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**Innovative approaches to autologous bone marrow transplantation (BMT) –  
The Royal Hobart Hospital (RHH) experience**

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The RHH's bone marrow (BM) or haematopoietic stem cell (HSC) transplantation program was established in 1979. We have since performed over 250 transplants. Our programme for autologous transplantation has distinguished itself from those of many other hospitals in three aspects: 1) *Outpatient transplantation*. In 1997 we introduced a system whereby the entire transplant process (pre-transplant chemotherapy, stem cell reinfusion, post-transplant care) is carried out on the day ward, so far as possible, aiming to minimise hospital admission. With a 30-day mortality rate of <2%, our program has proved to be safe and is much preferred by most patients. Our average length-of-stay is 8 days compared with national figures of around 21 days and about 10% of patients avoid hospital admission altogether. 2) *Continued use of bone marrow as a stem cell source*. While most BMT services have switched to peripheral blood (PB) as the sole source of haematopoietic stem cells, we have continued to use BM in certain circumstances. However in contrast to historical collection methods, we use G-CSF-stimulated BM which, compared with unstimulated BM results in more rapid engraftment. The advantages over PB HSCs include collection on a predefined single day, avoidance of need for insertion of large-bore central cannulas, and smaller volume for cryopreservation. 3) *Rainy day HSC harvesting*. When patients with lymphomas and some other malignancies relapse, cure may still be possible with a transplant using HSCs collected at that time. However there are theoretical and in some cases practical advantages in having HSCs collected and cryopreserved at the time of first remission. Our routine policy is to offer this to all our patients with NHL and high-risk Hodgkin's disease in first remission. We have cells stored from over 400 such patients. Our studies have shown that in vitro the cells retain adequate viability for safe transplantation for at least 14 years. We have reinfused HSCs from nearly 40 patients after storage of >1 year (maximum 9.3 years) without engraftment failure. The engraftment kinetics of cells reinfused after long-term cryopreservation appear similar to those after short-term storage. In summary, our innovative approaches to autologous BMT are potentially of benefit to a large number of patients requiring HSC services.

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**Umbilical cord blood transplants – state of the art**

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**3**  
**Prospects for improving graft vs leukaemia effect post-transplant**

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Queensland Institute of Medical Research

**4**  
**Neonatal transfusion practices**

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The diagnosis and treatment of sick infants and children requires a broad knowledge of physiology, biochemistry, genetics and the application of sophisticated testing and treatment options. One of these options is transfusion of blood and blood products. Transfusion of the infant, especially the premature infant, and sick child, especially those with major organ dysfunction, requires careful consideration of their unique metabolic, hepatic and renal clearance mechanisms. Guidelines that direct the indications for transfusion differ from those in adults. Non-invasive measures of oxygen delivery and oxygen offloading may assist in guidelines for red blood transfusion. Metabolic complications from massive transfusion and/or the manipulation of blood products must also be considered. Dosing guidelines and apheresis techniques require special attention to the growing child's changing body mass and plasma volumes. Lastly, there are both infectious and noninfectious risks of transfusion unique to the pediatric patient. Neonatal transfusion practices must be guided by the unique physiology of the infant.

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### Novel platelet therapies and platelet additive solutions

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Platelet transfusions are intended to prevent or treat bleeding during episodes of thrombocytopenia. However, few clinical studies have been performed to establish platelet transfusion triggers or the increments that must be obtained to reach this aim. Bleeding time measurements to evaluate the function of platelets are considered unreliable and are generally abandoned. Consequently, the indication and evaluation of platelet transfusion treatment is based on surrogate parameters such as platelet count and post-transfusion platelet increment.

Similarly, products prepared for platelet transfusions ideally should contain viable platelets surviving in the recipient and exerting haemostatic function. Platelet survival studies, which require radioactive tracing, have been incidentally performed to validate product quality. Rather, many countries have developed national (European guidelines require pH and swirling) guidelines with surrogate criteria that should be fulfilled to consider a platelet product acceptable for transfusion. Again, very limited data on the relationship between pH and swirling capacity of platelet products and the post-transfusion recovery and survival are available, whereas in vitro-assays predicting platelet function of stored products are completely lacking.

In this virtual surrogate world of platelet supportive care we tried to answer some real questions: on the quality of innovative products, on allo-immunization and donor selection, on non-radioactive tracing of transfused allogeneic platelets and above all, why are patients bleeding.

## HSANZ – New Insights into Old Problems

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### Iron metabolism and anaemia of chronic disease

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<sup>1</sup>

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The anemia of chronic disease (also called the anemia of inflammation) is an acquired disorder affecting patients with a variety of medical conditions, and is characterized by changes in iron homeostasis and erythropoiesis. Mounting evidence suggests that hepcidin antimicrobial peptide plays a primary role in the pathogenesis of this disorder. Hepcidin functions by interrupting cellular iron export through the iron transporter ferroportin. Hepcidin expression is induced in response to the inflammatory cytokine interleukin-6 (IL-6). We have determined that IL-6 induces hepcidin expression by activating classical JAK/STAT signaling, culminating in binding of STAT3 to a key transcriptional regulatory element in the hepcidin promoter. To evaluate which features of the anemia of chronic disease can be attributed to hepcidin, we generated mice carrying an inducible hepcidin transgene. The transgenic mice developed a mild to moderate anemia associated with iron deficiency and iron-restricted erythropoiesis. Similar to the anemia of chronic disease, iron accumulated in tissue macrophages, while a relative paucity of iron was found in the liver. Circulating erythrocytes in transgenic animals had normal survival rates, but transgenic animals had an impaired response to erythropoietin. Thus, hepcidin transgenic mice recapitulated each of the key features of anemia of chronic disease in human patients, providing a useful model of this prevalent disorder.

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### The pathogenesis and management of acute promyelocytic leukaemia

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Acute promyelocytic leukaemia (APL) is characterised by chromosomal translocations involving the gene encoding Retinoic Acid Receptor Alpha (*RARA*) at 17q21, which is fused to one of five potential partner genes i.e. *PML* (~98%), *PLZF* (~1%), *NPM1* (<0.5%), *NuMA* (<0.5%) or *STAT5b* (<0.5%)<sup>1</sup>. Determining the underlying molecular lesion is critical for patient management, identifying those likely to benefit from molecularly targeted therapy in the form of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), as well as defining targets for monitoring of minimal residual disease (MRD) in order to direct treatment approach.

While the role of the APL fusion proteins in disease pathogenesis is firmly established, the mechanisms by which the causative chromosomal translocations are formed and the haematopoietic progenitors in which they arise have been unclear. Some insights into the former can be gained through the characterisation of translocation breakpoints in cases of APL arising as a complication of treatment for a prior tumour, typically involving exposure to drugs targeting DNA topoisomerase II. Recent studies suggest that the incidence of therapy-related APL (t-APL) has increased over the last two decades, tracking with the use of mitoxantrone and epirubicin for the treatment of breast cancer<sup>2</sup>. Characterisation of t(15;17) translocation breakpoints has shown that propensity to APL as a result of mitoxantrone  
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exposure reflects a breakpoint “hotspot” within the PML locus; with the drug inducing topoisomerase II-dependent cleavage of DNA, which is aberrantly repaired by the non-homologous end-joining pathway to generate the chromosomal translocation<sup>3</sup>. The cellular target for the t(15;17) has traditionally been considered to be committed myeloid progenitors<sup>4</sup>. However, we have found that APL cases, particularly those with the hypogranular variant (M3v) form, exhibit T-lineage associated chromatin features with expression of CD2 and *pre-T*; moreover, in some instances immature T-cell receptor rearrangements can be detected<sup>5,6</sup>. These findings lend support for the existence of haematopoietic progenitors with lymphomyeloid potential<sup>7</sup>, which could provide a plausible cellular target for the t(15;17) and raise the possibility that classical and variant forms of APL may have distinct cellular origins<sup>6</sup>.

The key challenge in the management of PML-RARA+ APL is to improve on the relatively favourable outcomes already achieved with ATRA and anthracycline-based chemotherapy, with survival rates currently exceeding 70% at 5 years. One issue that needs to be addressed is the persistently high rate of induction death due to haemorrhage, which still leads to the demise of over 10% of patients with higher presenting white cell counts ( $>10 \times 10^9/l$ )<sup>8</sup>. A further means by which outcomes could be improved, is being considered in the current Medical Research Council AML15 trial ([www.aml15.bham.ac.uk/trial/index.htm](http://www.aml15.bham.ac.uk/trial/index.htm)), whereby rigorous MRD monitoring by quantitative PCR is used to enable additional therapy (in the form of ATO and transplantation) to be targeted specifically to the small subgroup of patients who would otherwise be destined to relapse following first line treatment. Future studies will address whether treatment approaches that rely entirely upon molecular targeted therapies involving ATO, ATRA and gemtuzumab ozogamicin, which have been pioneered by Estey et al at MD Anderson<sup>9</sup>, can enable conventional chemotherapy to be dispensed with in a significant proportion of patients. While this is an attractive approach, potentially reducing treatment related morbidity and mortality, as well as time in hospital, its success will depend heavily upon optimized monitoring of MRD to allow early treatment intervention in those patients requiring additional therapy.

