such as HSV, VZV, ADV, EBV, CMV, HHV-6 and HIV was excluded by PCR. The blood lymphocyte subset showed lymphopenia, however with normal CD4/CD8 ratio. Finally CNS biopsy revealed T-cells close to blood vessels, a pattern typical for cerebral GvHD. Immunosuppressive treatment was started with high dose steroids in combination with mycophenolatmofetil (MMF). She recovered rapidly and became completely awake within one week. After 9 months of immunosuppression the patient is competent in activities of daily living.
Conclusions: GVHD of the central nervous system (CNS) is a rare disease after allogeneic stem cell transplantation. The absence of lymphocytes in the cerebrospinal fluid and normal CD4/CD8 ratio in peripheral blood does not exclude GvHD of the CNS. CNS biopsy should be considered to confirm the diagnosis. This case demonstrates that CNS involvement can be the only manifestation of chronic GvHD. Immunosuppressive therapy may provide an impressive benefit in these patients.

9230 POSTER

Can Allogeneic Peripheral Blood Stem Cells Be Safely Cryopreserved for Use in Patients Undergoing Transplant

M. Webber[1], M. Patel[1]. [1]*University of Birmingham, Bone Marrow Transplant Centre, Birmingham, United Kingdom*

Background: Peripheral blood cells (PBC) are now widely used over bone marrow transplantation in patients with haematological malignancy. To date, there has been no analysis as to whether cryopreservation is associated with delayed stem cell engraftment. We therefore decided to perform a retrospective study to observe the outcomes of patients post PBC transplantation.
Materials and Methods: 154 patients who underwent PBC transplantation over a 5 year period at the Queen Elizabeth Hospital were divided according to the type of stem cell transplantation; fresh allogeneic or cryopreserved allogeneic. Data was sourced from an automated Patient Information Communication System (PICS). The main outcome measure was defined as the time taken for primary stem cell engraftment (neutrophil count recovery to 1×10^9/l and platelet count recovery to 30×10^9/l). Any differences were compared whilst adjusting for age, diagnosis, transplant intensity and stem cell number.
Results: The mean time taken for neutrophil count to reach 1×10^9/l was greater in the cryopreserved group (14.5 days, 95% CI 11.9–12.9) when compared to the fresh group (12.4 days, 95% CI 13.6–15.4) ($p < 0.05$ for difference). The mean time taken for platelet count to reach 30×10^9/l was also greater in the cryopreserved group (19.36, 95% CI 16.2–22.6) when compared to the fresh group (11.72, 95% CI 10.9–12.5) ($p < 0.05$ for difference). Similar results were found when adjusting for age, diagnosis, transplant intensity and stem cell number.
Conclusions: For the first time, we have shown that cryopreservation of haemapoeitic stem cells does delay both platelet and neutrophil engraftment. We recommend that a cautious approach should be considered when choosing cryopreservation over fresh stem cell transplants. In patients requiring such methods there may be a delay in engraftment; increasing hospital associated morbidity and the necessity for greater supportive care.

9231 POSTER

BEACOPP-14 Vs. BEACOPP-esc in Patients With Hodgkin's Disease From Poor-prognosis Group − Updated Results of Prospective Randomized Multicenter Study

I. Titorenko[1], I. Kriachok[1], K. Filonenko[1], O. Novosad[1], O. Alexik[1], T. Kadnikova[1], A. Martynchik[1], T. Kadnikova[1], E. Kuscshevyy[1], Y. Pastushenko[1]. [1]*National Cancer Institute, Oncohematology, Kiev, Ukraine*

Background: The efficacy and toxicity of the treatment with beacopp-14 and beacopp-esc regimens in patients with Hodgkin's disease (HD) from high risk group are compared in prospective randomized study.
Materials and Methods: Since September 2008 81 patients in 6 Ukrainian centers from 18 to 65 years old (median 28 years), 38 male and 43 female with stage IIB with $\geqslant$1 unfavorable factors and stage III-IV were randomized to receive the treatment with beacopp-14 (36 pts, 5.88 cycles per patient) and beacopp-esc (45 pts, 5.61 cycles per patient). The treatment efficacy in both groups was evaluated after 4, 6 and 8 cycles by Cheson criteria (1999, 2007). Toxicity rate was evaluated with NCI-CTC. After completion of chemotherapy patients with initial sites >5 cm, residual lymph nodes >2 cm and PET-positive sites received radiotherapy (30−36 Gy). The similar group of patients, who received the therapy with ABVD, was selected for the historical control.
Results: The therapy efficacy in both groups was higher than in the group of ABVD treatment; the difference in the efficacy in the groups of beacopp-14 and beacopp-esc was insignificant (Table). 2 patients after the treatment with beacopp-esc have early relapse (after 3 months and 1 year). There were no relapses detected in the group of beacopp-14; $p > 0.05$. All patients are alive; maximal observation period is 26 mnths. In the group of historical control overall response rate (ORR) after the completion of the treatment was 80.39%; that is significantly lower than in the both groups treated with beacopp-esc or beacopp-14. The most frequent toxicity type in both groups was hematological toxicity (Table) of different grades. In 7.5% the beacopp-14 cycles were not completed due to neutropenia of 4 grade. The most frequent nonhematologic complications were nausea and vomiting.
Conclusion: Both comparative regimens show almost equal treatment efficacy and toxicity rate in patients with HD of the poor prognosis group (100% ORR after 6−8 cycles). The efficacy of ABVD treatment in the similar group of patients with HD was significantly lower. However, the results are preliminary and should be confirmed in larger number of patients and with a longer follow-up.

