.25 mCi/kg</td>
<td>1.0 mg/m²</td>
</tr>
<tr>
<td>Cohort 2</td>
<td>0.5 mCi/kg</td>
<td>1.0 mg/m²</td>
</tr>
<tr>
<td>Cohort 3</td>
<td>1.0 mCi/kg</td>
<td>1.0 mg/m²</td>
</tr>
</tbody>
</table>

Results. Cohorts 1, 2 and 4 have been enrolled (3 patients per cohort). Two patients (in Cohort 4) have shown responses, partial (n=1) and minor (n=1), and have received two cycles of treatment to date. Four patients progressed including one patient who showed a transient immunofixation+ complete response. Three patients in Cohort 2 have not completed their first cycle of therapy. No significant hematologic toxicities have been observed. Only one patient experienced transient fever, headache and vomiting. There have been no dose limiting toxicities to date. Conclusions. This dose escalation Phase I trial of the combination of Vel and Sam demonstrates responses in relapsed and refractory MM without significant toxicity and continues to enroll patients. Updated results from the trial will be presented at the meeting.

0739
ANTI-THYMOCYTE GLOBULIN INDUCES APOPTOSIS IN MYELOMA CELLS: A BASIS FOR MYELOMA SERO- THERAPY?
F. Ayuk, L. Fang, S. Mina, B. Fehse, N. Atassi, A.R. Zander, N. Kroger University Medical Center Hamburg, HAMBURG, Germany

Background. Monoclonal antibody based strategies have so far been unsuccessful in the treatment of myeloma. Polyclonal anti-thymocyte globulins (ATG) are used for in vivo T-cell depletion and have been reported to have cytotoxic activity against other cells including B-cells, dendritic cells and plasma cells. ATG is produced by immunization of rabbits or horses with thymocytes or T-lymphoblasts. Aim. We investigated the effect of ATG on myeloma cell lines and bone marrow samples from myeloma patients. We also studied the mechanisms behind ATG-induced myeloma cell death. Methods. Apoptosis was detected by flow cytometry after staining with Annexin V. ZVAD-fmk was used for caspase inhibition, N-acetyl-L-cysteine (NAC) served as ROS scavenger. Results. We observed strong cytotoxic activity of ATG against myeloma cell lines and primary myeloma cells. Complement-dependent cytotoxicity (CDC) was observed in 5 of 5 myeloma cell lines (RPMI-8226, U266, KMS-12-BM, EJM and NCIH929) and bone marrow samples from 6 myeloma patients. In the absence of complement ATG still induced up to 50% apoptosis in 4 out of 5 myeloma cell lines and up to 80% apoptosis in all primary myeloma samples. Preincubation of myeloma cells with a general caspase inhibitor (ZVAD-fmk) abrogated ATG-induced apoptosis but had no effect on CDC. Precinubation with N-acetyl-L-cysteine (NAC), a ROS scavenger, blocked ATG-induced CDC but had no effect on ATG-induced apoptosis. Absorption of ATG on primary T-cells completely removed anti-myeloma cytotoxicity. Conclusions. ATG induces complement mediated ROS-dependent lysis and caspase-dependent apoptosis in myeloma cells. This effect is probably due to...
to antibodies against epitopes also expressed by peripheral blood T-cells and not specific for myeloma cells or thymocytes and lymphoblasts used for the production of ATG.

0740

IMMUNOGLOBULIN-LIKE TRANSCRIPT 2 IS NOT DIFFERENTIALLY EXPRESSED IN MGUS AND MYELOMA, BUT APPEARS TO BE DOWNREGULATED AT AN EARLIER STAGE OF PLASMA CELL DISEASE