Overall, it is clear that study of APL has provided valuable insights into mechanisms of leukaemogenesis; moreover, this disease highlights the promise of molecularly targeted therapies and the importance of molecular diagnostics and MRD monitoring to direct treatment approach, concepts that are steadily being extended to other subsets of acute leukaemia.

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## HSANZ – Myeloma & Lymphoma

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#### Management of plasma cell dyscrasias other than symptomatic myeloma

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Plasma cell dyscrasias are produced as a result of malignant proliferation of a monoclonal population of plasma cells that may or may not secrete detectable levels of monoclonal immunoglobulin (M protein). Although symptomatic multiple myeloma (MM) and Waldenstrom's macroglobulinemia are the most common of the malignant plasma cell disorders that require treatment, there is a wide range of other plasma cell disorders including monoclonal gammopathy of unknown significance (MGUS), solitary extramedullary or bone plasmacytoma, asymptomatic MM, POEMS syndrome, and primary amyloidosis. This lecture will cover the pathophysiology and management of these less common, but important clinical problems.

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### Management of cutaneous T cell lymphoma

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The treatment of advanced-stage cutaneous T-cell lymphoma remains a therapeutic challenge. Patients universally relapse after chemotherapy and the disease remains incurable. Fortunately a number of new approaches are being investigated. These include immunotoxins such as denileukin diftitox (Ontak), monoclonal antibodies such as alemtuzumab (Campath 1-H) and novel retinoids such as bexarotene (Targretin). An exciting new approach is the family of histone deacetylase inhibitors which include vorinostat (SAHA), romsidepsin (depsipeptide) and LBH589. Immunomodulatory approaches are being explored including combination therapies with extracorporeal photophoresis, iMIDs such as lenolidomide, and reduced-intensity allogeneic transplantation. Gemcitabine, liposomal doxorubin and AraG appear more effective than other classic chemotherapy regimens. Thus the treatment paradigm is widening and the choice of therapy becomes a balance between efficacy and toxicity profile.

## HSANZ – Diagnostic Haematology

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### Measurement of uncertainty in haematology

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Considerable confusion has arisen in pathology laboratories regarding how to estimate the measurement uncertainty (MU) of testing procedures in order to comply with ISO 15189 regulatory requirements. The concept of uncertainty is not new but until recently the formal estimation of uncertainty has been restricted to the field of metrology. However it should be recognised that for many years pathology laboratories have used equipment and methods which have a clearly defined uncertainty. For example a pipette might be calibrated as  $500 \pm 2\mu\text{L}$ . It is unfortunate that the broadening of this simple concept from the physical sciences into medical testing laboratories has created such confusion.

One cause of the current confusion is that the standard “bottom-up” approach used in the reference procedures of the physical sciences is not appropriate for medical testing laboratories. For practical purposes a “top-down” approach should be used and only three individual uncertainties need to be considered. These are imprecision, bias and traceability. For many assays, imprecision is the only significant uncertainty because if a significant bias does exist attempts should be made to either eliminate or minimize it. The recently released second draft of the NPAAC standard and guidelines contains a collection of worked examples for haematology testing procedures.

In addition to compliance with regulatory requirements, there are also legal implications and ethical responsibilities involved with the reporting of test results. It should also be realized that the estimation of MU can provide real benefits to the laboratory and the patient.

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### Diagnostic utility of a JAK2 assay in patients presenting with a myeloproliferative disease

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The chronic myeloproliferative diseases are clonal stem cell disorders characterised by the proliferation of one or more myeloid lineages. The JAK2 V617F mutation is considered to contribute to the myeloproliferative phenotype by causing constitutive activation of signal transduction pathways normally triggered by haemopoietic cytokines binding to cell surface receptors. Current estimates would suggest that this mutation is present in approximately 90% of patients with polycythemia vera and 50% of patients with essential thrombocythemia and idiopathic myelofibrosis, although this incidence rate appears highly dependent on the sensitivity of the molecular assay used for mutation detection. The presence of the JAK2 V617F mutation in the peripheral blood appears highly specific for myeloproliferative disease as it has only rarely been reported in other haematological malignancy. The presence of the mutation appears to correlate with other biological features of myeloproliferation including cytokine independent growth of blood progenitor cells, granulocyte PRV-1 expression and expression of Mpl on platelets. In essential thrombocythemia and idiopathic myelofibrosis the presence of the JAK2 V617F mutation has been associated with an increased white cell count and older age of patient compared to JAK2 negative disease and clinically it appears to predict increased sensitivity to therapy with hydroxyurea.

# Lymphoma

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## **Molecular staging of diffuse large B-cell lymphoma (DLBCL) is best performed with freshly frozen marrow specimens**

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### Aim

To compare the use of freshly frozen trephine biopsy specimens and formalin-fixed, decalcified, paraffin embedded (FFPE) specimens, as sources of template DNA for performing Immunoglobulin heavy chain (IgH) and Immunoglobulin light chain (IgL) gene clonality studies by Polymerase chain reaction (PCR).

### Method

DNA was manually extracted using the Roche High Pure PCR Template Preparation Kit from 14 FFPE bone marrow trephine biopsies and 13 fresh frozen trephine biopsies.

DNA quality was assessed by two methods:

1. Amplification of a control size ladder from the IgH gene clonality kit from Invivo Scribe Technologies based on the BIOMED2 protocol. This master mix creates 5 amplicons of 96, 200, 300, 400 and 600 base pairs.
2. Real-time amplification of the beta globin gene using the Roche Lightcycler Control DNA kit.

Statistical analysis for comparing means using ANOVA was performed using SPSS version 12.0 software

### Results

All fresh frozen bone marrow trephine samples generated amplicons up to 600 bp in length on the specimen control size ladder. The mean maximum length of amplicons generated from FFPE trephines was statistically lower at 300 base pairs.

On the Lightcycler, the mean crossing threshold with fresh and paraffin-embedded bone marrow trephines was significantly different at 23.48 (95% CI 22.47, 24.48) and 33.64 (95% CI 32.15, 35.12) respectively.

### Conclusions

1. Amplifiable DNA can be extracted both from fresh-frozen and FFPE bone marrow trephines for IgH/ IgL analysis.
2. Freshly frozen specimens are superior as a source of template DNA, especially for higher base pair PCR products
3. Use of fresh bone marrow trephine for IgH/ IgL analysis is recommended given the prognostic utility of molecular staging in DLBCL (1),

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## **The significance of surface light chain negative B cells and its relation to lymphoproliferative disease**

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### Aims

1. To establish a "normal" kappa: lambda light chain ratio for fine needle and excision biopsies of lymphoid tissue.
2. To investigate the prevalence of surface light chain (SLC) negative B cells (defined as >25% of B cells failing to express SLC) and its correlation with tissue histology.

### Method

A retrospective review of flow cytometry data was performed at two institutions in Western Australia over 3 and 4 years respectively with correlation with the cytologic/ histological diagnosis of the samples. The light chain ratio and proportion of B cells lacking SLC was calculated for each sample. Both institutions utilized the Coulter EPICS XL flow cytometer with similar lymphoid panels. Samples with B cells failing to express SLC had the test performed with monoclonal and polyclonal antibodies from two different manufacturers to ensure reproducibility of the results.

## Results

858 fine needle biopsies and 696 excision biopsies were included in the final analysis. The kappa: lambda ratios for benign samples ranged from 0.6-5.5 with 98% between 0.6-4. A limited number of benign spleen and tonsil tissue was analysed with similar results. Whilst a kappa:lambda ratio outside the 0.5-6 range excluded a benign sample, the converse did not hold true. Large proportions (>40%) of SLC negative B cells correlated with a diagnosis of lymphoma whilst the majority of reactive samples had <25% SLC negative B cells. SLC negative B cell populations were also confirmed in bone marrow demonstrating it was not a site specific feature.

## Conclusions

1.

The "normal" kappa:lambda range applicable to benign tissue was 0.6-4, however this range cannot be used to exclude a malignant population.

2.

There is a strong correlation between the absence of SLC on large numbers of B cells and B cell lymphoma, and a diagnosis of lymphoma should be pursued in samples with such populations.

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### Comparison of prognostic indices in post-transplantation lymphoproliferative disorders (PTLD) after renal transplantation

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#### Aim

Recently proposed prognostic indices specific for PTLD after solid-organ transplantation have aimed to stratify patients into risk groups more effectively than the non-Hodgkin's lymphoma International Prognostic Index (IPI). Leblond *et al*<sup>1</sup> applied performance status (PS) and number of involved sites; while Ghobrial *et al*<sup>2</sup> used PS, monomorphic disease and graft involvement. The objective of this study was to compare the IPI to these two proposed PTLD-specific prognostic models, in patients with PTLD post-renal transplantation.

#### Methods

We performed a retrospective audit of 41 consecutive adult renal transplantation patients who developed PTLD at our institution. Survivals were compared by logrank test.