Table. Efficacy and toxicity rate

	BEACOPP-14, %	BEACOPP-esc, %	p-value
ORR, 6 cycles	100	97.4	>0.05
ORR, 8 cycles	100	100	>0.05
CRR, 8 cycles	82.8	86.8	>0.05
CRR, 8 cycles	88.9	87.5	>0.05
Relapses	2 pts	0 pts	>0.05
Hematological toxicity	72.8	67.6	>0.05
Anemia	25	12.5	<**0.05**
Neutropenia	35.5	37.3	>0.05
Febrile neutropenia	8.5	6.7	>0.05
Nausea and vomiting	31.3	44.6	>0.05
Mucositis	11.8	6.1	>0.05

9232 POSTER

B Cell-activating Factor of TNF Family (BAFF) Signaling Pathway is Associated With Helicobacter Pylori-independent Growth of Gastric MALT Lymphoma Without T(11;18)(q21;q21)

S. Kuo[1], L. Chen[2], K. Yeh[1], C. Lin[3], M. Wu[4], P. Hsu[4], Y. Tzeng[1], H. Jeng[1], A. Cheng[1]. [1]*National Taiwan University Hospital, Department of Oncology, Taipei,* [2]*National Health Research Institutes, National Institute of Cancer Research, Tainan,* [3]*National Taiwan University Hospital, Department of Pathology, Taipei,* [4]*National Taiwan University Hospital, Department of Internal Medicine, Taipei, Taiwan*

Background: We have recently discovered that nuclear expression of BCL10 or NF-κB is closely associated with *Helicobacter pylori* (HP)-independent status of low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma with or without t(11;18)(q21;q21) (Blood. 2005;106:1037–1041). In this study, we examined the role of B cell-activating factor belonging to the TNF family (BAFF) in mediating BCL10 nuclear translocation and in activating NF-κB, and HP-independence of gastric MALT lymphoma without t(11;18)(q21;q21).
Materials and Methods: Sixty-six patients who underwent successful HP eradication for localized low-grade gastric MALT lymphomas were included. Status of t(11;18)(q21;q21) was determined by reverse transcriptase polymerase chain reaction for API2-MALT1 transcript, while expression of BCL10, NF-κB, and BAFF was detected by immunohistochemistry. The primary MALT lymphoma cell was obtained from fresh marrow aspiration-derived lymphoma of a t(11;18)(q21;q21)- and t(1;14)(p22;q32)-negative gastric MALT lymphoma patients who had failed antibiotics treatment and standard chemotherapy. Phospho-Akt (Ser473 and Thr308), BCL3, BCL10, NF-κB (p65), NF-κB (p52), cyclin D3, c-Myc, and BAFF protein expression were assessed by immunoblotting. Transactivity of NF-κB was measured by electromobility shift assay,
Results: Fifty-two (78.8%) patients were negative for t(11;18)(q21;q21); among them, 34 (65.4%) were HP-dependent and 18 (34.6%) were HP-independent. Furthermore, in t(11;18)(q21;q21)-negative patients, BAFF expression was significantly higher in HP-independent than in HP-dependent tumours (13 of 18 [72.2%] vs 10 of 34 [29.4%]; $P = .003$). BAFF overexpression was associated with nuclear expression of BCL3 ($P = .014$), BCL10 ($P = 0.006$), and NF-κB ($P = 0.008$). In MALT lymphoma cell line, BAFF activated NF-κB and AKT; the activated NF-κB up-regulated BCL10, c-Myc, and cyclin D3, and the activated AKT caused formation of BCL10/BCL3 complexes that translocated to the nucleus. Inhibition of AKT by LY294002 (a PI3K inhibitor) blocked BCL10 and BCL3 nuclear translocation, NF-κB transactivity, and BAFF expression. The BCL3 nuclear translocation and NF-κB activation were inhibited by silencing BCL10 (BCL10 SiRNA). In addition, knockdown of BCL3 expression by SiRNA influenced the nuclear translocation of BCL10.