M. Schrader, W. Hüb, G. Koch, K. Strasser-Weiβpl, N. Zojer, H. Luβwig

Wilhelminenspital, VIENNA, Austria

Background. Immunoglobulin-like transcript 2 (ILT2) belongs to the Ig superfamily and has homology to the killer cell inhibitory receptors (KIRs). Like KIRs, ILT2 delivers an inhibitory signal upon interaction with MHC class I ligands. It is expressed on natural killer (NK) cells, which lyse transfomed or virally infected cells that have lost or downregulated expression of self MHC class I molecules. ILT2 is also known to be expressed on monocytes, macrophages, dendritic cells, and (naive) B lymphocytes. A differential expression of ILT2 was described for monocolonal gammopathy of undetermined significance (MGUS) and myeloma (Dave et al, 2008). Seven MGUS patients and 24 newly diagnosed myeloma patients were studied by gene expression profiling using Affymetrix GeneChip arrays. ILT2 was downregulated 8.26 fold in myeloma as compared to MGUS, being the most differentially expressed gene between these two subsets. However, as RNA from CD138+ cells was used in this analysis, a varying percentage of normal, non-malignant plasma cells will impact on the results, especially in MGUS cases. We also wanted to determine ILT2 expression in different plasma cell subsets (normal vs. malignant) in MGUS and myeloma and the eventual prognostic impact of a differential expression level. Methods. ILT2 expression was measured by flow cytometry using a PE conjugated antibody (clone HP-E1) Beckman Coulter, Inc.) in a series of 30 MGUS patients and 91 myeloma patients. Phenotypically normal and malignant plasma cells were defined by differential expression of markers CD38, CD45, CD19, CD56. Expression levels are given as mean fluorescence intensity (MFI) after correction for background staining. Results. ILT2 is not differentially expressed between MGUS (MFI median 112.0 1 range 13.45-274.42) and myeloma cells (MFI median 96.64, range 0.4-254.46). In contrast, MGUS/myeloma cells showed a lower expression of ILT2 as compared to phenotypically normal plasma cells in the majority of samples. An intranidividual comparison revealed a decrease in MFI in 70% of cases by a median of 63.5%, while in 30% of cases, there was an apparent upregulation of ILT2 in malignant cells (median increase in MFI of 510%). For myeloma, the variable level of ILT2 expression was confirmed by quantitative real time PCR in 26 cases. ILT2 levels did not vary with stage of disease (newly diagnosed versus progressive disease). Also, we found no correlation of ILT2 expression with clinical parameters or prognosis in our series of myeloma patients, although, interestingly, 5 myeloma cell lines were completely ILT2 negative. Summary/Conclusions. ILT2 seems to be downregulated in the majority of cases at an early stage of plasma cell disease, i.e. upon transformation from a normal plasma cell to the MGUS/myeloma stage. The level of residual ILT2 expression in malignant plasma cells is neither correlated to the state of disease (MGUS versus newly diagnosed myeloma versus advanced disease), nor to prognosis of myeloma patients or other clinical parameters.

0741

A NEW MODEL PREDICTING AT LEAST A VERY GOOD PARTIAL RESPONSE IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER 2 CYCLES OF BORZETMBOD-THERAPEUTIC BASED TREATMENT

J.F. Friedman, A. Al-Zoubi, M. Kaminski, T. Kendall, A. Jakubowiak

University of Michigan, ANN ARBOR, USA

Background. We recently reported that Velcade, Doxil, and Dexamethasone (VDD) is very active in both newly diagnosed and relapsed MM producing overall response rate up to 94% and complete and near complete response rate (CR/nCR) up to 33%. Despite these excellent response rates, the majority of patients do not achieve ≥90% reduction of disease (or ≥VGPR) which is considered a predictor of a longer remission and survival. Several recent studies showed that modification of therapy intensity in treatment in poor responders can improve quality of response. However, there are no established models to make early prediction of failure to achieve ≥VGPR. Aims. Using VDD as a model of Velcade-based therapy, we analyzed whether combination of normalization of free light chain (FLC) ratio and reduction of serum M-protein can be used as an early predictor of ≥VGPR response in MM. Methods. Three-ty-six patients who were enrolled on IRB approved phase II trials with VDD in newly diagnosed and relapsed MM were eligible for analysis. Ultimate responses were assigned using the EBMT criteria after 6 cycles or if after 2 cycles of chemotherapy the patient exhibited at least VGPR. Patients received a score of 1 if they had either normalization of FLC ratio (provided there was a reduction in involved FLC by ≥90% from the baseline) or ≥90% reduction in serum monoclonal protein by ≥90% after 2 cycles. If both criteria were met a score of 2 was assigned. If neither were met a score of 0 was given. The Fisher’s exact test was used to compare the score in patients exhibiting a ≥VGPR to those with ≤VGPR. Results. Of the 22 evaluable patients with VDD in relapsed disease, 7 exhibited a ≥VGPR, with 15 ≤VGPR. Of the 14 evaluable patients with VDD as first line, 7 demonstrated a ≥VGPR, and 7 ≤VGPR. All patients with ≥VGPR except for one in relapsed VDD protocol, had a score of 1 or 2 compared to those with ≤VGPR, who all had a score of 0 (p<0.0001). Summary/Conclusions. In both relapsed and first line therapy, a normalization of FLC ratio or reduction of serum monoclonal protein by ≥90% after 2 cycles of chemotherapy accurately predicts at least VGPR response to chemotherapy. In future trials with Velcade-based regimens, early modification of therapy could be planned if a patient does not demonstrate a normalization of FLC or ≥90% reduction of serum monoclonal protein after the initial 2 cycles.