#### Results

Median age at time of diagnosis was 42 years (range, 17-71 years). Thirty patients (73%) had monomorphic and 10 (24%) had polymorphic PTLD. Twenty-seven patients (66%) received anthracycline based-chemotherapy. Other modalities were utilised including rituximab, radiation therapy, reduction in immunosuppression, EBV-specific cytotoxic T-cells, anti-viral therapy and surgery. Median overall survival (OS) for all patients was 101.9 months and the median follow-up of survivors was 67.2 months (range, 18.9-268.4). Nineteen patients (46%) had died. IPI identified low-risk (32.5%), low-intermediate (35%, OS 101.9 months), high-intermediate (25%, OS 0.7 months) and high-risk (7.5%, OS 0.6 months) prognostic groups ( $P < 0.0001$ ). The Leblond PTLD prognostic index also distinguished patients according to survival into low-risk (27%), intermediate-risk (38%) and high-risk (35%, OS 7.6 months) but to a lesser extent than IPI ( $P = 0.0009$ ). The Ghobrial PTLD prognostic model failed to significantly discriminate patients with worse survival ( $P = 0.2411$ ). In contrast to this model, a trend towards worse survival with polymorphic disease was shown (HR 2.3, 95% CI 0.9-10.3).

#### Conclusions

Our study suggests that the IPI is more discriminating than both proposed PTLD-specific prognostic indices in this renal transplant population. The relative prognostic value of polymorphic versus monomorphic disease requires further clarification. These indices require further validation and confirmation with larger prospective multicentre studies with uniform treatment strategies.

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### Prolonged hematologic toxicity from the hyper-CVAD regimen; manifestations, frequency, and natural history in a cohort of 125 consecutive patients

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Hyper-CVAD is a dose-intensive regimen which has been employed in the last decade to treat patients with a range of hematologic malignancies, with impressive efficacy. This regimen has been associated with considerable short-term hematologic toxicity, which has been mostly described as transient and reversible. However, recent reports have highlighted a concerning early rate of MDS or AML on medium-term follow-up. While cases of MDS/AML may be the most extreme end of the spectrum of hematopoietic stem cell damage, in the current retrospective study we describe other prolonged hematologic sequelae of this regimen. All patients were previously untreated and received Hyper-CVAD for the treatment of NHL or ALL. 125 patients (pts) with a median age of 47 (range, 15-76) were followed up for a median of 23 months (range, 1-84). The median number of Hyper-CVAD cycles was 6 (range, 2-9). Follow-up for blood counts was censored at the next cytotoxic therapy. At 3 months post therapy, 78 patients were evaluable. Thirty-two (41%) had achieved full blood count recovery and 45 (59%) had persisting cytopenias. Dose intensity delivered was significantly associated with persisting cytopenias ( $p = 0.028$ ), but age ( $p = 0.61$ ), underlying disease (0.49) and bone marrow involvement at diagnosis ( $p = 0.81$ ) were not. Of the patients who had not normalised all counts by 3 months, anemia was present in 56% (median 106 g/L, range 76-119), neutropenia in 47% (median  $1.27 \times 10^9/L$ , range 0.03-1.75) and thrombocytopenia in 76% (median  $96 \times 10^9/L$ , range 9-120). One, two and three lineage cytopenias were present in 42%, 42% and 16% of pts, respectively. The median time to normalisation of counts for those with post-treatment cytopenias in the respective lineages was 9 months (range, 6-12) for Hb, 6 months (range, 6-30) for neutrophils, and 6 months (range, 6-30) for platelets. The median time to normalisation of the lowered counts was 9 months ( $n = 18$ ) in those with multi-lineage cytopenias and 6 months ( $n = 12$ ) in those with one lineage cytopenia. At 1 year following Hyper-CVAD with no further therapy, 49 patients were evaluable and 3 (6%), 7 (14%), and 9 (18%) still had anemia, neutropenia or thrombocytopenia respectively. MDS/AML was diagnosed in 3 patients at 4, 21, and 21 months after therapy. Of these patients, one received an autologous stem cell transplant 2 months after Hyper-CVAD, one was cytopenic at 3 months, and one was lost to follow-up until re-presenting with AML. These results indicate a considerable rate of prolonged hematologic toxicity after Hyper-CVAD, and a minor rate of MDS at this limited follow-up. These findings likely reflect cumulative damage to hematopoietic stem cells.

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### **Outcome of autografts for patients with relapsed diffuse large B cell lymphoma (DLBCL) is influenced by IPI at relapse, type of salvage therapy and remission status by functional imaging**

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#### Introduction

The outcome of autografting for patients with DLBCL is known to be consistently adversely affected by disease resistance to salvage chemotherapy. Other previously reported adverse variables are age >50, international prognostic index (IPI) >1 at *transplant*, number of prior therapies, and bone marrow involvement.

The influence of duration of first CR, IPI at *relapse* and the role of nuclear imaging in determining risk of relapse post autograft have been less well studied. Functional imaging by PET scanning in DLBCL has shown to be predictive for relapse risk in patients undergoing initial therapy. We therefore explored the influence of these factors in a cohort of patients undergoing autografting for relapsed DLBCL.

#### Methods

We analysed the IPI, first remission duration, type and response to salvage chemotherapy and functional imaging results of 60 sequential patients undergoing autologous SCT for treatment of first relapse of DLBCL at RMH and PMCC from date - date. Response to salvage was determined as absence/presence of disease using the worst result of available imaging post salvage

(No PET+CT<sup>CR</sup> =CR; PET<sup>CR</sup>+CT<sup>CR</sup>=CR; PET<sup>PR</sup>+CT<sup>CR</sup>=PR etc). Differences in EFS and OS for each of these variables were calculated by Kaplan-Meier analysis.

#### Results

Of the 60 patients, 55% male, median age 52 (range 18-66). All patients had de novo DLBCL. At relapse the median IPI was 1 (range??). Salvage regimens included MADEC, ESHAC, DHAP, ICE and CHOP. Rituximab (R) was included in the salvage therapy in 30%. Post salvage functional imaging (gallium (n=16) or PET (n=26)) was performed in 70% of cases (of which 33% were negative). The actuarial 5 year OS and EFS were 70% and 50% respectively. An inferior EFS was observed in patients with no response vs any response to salvage therapy (38 vs 70%), non-R containing salvage (39% vs 80%) and in those with persistent gallium/PET positivity (44% vs 82%) post salvage (each  $P < 0.05$ ). IPI>1 at relapse was associated with and inferior 5 year OS (53% vs 78%). No significant differences were seen in CR vs PR to salvage or according to first remission duration.

#### Conclusions

Our findings reinforce the importance of chemosensitivity on outcome of autografting for DLBCL. In addition we show striking differences in outcome when R is utilized as part of salvage therapy and the strong negative predictive value of absence of radionuclide avid disease.

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## Abbreviated dose rituximab in auto-immune haematological disorders is associated with a high response rate

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### Aim

To evaluate the efficacy of rituximab in auto-immune haematological disorders resistant to or relapsing after conventional immunosuppressive therapy

### Methods

Patients receiving rituximab for this indication between July 2004 and present were retrospectively identified through Pharmacy prescribing records. A review of medical and pathology records was undertaken.

### Results

Eleven patients, 8 female and 3 male with a median age of 46 years (range: 21-72), were identified. Disease categories included: ITP-6 (3 - 1°, 3 - 2°), TTP-3, AIHA-1 and FVIII inhibitor-1. Median number of doses of rituximab was 1 (range: 1-3) with a median dose of 600mg (range: 500-850mg). All patients had relapsed or refractory disease with a median number prior therapies of 3 (range 1-4) and concurrent therapies of 1 (range 0-4). 6 patients had stable therapy at the time of rituximab and 5 had an additional therapeutic intervention within 2 weeks of rituximab. Responses, coded used published criteria, included 9 complete responses, one partial response and one stable disease. With a short median follow up of 7 months (range: 2-24), 3 patients have relapsed at 1, 7 and 8 months. These patients have had a complete response on re-treatment.

### Conclusion

Abbreviated dose rituximab is an effective therapy, at least in the short term, for patients with relapsed / refractory auto-immune haematological disorders. Longer follow-up will be needed to establish if duration of response is comparable to 'traditional' 4 dose schedules. A prospective study of short course rituximab in this context is underway.