Conclusions: Our results indicate that BAFF-induced inflammation-related signal transduction can lead to BCL10 nuclear translocation and NF-κB activation. The autocrine BAFF signal transduction pathway may contribute to the HP-independence of gastric MALT lymphoma without t(11;18)(q21;q21).

9233 POSTER

Pharmacokinetic Intra-individual Variability of Imatinib − Consequences for Therapeutic Drug Monitoring in Chronic Myeloid Leukaemia

C. Bardin[1], X. Declèves[1], A. Vekhoff[2], L. Labat[1], F. Chast[1]. [1]*Hôtel-Dieu Hospital, Pharmacy-Pharmacology-Toxicology, Paris Cedex 04,* [2]*St-Antoine Hospital, Clinical Hematology, Paris, France*

Background: Imatinib is a competitive inhibitor of protein tyrosine kinase Bcr-Abl and is currently used for the treatment of chronic myeloid leukaemia (CML) and other digestive malignant pathologies. Trough imatinib plasma levels are associated with major molecular response in CML. Due to important interpatient variability, monitoring of imatinib plasma levels can be very useful especially in the case of treatment failure. The aim of this work was to determine intra-individual variability in imatinib plasma concentrations to a better knowledge of therapeutic drug monitoring (TDM) of imatinib in the real life.
Methods: Imatinib plasma concentrations were determined by high-performance liquid chromatography with UV detection at 262nm after liquid-solid extraction. Limit of quantification was set at 200 ng/mL. Blood samples were collected at steady-state (trough values before drug administration). Samples were collected and analysed over a period of 3 years with an interval of at least 3 months between each sample. Dosage regimens ranged from 200 mg/day to 600 mg/day. Results were expressed as mean ± standard deviation and variability in plasma concentrations were presented as a CV expressed in percentage. A minimum of three measurements was needed to calculate CV%.
Results: 21 patients were evaluated with a mean number of 5 measurements per patients (3−10) and a total of 108 samples. Sex-ratio (M/F) was 0.48, mean age and weight were respectively 55±16 years and 76±19 kg. Concerning the 400 mg/day group of patients (61 samples), mean imatinib plasma concentration was 1130±640 ng/mL, ranging from 250 to 2800 ng/mL(total CV of 57%) with 18 subtherapeutic concentrations (a plasma threshold of 1000 ng/mL is associated with major molecular response). For all dosage regimens (21 patients), mean intra-individual variability of imatinib was 31%, ranging from 9.7% to 70%. Possible causes for pharmacokinetic variability are multiple: drug/drug interactions (due to metabolism of imatinib with CYP3A4 and transport by Pgp), poor compliance, genetic polymorphisms. A value of 31% for intra-individual variability can be considered low.
Conclusion: The high interindividual and relatively low values of intrapatient variability in plasma support therapeutic drug monitoring. Nevertheless mean plasma concentrations were often closed to therapeutic threshold and our data suggest the need of regular TDM measurement.

9234 POSTER

Cytogenetic Abnormalities in the Spleen Detected by FISH in Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

S. Norin[1], A. Wallblom[1], B. Sander[2], E. Kimby[1]. [1]*Karolinska University Hospital, Hematology Center, Stockholm,* [2]*Karolinska University Hospital, Department of Laboratory Medicine, Stockholm, Sweden*

Background: In chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), the prognostic importance of cytogenetic abnormalities in blood and bone marrow is well established. However, only limited data has been published regarding frequency and prognostic impact of aberrations in other tissues.
Materials and Methods: We identified 62 patients with CLL (n = 57) and SLL (n = 5) who underwent splenectomy between 1989–2010. Fluorescence in situ hybridization (FISH) could be successfully performed on spleen tissue in 60 cases for detection of the cytogenetic abnormalities 11q-, 13q-, 17p- and trisomy 12. The results were compared with available FISH analyses on blood and/or bone marrow. To avoid false-positive results due to incomplete nuclei present in the tissue sections, the cut-off rate for the deletions was set to 40%. For trisomy 12 the cut-off was set to 5%.
Results: Cytogenetic aberrations were detected in 75% of the patients; in 31 cases a single abnormality and in 16 multiple aberrations. The most common aberration was 13q-, detected in 69% of cases. There was a significant correlation between the frequency of 13q and 11q deletions in spleen and blood/ bone marrow.
In 4 of 27 cases, new abnormalities were detected in blood/bone marrow samples after splenectomy. Two cases with a heterozygous 13q deletion in the spleen developed homozygosity for 13q- in blood and a new 11q- clone was found in two patients. One patient had both homo- and heterozygous 13q- clones in the blood before splenectomy as well as in the spleen, but in repeated blood samples taken nine to ten years later only a heterozygous clone remained.
After a median follow-up of 43 months after splenectomy, 23 of 62 patients are alive. Median time to next therapy was 9 (range 0–255+) months. Cytogenetic aberrations had a significant impact on overall survival dividing patients into three categories, (1) 13q- as a sole abnormality, (2) normal karyotype or trisomy 12 and (3) abnormalities involving 11q- or 17p- ($p < 0.05$). Patients with 11q- and/or 17p- did also have shorter therapy-free survival ($p < 0.01$).
Conclusions: In our study on splenectomized patients, cytogenetic aberrations could be reliably detected by FISH on paraffin-embedded sections and did influence overall and therapy-free survival. Clonal evolution could be found in a few patients.