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RARE OCCURRENCE OF T(4;14) AND P53 DELETION BUT HIGH INCIDENCE OF OTHER MYELOMA HIGH-RISK FEATURES (+1q, +9q, AND +11q) IN MGUS ANALYSIS USING FISH AND DNA PROBES FOR THE DETECTION OF GENOMIC ABNORMALITIES INVOLVING 10 CHROMOSOMAL LOCI

C. Wendl, A. Greiner, H. Döhner, P. Liebisch

University Hospital of Ulm, ULM, Germany

Background. The biology of the transition of monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM) is poorly understood but one concept that is closely linked to the accumulation of clonal aberrations in the neoplastic cell. In MM, some chromosomal abnormalities (13q-, 17p-, +q9, t6;14;14) and amplification of CKS1B at 1q21.2 are of prognostic relevance as they are associated with shorter survival. Methods. So far, bone marrow specimens from 48 patients diagnosed with MGUS at our institution were analyzed by FISH and a DNA probe set originally designed for the evaluation of MM. The probe set comprises probes mapping to chromosome bands 1p22, 1q21.2, 6q21, 8p11, 9q34, 11q25, 13q14, 17p15, 22q11, and 14q32 (including probes for the detection of t[11;14] and t[4;14]). Purification of PC by immunomagnetic separation (CD138) was performed in 56 of 45 cases. Results. The most frequent chromosomal imbalances in the entire cohort were: +q9 (13/44-29%), t(11;14) (8/28-28%), +1q (10/48-21%), 13q- (10/48-21%), and +1q (9/46-19%). No p53 deletion was detectable in 48 patients. Chromosomal extra copies were significantly more prevalent in patients lacking an IgH translocation (p=0.047). Conclusion. Our findings for that follow-up samples are available, there was no evidence for clonal evolution by means of occurrence of additional abnormalities or increasing size of aberrant clones (analysis ongoing). To date, only one patient with +6q, +q9, +11q, and (t11;14) progressed to MM. Conclusions. The vast majority of patients with MGUS exhibit chromosomal abnormalities. +1q9, +1q, +13q-, +17p- and t[4;14] are more frequently found in MM than t[6;14] which seems to be rare. No p53 deletion was found in the present series. Chromosomal extra copies were significantly more prevalent in patients lacking a t[4;14] translocation.

0743

SCREENING OF JAK2 V617F MUTATION IN MULTIPLE MYELOMA


Università Cattolica del Sacro Cuore, ROME, Italy

Background. JAKs tyrosine kinases are important mediator of cellular signals between cytokines, receptors and effector proteins. They have 7 structural domains ‘JAK homology regions’ (JH1-JH7) in particular JH1 and JH2. JH1 has kinase activity, while JH2 has a negative regulatory function on JH1. Recently a somatic mutation in exon 12 of JAK2 has been described in myeloproliferative diseases Philadelphia Chromosome negative as PV, ET and IMF and more recently this mutation has been investigated also in AML, MDS, aCML (BCR-ABL negative), ALL and CLL. JAK2 mutation was identified in a subset of CmML/ aCML, and