## Stem Cell Biology

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#### The uptake and retention of imatinib into haemopoietic cells is not uniform, varying between normal and CML cells, and between mature and primitive cells

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Excellent and durable responses make imatinib the treatment of choice for CML patients. Interpatient response is however variable, and the disease persists in the majority of patients. We have demonstrated, using <sup>14</sup>C imatinib, that intracellular uptake and retention (IUR) of imatinib into PB mononuclear cells (MNC) cells varies between patients, influencing sensitivity to imatinib, and hence response. Further, interpatient variability of IUR is largely determined by variable function of the influx transporter OCT-1 (organic cation transporter 1). We now examine differences in IUR between MNC from normal and CML samples, and between mature (CD34-) and more primitive (CD34+) cells. The imatinib IUR in CML is significantly greater than that seen in normal (IUR CML = 41.0 ng/200,000cells ; IUR Normal = 15.6  $p < 0.001$  ( $n=6$ ) at 2 $\mu$ M). In addition, experiments using a BCR-ABL transduced cell line demonstrate a markedly increased IUR compared to the control cell line (vector only), indicating a possible role for BCR-ABL in the control of IUR. Real time quantitative PCR (RQ-PCR) for expression of hOCT-1 and ABCB1 indicate that normal MNC express less OCT-1 (median 5 fold ) but more ABCB1 (median 20 fold) than CML cells. Isolating CD34+ and CD34- cells from 11 CML patients demonstrates IUR for CD34+ cells is significantly less than for CD34- cells (median IUR CD34+ 14.3; CD34- 36.3,  $p=0.004$  at 2 $\mu$ M). Thus intracellular imatinib concentration may be lower in more primitive cells, suggesting a role for influx/efflux transporters in leukaemia persistence. Current investigations using RQ-PCR aim to elucidate the role of transport proteins in primitive cells. We conclude that the IUR of imatinib varies with leukaemic status and cell maturity. This variability may in part, be controlled by OCT-1 and ABCB1, raising the possibility that drugs modifying the function of these transporters may improve the efficacy of imatinib against leukaemic stem cells.

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#### Human dendritic cells produce T lymphocyte stimulating cytokines

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HSANZ Oral Abstracts, HAA, 15-18 October, 2006

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#### Aim

The outcome of a specific immune event depends on both the dendritic cell (DC) subset initiating the response and the type of activation signal it receives. IL-12 derived from DC has been considered critical for the induction of cytotoxic and type 1 helper T lymphocyte responses. However, both responses can be induced by monocyte-derived DC (MoDC) that secrete low or un-detectable IL-12, suggesting that IL-12-independent mechanisms exist. Recent evidence suggests that macrophages and DC produce IL-2 and IFN- $\gamma$  which may influence the induction of T lymphocyte responses.

#### Methods:

We investigated production of IL-2, IFN- $\gamma$ , and IL-12p70 by CD1c<sup>+</sup> human blood DC (BDC) and MoDC generated in the presence of GM-CSF and IL-4 (IL-4 MoDC), or GM-CSF and IL-15 (IL-15 MoDC), in response to whole *E. coli*, polyinosinic-polycytidylic acid (PIC) or IL-12 and IL-18.

#### Results

IL-2 was not detected in the culture supernatants from any of the DC populations. IL-12 was secreted in high levels by IL-4 MoDC but in low levels by IL-15 MoDC. In contrast, IFN- $\gamma$  was secreted in high levels by IL-15 MoDC but in low levels by IL-4 MoDC. BDC secreted low levels of IL-12 and produced IFN- $\gamma$  only in response to IL-12/18. Levels of these cytokines were also influenced by different culture media or serum supplements. Both IL-4 and IL-15 MoDC activated with PIC were capable of stimulating proliferation of naïve allogeneic CD4<sup>+</sup> T lymphocytes and inducing cytotoxic T lymphocyte responses.

#### Conclusions

This data provides novel information on IFN- $\gamma$  production by human DC and suggests an important role for this cytokine in the induction of T lymphocyte responses. It also highlights the importance of choosing the right conditions for activating DC *in vitro* or *ex vivo*, as this can affect their functional capacity, and must be considered in clinical studies.

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### **B Cells suppress IL-2, IFN- $\gamma$ and TNF- $\alpha$ production from alloaggressive CD8<sup>+</sup> T cells in a mismatched murine model of bone marrow transplant**

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B cells have been variously shown to induce direct tolerance of antigen specific CD8<sup>+</sup> T cells in models of autoimmunity. We have previously shown that resting B cells also inhibit anti-tumor T cell function and suppress Graft versus host disease (GVHD) in a mismatched mouse model.

We have extended these findings to reveal that B cell depletion of the donor graft results in higher IL-2, IFN- $\gamma$  and TNF- $\alpha$  production by engrafting allogeneic T cells. In turn the degree of cytokine production was highly correlated with the degree of weight loss in mice developing GVHD. Conversely, those mice treated with additional resting B cells at the time of marrow infusion showed lower levels of cytokine production from engrafting T cells and subsequently less GVHD. The amount of IFN- $\gamma$  and TNF- $\alpha$  production from alloaggressive T cells was substantially greater when a donor splenocytes model was used as compared to a marrow only model. The clinical score and histological examination of mice undergoing splenocyte engraftment was also substantially different from that observed in the bone marrow model and reflected a hyperacute GVHD syndrome as opposed to clinically relevant, histologically proven acute GVHD.

These findings indicate that resting B cells may regulate T cell activation through regulating T cell homeostasis and suppression of inflammatory cytokines. B cells therefore be used therapeutically to limit GVHD mediated by alloaggressive T cells, whilst pre-transplant B cell depletion may be detrimental to transplant outcome.

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### **Clinical CMRF-56 blood dendritic cell preparations for immunotherapy of multiple myeloma**

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#### Aim

Despite advances in therapy, such as stem cell transplantation and new drugs such as the proteasome inhibitors, multiple myeloma (MM) remains incurable for most patients. Immune therapy, which uses dendritic cells (DC) to generate cytotoxic T lymphocyte (CTL) responses targeting residual disease, represents a promising strategy, compatible with other therapies. We have shown that blood DC can be isolated from patients with MM and that they induce CTL to control antigens (J Immunotherapy 2005;28:322.). The CMRF-56 HSNZ Oral Abstracts, HAA, 15-18 October, 2006

monoclonal antibody (mAb) detects an antigen expressed on blood DC, which is expressed after a short *in vitro* culture period. To create a clinically feasible DC purification platform for clinical investigation of DC immunotherapy, we developed a method for single step purification of BDC using CMRF-56 monoclonal antibody (mAb) immunoselection.

#### Methods and Results

Positive immunoselection using the CMRF-56 with antibody produces a blood DC enriched population that contains some additional CMRF-56 positive B cells and monocytes. We have now developed and validated a procedure for isolation of CMRF-56+ blood DC using the CliniMACS device. Apheresed mononuclear cell preparations were cultured, labelled with antibody and DC immunoselected in a closed system, free of human or animal serum supplements. This system is compatible with regulatory requirements for cell therapy. Simultaneously, our team has developed a bioprocess for production of the murine biotinylated CMRF-56 antibody compatible with large scale synthesis required for clinical trials. The bioprocess incorporates components that remove contaminating DNA, host cell proteins, viral particles and endotoxin. We have now successfully tested the method and our clinical grade CMRF-56 mAb preparation for blood DC isolation. We have completed and report on our experience with clinical scale DC preparations from healthy donors (n=10) and MM patients (n=2). There were sufficient BDC isolated from both the healthy donors and MM patients for the planned vaccination schedule. These immunoselected DC can be loaded with antigen, either peptide, protein or messenger RNA to induce immune responses in MM patients. CMRF-56 DC can stimulate cytotoxic T cell responses against MM tumour associated antigens (HM1.24, MUC-1) and tumour cell lysates.

#### Conclusions

These studies, when additional studies on MM patients are completed, provide the necessary pre-clinical validation data to commence a Phase I Clinical Trial.

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### **Mesenchymal stromal cells can be mobilised and augmented in the bone marrow following cytokine administration**

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#### Background and Aim

Mesenchymal stromal cells (MSCs) are multipotential cells that could be used for a variety of therapeutic opportunities including facilitation of haemopoietic stem cell transplantation. Although a potential therapeutic advantage of MSCs is their ready propagation *in vitro* using relatively simple and reproducible techniques, there is concern that *ex vivo* culture may alter phenotype or genotype. Therefore, we have explored the potential to increase the number of marrow MSC *in vivo*, and to induce mobilisation into the peripheral blood, using various cytokine regimens in a nonhuman primate model.

#### Methods

Male baboons received subcutaneous cytokines as follows: 1. G-CSF 100mcg/kg/day for 5 days; 2. pegylated G-CSF (pegG-CSF), single dose 300mcg/kg day -5; 3. G-CSF 100mcg/kg/day + stem cell factor (SCF) 50mcg/kg/day for 5 days; and 4. pegylated megakaryocyte growth and development factor (pegMGDF) 1mcg/kg second daily for 10 days + G-CSF 100mcg/kg/day for 5 days starting day -5 before the harvest. Bone marrow was aspirated from the iliac crest at baseline and on the final day of cytokine administration. Mononuclear cells were isolated on Ficoll-Paque Plus, and plated in triplicate in alpha-MEM plus 20% FCS for fibroblast colony forming cells (CFU-F). Peripheral blood mononuclear cells harvested using leucapheresis were assessed to detect possible mobilisation of CFU-F.