9235 POSTER

BCL2 Expression Correlates With Surface Immunoglobulin Levels and Prognosis in Follicular Lymphoma Patients

A. Barreca[1], C. Martinengo[2], L. Righi[3], A. Chiappella[4], M. Ladetto[5], L. Chiusa[4], A. Stacchini[4], R. Chiarle[2]. [1]*University of Turin, Department of Biomedical Sciences and Human Oncology, Turin,* [2]*University of Turin, Department of Biomedical Sciences and Human Oncology Center for Experimental Medicine and Clinical Studies (Cerms), Turin,* [3]*University of Turin at S. Luigi Hospital, Department of Clinical and Biological Sciences Division of Pathology, Orbassano,* [4]*ASO San Giovanni Battista Hospital, Department of Biomedical Sciences and Human Oncology, Turin,* [5]*University of Turin, Department of Experimental Medicine and Oncology, Turin, Italy*

Background: Almost 90% of Follicular Lymphoma (FL) carry a t(14;18)(q32;q21), which juxtaposes the immunoglobulin heavy chain (IgH) enhancers to the Bcl2 gene and results in an overexpression of the BCL2 protein. Most cases of FL show heterogeneous intensity of IgH or BCL2 protein. The aim of this study was to correlate IgH and BCL2 expression and to ask whether different levels of BCL2 were associated to resistance to therapy and prognosis in FL patients.
Matherial and Methods: We analyzed 103 freshly isolated FL cases by flow cytometry and immunohistochemistry to asses BCL2 levels, and correlate BCL2 expression to survival. Isolated cells from FL patients and cell lines were studied for BCL2 regulation and resistance to apoptosis.
Results: We found a strong positive correlation between IgH and BCL2 expression in FL cases ($P < 0.0001$) as well as in subpopulations of FL cells within individual patients. A concordant regulation of both IgH and BCL2 in lymphoma cells carrying a t(14;18) translocation was found. Remarkably, primary FL cells expressing high BCL2 were more resistant to Rituximab or doxorubicin than FL cells with low BCL2 isolated from the same patient. Finally, patients with low BCL2 levels had significantly higher probability of survival as compared to patients expressing high levels of BCL2 ($P < 0.05$).
Conclusion: We show a strong correlation between IgH and BCL2 expression in FL cells, suggesting mechanisms that concordantly regulate both IgH and BCL2 transcription in translocated cases. Heterogeneous expression of BCL2 affects sensitivity to therapy and overall survival.

9236 POSTER

Phosphatidylinositol-3 Kinase I Inhibitor BKM120 Induces Cell Death in B-chronic Lymphocytic Leukemia Cells In-vitro

L. Amrein[1], M. Shawi[1], L. Panasci[1]. [1]*Segal Cancer Center, Oncology, Montreal QC, Canada*

Background: B-chronic lymphocytic leukemia (CLL) is characterized by the accumulation of CD5+ B-lymphocytes that are long-lived *in-vivo* but die quickly by apoptosis when cultured *in-vitro*. The phosphatidylinositol-3 kinase (PI3K) cascade is a critical component of survival signalling including PI3K-activated Akt (phosphorylated Akt) which inhibits cell death pathways by inactivating pro-apoptotic proteins. However,PI3K has increased activity in CLL lymphocytes as compared to normal B lymphocytes. There are three classes of PI3Ks of which class I is the most clearly implicated in human cancer. The PI3K-δ inhibitor, CAL-101, promotes caspase-dependent apoptosis and abrogated protection from spontaneous apoptosis induced by CD40 in primary CLL lymphocytes *in-vitro*. Thus PI3K pathway appears to play a critical role in B-CLL cell survival. BKM120 is a pan class I PI3K inhibitor developed by Novartis. Phase I trials demonstrated that plasma concentrations of 3−5 μM can be obtained. In view of the critical role of PI3K in CLL homeostasis, the activity of BKM120 was examined in CLL lymphocytes.
Material and Methods: BKM120 cytotoxicity was assessed by the MTT assay in primary B-CLL lymphocytes and the MEC-2 B-CLL cell line.