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We investigated the presence of JAK2 V617F mutation (EC). A. Agrillo, (1) has recently been approved for treatment of a subset of myelodysplastic syndromes, and is being evaluated as treatment for a broad range of other hematologic and oncologic conditions, including multiple myeloma (MM), chronic lymphocytic leukemia and solid tumor cancers. Data from Phase II and Phase III studies of lenalidomide plus dexamethasone in MM patients show significant benefits of combination therapy in the management of MM, in terms of median time to disease progression and overall survival. A subset of MM patients (10-20%) are known to have a t(4;14) translocation that results in the overexpression of oncogenes FGFR3 and MMSET/WHSC1. FGFR3 is not normally expressed in B cells but is overexpressed in MM cells and is sensitize to lenalidomide (IC50=0.78 µM). FGFR3 signaling may be one of the mechanisms of lenalidomide action. Aims. The present study examines the effects of lenalidomide on FGFR-induced signals in endothelial cells and t(4;14) MM cells. Methods. EC migration assay. HUVECs (5×10⁴ cells/insert) were assayed for migration in response to bFGF (0.1 ng/mL) using the BD BiocoatTM Angiogenesis Pore inserts. Cells were allowed to migrate overnight. Luciferase activity was measured in response to bFGF (0.1 ng/mL) using the BD BiocoatTM Angiogenesis Pore inserts. Cells were allowed to migrate overnight. Luciferase activity was measured using the BD Gaussia Luciferase Assay (Stratagene). Results. Lenalidomide was used to inhibit FGFR-induced signals in endothelial cells, reducing cell migration through fibronectin-coated membranes, and suppressing phosphorylation of the scaffolding protein Gab1 and the serine-threonine kinase Akt. However, in a FGFR3-stimulated F9 cell line, FGFR3 signaling is not inhibited by lenalidomide (IC50=0.78 µM). Lenalidomide has no effect on the phosphorylation of Shc, Erk1/2, MEK, or Erk1/2. Conclusions. These data suggest that lenalidomide’s effects in endothelial cells and multiple myeloma cells involve inhibiting FGFR signaling, albeit by different mechanisms.

0745 EFFECTS AND MOLECULAR MECHANISM OF LENALIDOMIDE ON FGFR SIGNALING IN ENDOTHELIAL CELLS AND FGFR3+ MULTIPLE MYELOMA CELL LINES

A. Gandhi, (1) L. Zhang, (1) L. Lu, (1) B. Bartlett, (1) J. Kang, (1) P. Schafer, (1) W. Sherman, (1) D. Stirling (1)

(1) Celgene Corporation, SUMMIT, USA; (2) Columbia Presbyterian Medical Center, NEW YORK, USA

Background. Lenalidomide (Revlimid®) has recently been approved for the treatment of a subset of myelodysplastic syndromes, and is being evaluated as treatment for a broad range of other hematologic and oncologic conditions, including multiple myeloma (MM), chronic lymphocytic leukemia and solid tumor cancers. Data from Phase II and Phase III studies of lenalidomide plus dexamethasone in MM patients show significant benefits of combination therapy in the management of MM, in terms of median time to disease progression and overall survival. A subset of MM patients (10-20%) are known to have a t(4;14) translocation that results in the overexpression of oncogenes FGFR3 and MMSET/WHSC1. FGFR3 is not normally expressed in B cells but is overexpressed in multiple myeloma cells with t(4;14). FGFR3 is an oncogenic tyrosine receptor kinase that is activated by the pro-angiogenic growth factors aFGF and bFGF. FGFR3 signals activate the MAPK pathway via Shc and Grb2 scaffolding complexes in FGFR3+ MM cells, while FGFR signals activate the Akt pathway in ECs. We hypothesized that inhibition of FGFR3 signaling may be one of the mechanisms of lenalidomide action. Aims. The present study examines the effects of lenalidomide on FGFR-induced signals in endothelial cells and t(4;14) MM cells. Methods. EC migration assay. HUVECs (5×10⁴ cells/insert) were assayed for migration in response to bFGF (0.1 ng/mL) using the BD BiocoatTM Angiogenesis Pore inserts. Cells were allowed to migrate overnight. Luciferase activity was measured in response to bFGF (0.1 ng/mL) using the BD Gaussia Luciferase Assay (Stratagene). Results. Lenalidomide was used to inhibit FGFR-induced signals in endothelial cells, reducing cell migration through fibronectin-coated membranes, and suppressing phosphorylation of the scaffolding protein Gab1 and the serine-threonine kinase Akt. However, in a FGFR3-stimulated F9 cell line, FGFR3 signaling is not inhibited by lenalidomide (IC50=0.78 µM). Lenalidomide has no effect on the phosphorylation of Shc, Erk1/2, MEK, or Erk1/2. Conclusions. These data suggest that lenalidomide’s effects in endothelial cells and multiple myeloma cells involve inhibiting FGFR signaling, albeit by different mechanisms.
TUMOR ANGIogenesis AND SENSITIVITY TO THE IL-6 IN MULTIPLE MYELOMA: EXPRESSION OF THE MICROVEIN DENSITY AND GP-130 INTERLEUKIN-6 TRANSDUCER WITHIN THE BONE MARROW COMPARTMENT