#### Results

An increase in the number of bone marrow CFU-Fs was observed compared to baseline as follows: 4, 2.1, 7.6 and 11.2 after G-CSF (n=4, p<0.05), pegG-CSF (n=4, p>0.05), G-CSF+SCF (n=5, p<0.01) and G-CSF+pegMGDF (n=4, p<0.05) respectively. Neither immunophenotype nor differentiation potential was affected by the administration of cytokines. CFU-F were not detected in baseline peripheral blood mononuclear cells from any animal. However, CFU-F were detected in 3/5 animals after G-CSF+SCF at a frequency of 0.8/mL to 1.5/mL, but no other cytokine regimen.

#### Conclusion

These data confirm that cytokine regimens used to mobilise haemopoietic stem cells can be used to induce mobilisation of MSCs and augment their number in the bone marrow. Such regimens may facilitate the harvest and culture of these cells for a burgeoning number of potential therapeutic applications.

## Telomere length of donor cord blood cells predicts survival following cord blood transplantation in children

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### Aim

Cord blood (CB) transplantation is one approach currently used to treat leukemia, aplastic anemia and inherited genetic disorders. To date, the only way to predict a successful outcome for a CB transplant is based on the number of nucleated cells infused, however this method remains crude and many patients still die post-transplant. A more precise means is required to predict, *prior* to transplant, which CB units will be most suitable to provide successful hematopoietic recovery following transplant. Telomeres are found at the ends of chromosomes. Telomere length provides information about both the proliferative *history* and proliferative *potential* of a particular cell. The aim of this study was to investigate if telomere length of donor CB cells can predict transplant outcome.

### Methods

We used a flow cytometry method, flow-FISH, to retrospectively measure the telomere length of cells from 15 donor CB units that were transplanted into children, and correlated this information with transplant outcome.

### Results

All 7 patients transplanted with CB cells with long telomeres are surviving after 2 – 10 years. In striking contrast, of the 8 patients transplanted with CB cells with short telomeres only 3 are still alive. Despite the small cohort, this difference is statistically significant ( $p = 0.03$ ). Telomere length did not correlate with rate of engraftment. All of these patients were transplanted with at least  $2 \times 10^7$  nucleated cells / kg and yet, despite ensuring a sufficient cell dose, a successful transplant outcome did not always ensue.

### Conclusions

Our findings suggest that telomere length of transplanted CB cells may be a powerful predictor of transplant outcome, though not by the anticipated correlation with engraftment kinetics, but by an as yet unclear mechanism impacting on long term survival. Where there is a choice in CB units available for transplant, once cell dose and HLA-match have been considered, selecting a CB unit with longer telomeres may increase the likelihood of survival post-transplant.

## Stem Cell Transplant-Toxicity

## Severe non-haematological toxicity of fractionated total body irradiation and high dose etoposide (FTBI/VP16) as allograft conditioning is limited to mucositis

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### Aim

FTBI/VP16 (1320cGY in 11 fractions of 120cGY over 4 days; etoposide 60mg/kg) is a widely used conditioning for allografting, but detailed assessment of the non-haematological toxicities, particularly mucositis, has not been published. This retrospective audit aimed to address the non-haematological toxicities in patients receiving cyclosporin (CSA) and methotrexate (MTX) as GVHD prophylaxis.

### Methods

A retrospective review of patients undergoing FTBI-VP16 allografts at RMH between 1992-2006 who received CSA/MTX.

### Results

Toxicity data were available in 38 of 42 pts; adequate data could not be obtained for the remaining 4 patients. The majority underwent sibling peripheral stem cell allografts for advanced lymphoid malignancies. All except two patients experienced grade 3-4 mucositis and 50% required MTX dose reduction or omission. All except one patient required total parenteral nutrition (TPN) and IV narcotic analgesia for a median of 18 and 21.5 days respectively. Significant narcotic side effects occurred in 55% of patients. Platelet transfusion support above normal and/or anti-fibrinolytic therapy to reduce mucosal bleeding was required in 63% of patients. In contrast, other grade 3-4 non-haematological toxicities were uncommon; 2 patients developed steroid-responsive early onset pericarditis. The average inpatient stay was 35 days (range 25-84). There were no transplant-related deaths in the first 100 days. Analyses of the relationship between severity of mucositis and age, previous chemotherapy, MTX deliverability, duration of TPN and MTHFR C677T genotype will be presented.

### Conclusion

FTBI-VP16 is intensely mucositic and therefore, an ideal regimen to assess the efficacy of novel mucoprotective agents. A pilot protocol evaluating the mucosal protective effect of keratinocyte growth-factor (palifermin) in these patients is underway.

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### Cost analysis of bone marrow transplantation: impact of degree of mucositis

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#### Aim

To perform an analysis of the costs associated with bone marrow transplantation (BMT) and assess the impact of mucositis on these costs at a single institution in Australia.

#### Methods

The costs associated with patients undergoing BMT at the Alfred who were discharged from July 2004 to June 2005 were quantified. Data were compiled on patients with a BMT ICD Procedure Code and a BMT Diagnosis Group code of A07Z, A08A or A08B. Costs were determined through data extracts from the hospital's clinical costing system and through retrospective medical record review. Incidence, duration and grading of mucositis were quantified using NCI Common Toxicity Criteria for Adverse Events. Revenue offsets were calculated from coded Weighted Inlier Equivalent Separations (WIES), Section 100 and Section 85 revenue for these patients.

#### Results

There were twenty-two (22) patients included in the analysis. The principle diagnosis was AML (15 patients, 68%) ALL (3 patients, 14%) and CML (3 patients, 14%). Twenty-one (21) patients experienced mucositis with 10 of these also experiencing bacteraemia, febrile neutropenia and/or fungal infections. Fourteen patients required total parental nutrition support. The total cost of the hospital admissions was \$1,817,496 (average \$82,613, range \$28,856-\$276,989) and average length of stay was 37.2 days (range 15 to 90 days). Five patients had ICU admissions ranging from 3 to 24 days with total ICU costs of \$162,242 and total episode costs of \$809,945 (average:\$161,989). Total pharmaceutical costs for all 22 patients was \$767,520 which was 42% of total costs. The average pharmaceutical cost was \$36,334 for patients with oral mucositis compared to \$4,516 without.

#### Conclusion

The average cost of the BMT episode was \$82,613 with pharmaceuticals accounting for 42% of these costs with the incidence of mucositis having a major impact. The magnitude of the economic impact of mucositis requires further evaluation.

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### Thromboembolic complications post allogeneic haematopoietic stem-cell transplantation

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#### Aim

Venous thromboembolic (VTE) events are frequently observed in patients undergoing haematopoietic stem cell transplants (HSCT), especially in patients with Graft-versus-Host Disease (GVHD) – the reason for which is unclear. We performed a retrospective analysis of patients undergoing allogeneic HSCT in a single centre to determine the incidence of VTE events, in particular those events that were not line-related, and evaluate possible determining factors including prothrombotic markers including elevated FVIII.

#### Method

Retrospective analysis of all allogeneic HSCT recipients from The Royal Melbourne Hospital Bone Marrow Transplant Unit over a seven year period. VTE events were recorded, as well as type of conditioning, sibling versus unrelated and presence or absence of GVHD, both acute and chronic. Review of all prothrombotic markers performed at the time was done.

#### Results

Of 301 allogeneic HSCTs (109 matched related, 106 reduced intensity, 70 matched unrelated, 12 reduced intensity matched unrelated and 4 syngeneic) there were 17 episodes of VTE (incidence 5.64%). Seven were line-related. Of non-line-related episodes, only 1 occurred before Day 100. Of the late occurring episodes, only 1 did not have active GVHD. 2 patients were heterozygous for Prothrombin Gene Mutation, 1 activated Protein C resistance and 1 anticardiolipin antibody positive. Of the 8 patients who had FVIII levels checked, 7 were elevated.

#### Conclusions

VTEs are not uncommon events in this patient population. Chronic GVHD does appear to be a significant risk factor for VTE. FVIII appeared to be elevated in the majority of cases in which it was tested. To determine whether it is causative requires further study.

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### Lung transplantation after allogeneic stem cell transplant

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HSANZ Oral Abstracts, HAA, 15-18 October, 2006

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#### Aim

Pulmonary complications cause significant morbidity and mortality after allogeneic stem cell transplant. We aim to describe local experience with lung transplantation as a therapeutic option in allogeneic stem cell transplant (SCT) patients with end-stage lung disease

#### Methods

Cases were identified via search of database information from St Vincent's Hospital and personal contact with lung transplant units in Australia. Information from medical records of identified cases was analysed. Pathology specimens of the end stage lung were reviewed.