M. Perunovic-Jovanovic, J. Bila, I.J. Jakovic, D. Tomin, V. Cemenickic-Martovic, T. Terzic, M. Gotic, D. Boskovic
Institute of Hematology, CCS, BELGRADE, Serbia and Montenegro

The functional interplay between the myeloma cells and the surrounding microenvironment within the bone marrow (BM) includes increased activity of endothelial cells resulting in neovascularisation, and enhanced sensitivity to the IL-6 as a main growth factor in multiple myeloma (MM). This cytokine, as a member of gp130 family, binds on the surface of myeloma cells to the IL-6 receptor α chain that associates with the gp130 transducer chain (CD130), providing the proliferation signal to the tumor cells. The aim of study was to investigate the correlation between expression of BM angiogenesis estimated as microvein density (MVD), and expression of the transmembrane signal transducer, gp130, in the bone marrow of MM patients (pts). The study included 60 newly diagnosed MM pts (33 male and 27 female pts, mean age 60 years, range 35–75). According to the clinical stage (CS, Salmon&Durie), distribution of MM pts was as follows: I pts, II 22pts, III 30pts. There were 53pts with IgG monoclonal (m) protein, 12pts with IgA, and 12pts with secretion of kappa/lambda chain. None secretory MM was diagnosed in 1pts. All pts were treated with standard chemotherapy regimens. BM vessels were visualized by immunohistochemical staining for CD34 (B1-3CS, Santa Cruz Biotechnology, USA) on slides of formalin-fixed, paraffin-embedded BM biopsies. MVD was calculated by the number of vessels per high microscopy field in the area of the most dense vascularization. All samples were further analyzed for the immunohistochemical expression of the gp130 (AN-H2, Santa Cruz Biotechnology, USA) which showed cytoplasmic and membrane localization. The intensity of these stainings was graded as weak (0–30% myeloma cells), moderate (31–60% myeloma cells), and strong (>60% myeloma cells). Control specimens were obtained from pts without hematological malignancy. According to the CS of myeloma, positive correlation was found between MVD and expression of GP130 in myeloma cells. The expression of MVD was significantly higher in MM pts in III CS than in pts in I CS of myeloma (15 vs. 7.5±0.60 field, p<0.01). Similarly, significantly higher expression of gp130 was found in pts in III CS of myeloma comparing to the MM pts in I CS (32 ±15%, p<0.05). These findings of increased angiogenesis in correlation with high IL-6 sensitivity found in IIICS of myeloma pointed out significantly shorter survival of those pts (26 vs. 45.5 m, log rank, p<0.05). In conclusion, strong activity of angiogenesis in myeloma, combined with high IL-6 sensitivity by immunohistochemical expression of gp130 represents possible predictive factors of poor prognosis.

CORRELATION BETWEEN THE CYTOGENETIC FINDINGS AND THE PROGNOSTIC FACTORS IN THE GROUP OF PATIENTS FROM THE CMG 2002 CLINICAL STUDY

J. Smejkalova,’ P. Kuglik,’ H. Filipka,’ A. Oltova,’ Z. Adam,’ L. Pour,’ M. Krejci,’ M. Penka,’ R. Hajek’
‘University Hospital, BRNO, Czech Republic; ‘University Hospital, Brno, BRNO, Czech Republic