#### Results

Five cases have been identified. All received HLA matched allogeneic transplant from related donors (four sibling, one maternal) for haematological disease (three CML, one AML, one aplastic anaemia). Conditioning was with Cy/Bu in four patients and Cy/TBI in one. Graft versus Host Disease (GVHD) prophylaxis was with CsA/MTX. Acute GVHD (grades II-III) occurred in two patients. Chronic GVHD other than lung occurred in three. Pathology review confirmed the diagnosis of bronchiolitis obliterans (BO) in three patients, interstitial fibrosis in a fourth, and a mixed process in the fifth. Time from SCT to lung transplant ranged from 23 to 125 months. Four of five patients are alive after lung transplant with no evidence of relapse of haematological malignancy (range +8 to +74 months). One patient died three years after lung transplant from PTLN. The remaining patients maintain normal pulmonary function. Morbidity in the living patients includes GORD, osteoporosis, hypertension, mild renal impairment, and opportunistic infections (CMV, aspergillus colonization, MAC reactivation, herpes zoster and simplex). Marrow function is normal and performance status ECOG 0-1 in the surviving patients.

#### Conclusion

Lung transplantation is a viable therapeutic option for patients with prior SCT and end-stage lung disease. Interesting questions requiring further research include those related to causative processes, HLA matching, stem cell dose, prevention and therapy of end stage lung disease. Ongoing immunosuppression does not appear to affect recipient bone marrow function or contribute to relapse.

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### **Haploidentical stem cell transplantation in children using CD3/CD19 depleted grafts: first report of novel treatment in Australia**

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#### Aim

To describe a novel form of T-cell depletion suitable for haploidentical stem cell transplantation using the CliniMACS device.

#### Methods

4 patients who had suffered a bone marrow relapse after mismatched or unrelated BMT for acute leukaemia were counselled on the highly lethal nature of their relapse and recommended palliative therapy. 3 had already had a CD34-selected T-cell depleted graft and we were not willing to run a high risk of severe GvHD with a second non-depleted procedure. With informed consent, a haploidentical relative was mobilised with G-CSF and the peripheral blood was depleted of blood CD3+ T-cells and CD19+ B-cells by large scale immunomagnetic depletion using the CliniMACS instrument. The balance was processed by CD 34 selection. BMT conditioning was fludarabine, thiopeta, melphalan and OKT-3.

#### Results

After CD3/CD19-depletion haploidentical grafts contained a median of  $13.6 \times 10^6$  (range,  $7.1-21.5 \times 10^6$ ) CD34+ cells/kg and  $6.9 \times 10^4$  (range,  $0.6-16 \times 10^4$ ) T-cells/kg. A mixture of cells from the CD34 selection and CD3/CD19 depletion were used to maximise CD34 dose reinfused and minimise T-cell contamination.

3 patients were transplanted; one patient died of systemic fungal infection during her reinduction therapy.

Two patients with ALL have been followed long enough to monitor their level of MRD in the marrow post BMT. The first patient achieved molecular remission for the first time in over two years of therapy, but then died of progressive neurological problems in haematological and molecular remission. The second patient is well in morphological remission at day +182 and has a reducing level of disease by MRD.

#### Conclusion

Patients relapsing after BMT have a poor prognosis. Use of a CD 3 depleted haploidentical graft provides significant numbers of CD34-

negative progenitors, NK cells, dendritic cells, and other facilitating cells. This may exert an effective graft versus leukaemia effect that can salvage this group of patients.

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### **Daclizumab has poor efficacy in steroid-refractory severe acute graft versus host disease: a single centre experience with 12 allograft patients**

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#### Aim

Effectiveness of Daclizumab in steroid-refractory acute graft versus host disease (aGVHD).

#### Methods

We performed a retrospective audit of the outcome of 12 consecutive allograft patients who had been treated with Daclizumab for steroid-refractory (defined as  $\geq 2$  mg/kg/d of iv Methylprednisolone for  $\geq 3$  days) grade III-IV aGVHD from year 2000 to 2004 in our transplant unit. All patients had received standard GVHD prophylaxis using Cyclosporin and Methotrexate (except one patient due to the absence of GVHD in a previous transplant). They were also given standard anti-microbial prophylaxis and pre-emptively monitored against cytomegalovirus. Clinical grading of biopsy-confirmed aGVHD was performed according to standard criteria. Daclizumab was given at a dose of 1 mg/kg iv on days 1, 4, 8, 15 and 22. Treatment response at day 43 of aGVHD was defined according to Przepiorka et al.

#### Results

Twelve patients developed severe grade III-IV aGVHD after HLA-matched blood stem cell allogeneic transplants, who consisted of 9 sibling (7 ablative, 2 reduced-intensity) and 3 unrelated (1 ablative, 2 reduced-intensity) allografts. Acute GVHD occurred at a median of 27 days (range; 11-128). 25% of patients with skin, 50% with gut and 33% with liver of grade III or more involvement. Daclizumab was commenced at a median of 8½ days after failing methylprednisolone (range; 3-28). Typically these patients also received numerous (3-7) concomitant GVHD therapies. The only complete responder and the single partial responder eventually died of progressive GVHD and sepsis. There was no long-term survivor with infections as the main contributing terminal events in 10 of the 12 patients.

#### Conclusions

In contrast to initial published reports, allograft patients with severe steroid-refractory aGVHD when treated with Daclizumab in our institution had poor response, high incidence of infective complications and dismal outcome. Although 58% of our patients survived at day 120, we had virtually no long term survivors with majority succumbing from infections. In fact, the data from Willenbacher *et al* also suggested similarly unfavourable long term outcome, a high incidence of infectious complications including CMV.

Possible factors are:

(a) Severity of baseline aGVHD - All our patients had severe grade III/IV aGVHD.

(b)

Timing of Daclizumab - Daclizumab was commenced at a median of 2.5-3.5 days later in our series compared with published studies.

(c) Concomitant use of corticosteroid - Whereas two previous studies had tried to wean patients off steroids 7 days after the start of Daclizumab therapy, we were uncomfortable to do so due to the severity of the aGVHD in our patients. Lee *et al* had reported that Daclizumab combined with steroids for the initial treatment of aGVHD increased mortality without benefit to aGVHD.

As a result of this audit, we have moved away from the use of Daclizumab and back to h-ATG as salvage aGVHD therapy since 2005. It was our major concern that the poor survival may have been contributed by the delay of more appropriate aGVHD therapy and that Daclizumab might have contributed to infectious complications.

## **Mouse Models of Human Disease**

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### **Mouse models of germinal center-derived B cell Non-Hodgkin lymphomas**

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Diffuse large B-cell lymphoma (DLBCL), the most common type of human B cell lymphoma, is a biologically and clinically heterogeneous disease which is still poorly understood in its pathogenesis and is incurable in over 50% of cases. This is partly due to incomplete knowledge about the role of *BCL6*, the gene most frequently altered in DLBCL, as well as to the lack of animal models that recapitulate both the genetics and the biology of the disease.

To investigate the role of BCL6 in DLBCL pathogenesis, we have recently engineered a mouse model where BCL6 is expressed constitutively in mature B cells, by knock-in insertion of a tagged (HA) murine BCL6 coding sequence downstream of the immunoglobulin heavy chain  $\mu$  promoter, thereby mimicking a chromosomal translocation which occurs frequently in human DLBCL. The resulting mice ( $\mu$ HA-BCL6) display increased germinal center (GC) formation and perturbed post-GC differentiation, characterized by a decreased number of post-isotype switch plasma cells. Subsequently, these mice develop a lymphoproliferative syndrome that culminates—in up to 70% of the animals—with the development of clonal B cell lymphomas displaying most of the critical features of the corresponding human DLBCL<sup>1</sup>. Importantly, analysis of the rearranged immunoglobulin V genes (IgV) from lymphoma biopsies revealed the presence of somatic mutations in 80% of the cases, documenting their GC/post-GC origin. In addition, SKY analysis showed complex clonal cytogenetic alterations, including translocations or trisomy of chromosome 15, observed in 13 of 16 tumors analyzed.

We then tested whether deregulated BCL6 expression can cooperate with other oncogenic lesions occurring in human lymphomas, such as cMYC deregulation, which is found in a sizable fraction of DLBCL. To this end, the  $\mu$ HA-BCL6 mice were crossed with mice ( $\square$ MYC) carrying a cMYC transgene under the control of  $\square$ chain regulatory sequences, which normally develop lymphomas of pre-GC B origin (IgV unmutated)<sup>2</sup>. When compared to  $\square$ MYC littermates,  $\square$ MYC/  $\mu$ HA-BCL6 mice displayed accelerated tumor development ( $p < 0.03$ ) and a phenotypic shift of the tumor to post-GC derived lymphomas, as defined by the presence of somatically mutated IgV genes and upregulation of post-GC markers (CD138), thereby indicating a cooperative effect between BCL6 and cMYC. Notably, both  $\mu$ HABCL6 and  $\square$ MYC/ $\mu$ HABCL6-induced oncogenesis could be dramatically modulated by disruption of activation induced cytidine deaminase (AID), the gene required for both CSR and SHM, and presumably implicated in the generation of genetic lesions accompanying GC-derived lymphomas.

These results demonstrate the oncogenic role of BCL6 in the pathogenesis of DLBCL and provide a faithful mouse model of this common disease, which recapitulates a genetic lesion occurring in human tumors and may be instrumental for testing novel therapeutic strategies targeted to BCL6.