Background. Cytogenetic abnormalities in multiple myeloma (MM) are one of the most important independent prognostic factors. Aims. To determine the correlation between the aberration of the chromosome 13 (detected by molecular cytogenetic methods) and the prognostic factors in the pilot group of patients from the CMG 2002 clinical study (only data from one clinical centre covers 1/4 of patients) using three various of cut off levels (9%, 20%, 80%). Methods. Interphase fluorescence in situ hybridization (ISH) and fluorescence in situ hybridization and clonotypism immunoglobulin staining (clg-ISH) were used to detect the aberration of the chromosome 13. Cytogenetic abnormalities were found in 65 newly diagnosed MM patients with MM, the median of follow up was 22.8 month. Results. The aberration was found in 40% (26/65) patients (cut off levels 9%, 20%) and in 21.5% (14/65) patients (cut off level 80%). We have correlated standard prognostic factors (MIG, LDH, β2M, Hb, platelet count, albumin), event free survival (EFS), and overall survival (OS) with the occurrence of the aberration of chromosome 13 using three variants of cut off levels (9%, 20%, 80%). Higher MIG and lower albumin concentrations and platelet counts (for cut off level 80%) were detected in patients with aberration of chromosome 13 (cut off levels 9%, 20%). This analysis will be extended for all centres of CMG 2002.

LOW-DOSE THALIDOMIDE AS MAINTENANCE THERAPY FOLLOWING SINGLE OR TANDEM AUTOTRANSPLANT IN ADVANCED MULTIPLE MYELOMA IMPROVES OVERALL RESPONSE WITH MILD TOXICITY

F.B. Benedetti
Centro Trapianto Midollo Osseo, VERONA, Italy

Background. Thalidomide has been introduced few years ago in the treatment of MM. At present is part of many clinical trials, especially as front line therapy in combination with desamethasone or chemotherapya. Although the activity of Thalidomide as monotherapy is widely accepted in relapsed or refractory MM, its role as maintenance therapy following autotransplant is still under investigation. The drug is effective, but the toxicity, i.e. the DVT, remains one of the main reasons of concern for many investigators, so that the schedule, dose and anti-angiogenic prophylaxis are still matter of debate. Methods. In 1999 we started a trial with conventional chemotherapy (3 cycles of VAD), followed by high-dose cyclophosphamide (7 g/m² iv.) and peripheral stem cells (PBSC) harvest, followed by single or tandem autotransplant with melphalan (200 mg/m² iv.), in patients affected by advanced MM (stage II-III Salmon-Durie). Thalidomide 100 mg a day was then given as maintenance to all patients regardless the type of response, and discontinued at the time of relapse or progression, or for toxicity. No anti-angiogenic prophylaxis has been administered. Patient characteristics. Between January 1999 and June 2005, 75 consecutive MM patients were enrolled. Seventy patients, median age 55 (range 46-66 years), M/F 43/27, are valuable. All these patients completed chemotherapy without major problems, and no toxic deaths occurred: 10/70 patients were in complete remission (CR) at the time of PBSC transplant, 54/70 reached CR after transplant (60/70 cases underwent tandem transplant), so that after chemotherapy 44/70 (62%) were in CR, defined as bone marrow plasmacytosis below 5% and absence of serum and urine paraprotein. Thalidomide was started when possible within 6 months following transplant: 21/70 patients could not be treated because of different reasons: progression of disease (6 cases), unacceptable side effects (3), performance status <70% (2), neurological problems (2), refusal (1). Three cases were followed in other Institutions. Only 4 patients discontinued the drug in few weeks because of mild neurological toxicity (WHO < 2). The remaining 49 patients (70%) continued the drug until relapse or progression, for a median time of 24 months after transplant. Results. The CR rate after PBSC transplant was 69% in the group treated with thalidomide and 52% in the remaining patients. With a median follow-up of 38 months we compared the number of relapses/progressions, the time to relapse/progression, the disease free survival (DFS) and the overall survival (OS) in the two groups of patients. Toxicity. Most patients reported peripheral neurophy, somnolence and constipation: when severe, a temporary adjustment of drug dose was able to control these symptoms. Despite the absence of anti-angiogenic prophylaxis, no DVT were observed. Conclusions. Low-dose Thalidomide following single or tandem autotransplant appears to be a safe and feasible maintenance treatment improving overall response rate without severe side effects. No anti-angiogenic prophylaxis is needed.

Table 1. In results, after/in the two groups of patients.