In addition, our studies provide direct support to the notion that AID-mediated mistakes in antigen receptor gene modification events (CSR and SHM) represent major contributors to B-NHL pathogenesis.

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### **Acute lymphoblastic leukaemia**

Richard Lock

## **ANZBT HSANZ ASTH Combined**

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### **Pregnancy and venous thromboembolism**

Saskia Middeldorp

The risk of venous thromboembolism is increased during pregnancy and the postpartum period and is estimated to be approximately 1 in 1000 pregnancies. In western countries, pulmonary embolism is the leading cause of maternal death. In pregnant women, risk factors for venous thromboembolism are similar as in non-pregnant patients and include surgery (i.e. caesarian section), immobilization, a personal or family history of venous thromboembolism, hereditary thrombophilia, and an increased body weight. Several aspects of venous thromboembolism in pregnant patients deserve attention. The diagnostic work-up for a suspected deep vein thrombosis or pulmonary embolism appears more complicated in pregnant women. For the treatment of antepartum venous thromboembolism, effects of anticoagulants on mother and fetus should be taken into account. Management of anticoagulants close to delivery requires a multidisciplinary approach to the pregnant patients with recent thrombosis. Finally, many uncertainties exist with respect to the optimal management of women who are considered at high risk for pregnancy-associated venous thromboembolism. These include women with a personal history of venous thromboembolism, as well as known carriers of inherited thrombophilia or women with a strong family history for venous thromboembolism. In this lecture, an overview of the available evidence of these various aspects as well as practical issues will be discussed.

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### **Control of critical bleeding in obstetrics**

Claire McLintock

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### **Iron homeostasis and the pathophysiology of haemochromatosis**

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Dean for Basic Sciences and Graduate Studies and the Leland Fikes Professor of Pediatrics, Harvard Medical School, Boston, Massachusetts

Genetic hemochromatosis is caused by mutations in genes encoding HFE, transferrin receptor-2 (TFR2), hepcidin, hemojuvelin (HJV) or ferroportin. Loss of function mutations in HFE and TFR2 cause adult onset hemochromatosis. Loss-of-function mutations in HJV and hepcidin cause severe, early onset hemochromatosis. We and others have shown that all of these diseases result from perturbation of a hepcidin/ferroportin regulatory axis that normal controls iron export from absorptive intestinal cells and tissue macrophages. Hepcidin is a circulating hormone that controls systemic iron homeostasis. Ferroportin is a key cellular iron transporter that is downregulated by hepcidin binding. Hemochromatosis-associated mutations in ferroportin are believed to interfere with its regulation by hepcidin. The functions of the proteins encoded by HFE, TFR2 and HJV were not obvious, but all were inferred to be involved in regulation of hepcidin production. With collaborators, we showed that HJV acts as a co-receptor for bone morphogenetic proteins (BMPs). HJV interacts with BMP receptors and BMP ligands to activate SMAD proteins that directly induce hepcidin expression. We asked whether HFE and TFR2 might also participate in this signal transduction pathway. We found that both HFE and TFR2 associate with HJV in a stable protein complex. HFE acts to amplify BMP signaling, presumably as part of a larger complex including the hemochromatosis-associated proteins and BMP receptors. TFR2 prevents the release of a soluble form of HJV that has been shown by others to inhibit hepcidin expression, suggesting that its function in the complex is to stabilize HJV. Our observations provide an explanation for the similar clinical features of genetic hemochromatosis disorders due to mutations in these five genes.

## CML and Myeloproliferative Diseases

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### Increased bone formation and secondary hyperparathyroidism following initiation of therapy with imatinib mesylate

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#### Aim

Imatinib mesylate is now first line therapy for the majority of patients with chronic myeloid leukaemia (CML). The molecular targets of imatinib are expressed in several tissues, including bone cells, with interruption of c-abl and c-kit signalling inducing osteopenia in mice. The objective of this study was to assess the effects of imatinib on bone and mineral metabolism in patients with CML.

#### Methods

Nine subjects with newly diagnosed bcr-abl positive CML treated with imatinib 400 mg daily were followed prospectively for 6 months. Fasting serum, plasma and urine samples were collected at baseline, 3 and 6 months and analysed for bone metabolic markers in addition to calcium, creatinine, albumin and phosphate levels.

#### Results

The bone formation markers, serum PINP and osteocalcin, increased significantly from baseline after 3 months imatinib therapy ( $33.2 \pm 3.5$   $\mu\text{g/L}$  to  $73.4 \pm 13.5$   $\mu\text{g/L}$ ,  $p < 0.05$  and  $11.2 \pm 1.9$   $\mu\text{g/L}$  to  $26.2 \pm 3.9$   $\mu\text{g/L}$ ,  $p < 0.01$  respectively), with the PINP similar to baseline at 6 months, whereas osteocalcin levels remained elevated. There was no change in serum BCTX, a marker of bone resorption, and no change in bone density at hip or spine after 6 months. Serum calcium levels were lower than baseline at 3 and 6 months, with a two-fold increase in intact PTH levels at 3 and 6 months. This was associated with a fall in the serum phosphate at both 3 and 6 months with an increase in urinary phosphate excretion.

#### Conclusions

These results show that initial imatinib therapy in patients with CML is associated with an increase in bone formation with no change in bone resorption. This is associated with the development of secondary hyperparathyroidism that promotes phosphaturia and a fall in the serum phosphate. Calcium supplements to prevent secondary hyperparathyroidism may need to be considered in patients starting imatinib therapy.

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### Intrinsic sensitivity to imatinib measured by $\text{IC}_{50}^{\text{imatinib}}$ is a better predictor of good molecular response than a functional assay of imatinib uptake and retention, and measurement of OCT-1 mRNA levels

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Assays of primary CML cells that assess cellular transport of imatinib and other ABL kinase inhibitors may facilitate individualized therapy in CML. Using <sup>14</sup>C imatinib and a 2 hour assay we demonstrated that intracellular uptake and retention (IUR) of imatinib into CML mononuclear cells is variable, and the main determinant of interpatient variation observed in the assessment of IC<sub>50</sub><sup>imatinib</sup>. Using inhibitors we also demonstrated the human organic cation transporter 1 (OCT-1) is the main influx protein involved in imatinib transport. We now confirm a strong correlation between the IC<sub>50</sub><sup>imatinib</sup> and the IUR ( $p < 0.001$ ). Using RQ-PCR we observe a strong correlation between the level of OCT-1 mRNA and the IUR for imatinib at 2 hours ( $p < 0.001$ ). Low OCT-1 expression has been associated with inferior molecular response, however not all patients with high OCT-1 mRNA expression achieve good molecular response, suggesting that high OCT-1 expression alone may be insufficient. Further, there was no correlation between the IC<sub>50</sub><sup>imatinib</sup> and OCT-1 expression. While IC<sub>50</sub><sup>imatinib</sup> is predictive of molecular response to 12 months (MMR by 12 months: low IC<sub>50</sub><sup>imatinib</sup> (n=32) 56% ; high IC<sub>50</sub><sup>imatinib</sup> (n=20) 25%  $p = 0.02$ ), the IUR and OCT-1 mRNA level are not predictive (low IUR (n=23) 48% vs high IUR (n=23) 43%  $p > 0.05$ ; Low OCT-1 (n=15) 47%; high OCT-1 (n=16) 56%  $p > 0.05$ ). This suggests that intrinsic sensitivity of patients to imatinib induced kinase inhibition is related to the functional activity of OCT-1 (IUR), but not directly related to the level of OCT-1 mRNA. Assays to detect expression (mRNA and protein) of the key efflux proteins are being performed. We conclude that interpatient variation in IC<sub>50</sub><sup>imatinib</sup> is primarily determined by variation in imatinib uptake and retention mediated by OCT-1, but other factors are also measured. Speculatively these factors may be related to the BCR-ABL imatinib interaction, but active efflux, or a drug-transporter interaction cannot be discounted, and are being investigated.

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## Sub acute Budd-Chiari syndrome associated with venous web in a young patient with essential thrombocythaemia

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### Introduction

Hepatic vein thrombosis is associated with a conglomeration of clinical and pathological findings collectively known as the Budd-Chiari syndrome. These include hepatic sinusoidal dilatation and congestion, ascites and abdominal pain, as a result of hepatic outflow block.(1) In a published study of 460 consecutive patients with essential thrombocythemia (ET), the incidence of abdominal vein thrombosis (AVT) was only 4 percent.(2). The overall risk of thrombosis in ET was 6.6%/patient-year, versus 1.2%/patient-year in the control population. (3) Although hepatic vein thrombosis is associated with ET, it has been rarely described in conjunction with a normal platelet count. (4) Here, we are reporting a patient with sub acute Budd-Chiari syndrome associated with ET and a unilateral hepatic vein web in a patient with normal platelet count.