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PEGYLATED LIPOSOMAL DOXORUBICIN, MELPHALAN AND PREDNISONE THERAPY FOR ELDERLY PATIENTS WITH MULTIPLE MYELOMA

R. Garcia-Sanz,1 H. Hernández,1 A. Sureda,1 J. García-Laraña,1 F. Prósper,1 A. Alegre,1 A. Bárez,1 M.V. Mateos,2 J. San Miguel2
1University Hospital of Salamanca, SALAMANCA, Spain; 2Grupo Español de Mieloma, SALAMANCA, Spain

Background. Melphalan & Prednisone (MP) is considered as the standard therapy for Multiple Myeloma (MM) patients not eligible for high dose therapy, but the addition of new drugs could result in better results. Aims. Here we report the results of a phase I-II study to evaluate the feasibility and efficacy of the association of PLD to the conventional MP regimen during the first 6 cycles of the front-line therapy for untreated MM patients older than 70.

Patients and Methods. Thirty patients were included in the study with a median age of 77 years (71-84) and a M/F ratio of 17/13 in a phase I/II study to determine the best dose of PLD and the response rate. Results. The phase I of the study demonstrated that the maximum tolerable dose of PLD in this setting was 30 mg/m², so it was the final dose evaluated in the study. 29 patients were valuable for response, which was: complete in 4 (14%), partial in 15 (52%), minor/no changes in 7 (24%) and progressive in 3 (10%). The median progression free survival (PFS) was 24 months. The median overall survival (OS) has not been reached yet, with a 3-year probability for OS and PFS of 52% and 37%, respectively. Hematological toxicity was frequent but usually weak/moderate (grades 1 & 2 of the WHO scale) and it was resolved only with dose delays. Infection was a relatively frequent event (30% of patients), but only in 4 cases it was of grade 3. No cases of palmar-plantar erythrodysesthesia were observed. Conclusions. Elderly MM patients can benefit from other more intensive therapeutic alternatives than MP as the addition of pegylated liposomal doxorubicin to this conventional regimen.

INCREASED INHIBITORY, CD158A, RECEPTOR EXPRESSION ON CD16+NK CELLS AND IMPAIRED NK CELL CYTOTOXICITY IN ADVANCED MYELOMA PATIENTS

G.K. Konjevic,1 V. Jurisic,2 M. Colovic,3 K. Mirjacic,1 A. Vuletic,1 T. Srdic,1 I. Minic,1 I. Spuzic1
1Inst of Oncol. and Radio of Serbia, BELGRADE, Yugoslavia; 2School of Medicine, Univ of Kragujevac, Kragujevac, Yugoslavia; 3School of Medicine, Univ. of Belgrade, BELGRADE, Yugoslavia

Background. The inability of the immune system to recognize and kill malignant plasma cells in patients with multiple myeloma (MM) has been attributed in part to the ineffective activation of natural killer (NK) cells. The activity of NK cells is regulated by opposing, activating and inhibitory, receptors and their balance, as well as the influence of cytokines, determines NK cell cytotoxicity. Aim. The aim of this study was to evaluate NK cell activity in the light of the expression of novel NK cell activating and inhibitory receptors in myeloma patients. Methods. In this study in 20 MM patients in clinical stage III and IV, prior to therapy, and in 15 controls NK cell activity, percent of innate cell subsets, expression of activating (CD161) and inhibitory (CD158a, CD158b) receptors on freshly isolated PBL and CD16+NK cells were evaluated using 51-chromium release assay and direct immunofluorescence by Flow cytometry. Results. We show significant impairment of NK cell activity without any change in the percent of innate immunity subsets (CD16+NK, NKT and CTLγδ). There is a significant increase in the CD16dim NK cell subset in PBL in MM patients compared to controls. There is no decrease in CD161 activating receptor (MFI of CD161 on CD16bright is significantly higher), or increase in CD158b inhibitory receptor, expression on fresh PBL or CD16+NK cells, while, there was a significant increase in the inhibitory, CD158a, receptor expression on CD16+NK cells in MM patients. Conclusion. We give novel results for advanced multiple myeloma patients that show that an increase in the immature CD16dim NK cell subset and an increase in the expression of KIR, CD158a inhibitory receptor, on CD16+NK cells has an adverse effect and is associated with impaired NK cell cytotoxicity. Aside from this, these findings may have implications in developing therapeutic approaches in multiple myeloma which use recombinant NK receptor ligands that aid in targeting NK cells to tumor cells.