### Case History

A 41-year-old woman presented with abdominal pain, bloating, nausea, fatigue and symptoms of breathlessness of 4 weeks duration. She previously had Hashimoto's thyroiditis on sertraline and was euthyroid. She had a brief period of postnatal depression two years previously. She used to smoke minimally and drink alcohol socially. One year ago, her platelets were 800 X 10<sup>9</sup>/L. Her platelet count was 340 X 10<sup>9</sup>/L when she presented to the hospital. ALT level was 71 U/L (N: < 37), AST was 20 U/L (N: < 36),  $\gamma$ -glutamyltranspeptidase level was 129 (N: < 36), Alkaline phosphatase was 104 U/L with normal LDH, fibrinogen and prothombin time. Haptoglobin was 2.06 g/l (N: 0.28–1.78). An abdominal contrast CT scan showed an invisible left hepatic vein, suggesting a thrombus in left hepatic vein and features of hepatic congestion in the territory of left and middle hepatic veins, consistent with sub acute Budd-Chiari syndrome.. An urgent hepatic venogram was done which revealed a thrombus and obliteration of the left hepatic vein. A thorough evaluation for a hypercoagulable state was performed: homocysteine level: 8.1  $\mu$ mol/l (N: < 15); antithrombin III: 86% (N: 75–125); protein C: 86% (N: 80–151); free protein S: 103% (N: 50–130); APC resistance was 3.1 (>2.0); factor V Leiden: not detected; anticardiolipin antibodies: negative; antinuclear antibodies were present in low titres only. Bone marrow biopsy was performed, even though the platelet count was normal at the time of presentation, considering the past history of transient high platelet count. The results were consistent with Essential thrombocythemia. The results of JAK-2 mutation studies on peripheral blood were positive, hence confirming the diagnosis of ET.

Using a transjugular and trans femoral approach, direct venography was performed after a difficult cannulation of the left and middle hepatic vein, demonstrating a "spider-web" network pattern, seen in the right hepatic vein. The hepatic venous pressure gradient showed moderate portal hypertension, with a value of 9 mmHg (N: < 4). Considering the clinical symptoms and the presence of a web in the right hepatic vein and thrombus at the left hepatic vein, an urgent balloon angioplasty was done. Low molecular weight heparin was commenced at a therapeutic level, and subsequently switched to oral Warfarin. Abdominal pain and ascites resolved. Her platelet count started increasing and was 453 X 10<sup>9</sup>/L 3 months after the procedure.

A trans jugular Liver biopsy done through right hepatic vein was essentially normal with no features of any pre-existing liver disease, and no evidence of any plasma cells infiltration or interface hepatitis that may suggest the presence of auto-immune hepatitis

At three months follow up, she is on a stable dose of warfarin and is symptom free.

#### Conclusion

Essential Thrombocythemia should be considered as a possible aetiology in Budd-Chiari syndrome even with a normal base line platelet count

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### **TaqMan quantitation of JAK2 V617F mutational load: A sensitive diagnostic tool that reflects clonal proliferation.**

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#### Aims

To establish a sensitive diagnostic tool for myeloproliferative disorders (MPD) and examine its relevance to clinical management.

#### Methods

A TaqMan PCR assay was designed for the specific and quantitative determination of DNA copy number of JAK2 V617F. Copy number was calculated in absolute units by comparing the JAK2V617F signal generated by the test samples to that generated by a set of external plasmid standards containing the sequence of interest. Samples from West Australian individuals referred for diagnosis of MPD (n=125) and among healthy controls (n=18) were typed and results assessed for possible associations between the occurrence and copy number of the mutation and clinical and laboratory co-variables.

#### Results

The assay demonstrated linearity over a 5 log range and was sensitive to less than 0.05% in clinical dilutions. Performance attributes included excellence in reproducibility (intra- and inter-assay coefficients of variation=34% and 36 %, respectively), PCR efficiency (99%) and correlation coefficients of standard curves (mean=0.97) (all over n=10 assays). Mutational load was <0.006 copies JAK2 V617F/cell among controls and ranged from <0.006 to > 3 among putative cases. The occurrence of the mutation correlated with increased WCC at the time of diagnosis (P=0.012; n=30) and on treatment (P=0.026; n=25). JAK2 V617F levels (above 0.08 copies per cell) positively correlated with haemoglobin (P value for slope =0.08, n=15), WCC at date of diagnosis (n=11) and PRV-1 over-expression (n= 16).

#### Conclusion

The high sensitivity and specificity of the assay to detect the JAK2 mutation provides a novel clinical tool for disease diagnosis and reflects proliferation of the mutant clone. Our findings suggest disease evolution involves gene duplication beyond 2 copies per cell. Further investigation is needed to determine whether variations in JAK2 V617F load may impart treatment relevant information.

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### **Predictive value of spontaneous erythroid colony formation for thrombotic events in Essential Thrombocytosis (ET)**

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#### Background

Essential Thrombocytosis (ET) is complicated by thrombotic events, which occur in both the arterial and venous circulations. At the present, thrombotic events in ET are difficult to predict with current risk stratification based on a simple combination of clinical factors. There is no routinely available laboratory test which is predictive of thrombotic events. We aimed to assess the predictive value of spontaneous erythroid colony (SEC) culture for thrombotic events in these patients.

#### Methods

A retrospective review of consecutive patients with ET who also had SEC testing at the Princess Alexandra Hospital was performed. ET was diagnosed according to the WHO criteria. SEC testing was performed at the time of diagnosis, using a previously validated, simplified method in routine use at our institution (Tey, SK et al, Clin Lab Haem, 2004). Data was gathered from the medical record regarding the clinical characteristics and presence of thrombotic events in these patients.

#### Results

37 patients with ET were identified: median age 56yrs; 56% female; median platelet count 770 x 10<sup>12</sup>/l at diagnosis; 8,5 and 23 being low, intermediate and high risk, respectively. 16 (44%) had a positive SEC assay. 13 (36%) and 4 (11%) developed arterial and venous

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thrombotic complications. Median follow up was 13 months (range, 1 to 260 months). Outcomes according to SEC results are as follows:

	SEC positive	SEC negative	P value
N	16	20	
Venous event	4	0	P= 0.03
Microvascular event	6	1	P= 0.03
Arterial event	5	8	P= 0.73

\*erythromyelgia

The venous and microvascular events were all diagnosed at or just prior to diagnosis of ET. No other clinical factor (age, sex, risk group, diabetes, smoking, hypertension, platelet count) was predictive of thrombotic events.

Conclusion

Patients with ET who have spontaneous erythroid colony growth are at increased risk of venous thrombotic and microvascular events. Updated results incorporating JAK2 mutational status will also be presented.

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### Assessing the relationship between JAK2 mutation status and platelet function abnormalities in myeloproliferative diseases

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Background

Myeloproliferative diseases are characterized by effective proliferation of one or more myeloid lineages and by thrombohaemorrhagic complications. Recently, the JAK2 (V617F) mutation has been described in 65-97% of patients with polycythemia vera (PCV), 23-57% essential thrombocythemia (ET) and 35-57% idiopathic myelofibrosis (IMF). Platelet function abnormalities are common in myeloproliferative diseases (75-83%). There is debate regarding the association between platelet function abnormalities and clinical outcomes. This study aimed to correlate JAK2 mutation status, platelet function and clinical phenotype.

Methods

Of the 52 patients with myeloproliferative disease evaluated, 25 patients had platelet function tests (PFT) available. We also studied 18 healthy controls. Granulocytic DNA was extracted and Taqman real time-PCR was performed to provide a quantitative expression of the V617F mutation. Standard platelet aggregometry and mepacrine labelling of platelet dense granules was used to evaluate platelet function.

Results

All 18 healthy controls were negative for the V617F mutation. The mutation was present in 15/24 (62%) patients with PCV, 13/23 (57%) patients with ET and 1/5 (20%) patients with IMF. Of the patients who had available results, 18(72%) patients had abnormal PFT. 67% of the PCV patients, 83% of the ET patients and 50% pts with IMF had abnormal PFT. There was no correlation between JAK2 mutation status and the PFT results. Of the patients with the V617F mutation, 73% had abnormal PFT compared with 72% amongst the patients without the V617F mutation. There was no correlation between JAK2 mutation status and clinical symptoms of thrombosis or haemorrhage. However amongst the patients with PCV and ET, 50% who had abnormal PFT had symptoms whilst none of the patients with normal PFT had symptoms.

Conclusion

We found no correlation between JAK2 mutation status and results of PFT. However, amongst patients who express the wild type (WT) JAK2 gene, platelet aggregometry and mepacrine labeling of the platelets still serve as useful additional diagnostic tools. The relatively high percentage of patients with WT JAK2 gene having abnormal platelet function suggests that the JAK2 signalling pathway is not the primary pathway involved in the platelet function abnormalities that characterize myeloproliferative disorders.

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There was no correlation between JAK2 mutation status and symptoms but we did find a significant correlation between abnormal platelet function tests and symptoms.

## Myeloma

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### Serum free light chains as a predictor of disease response to autologous stem cell transplantation in myeloma

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#### Purpose

The serum free light chain assay (FLC) is a new method for FLC measurement in patients with plasma cell dyscrasias. This study retrospectively evaluates whether a reduction in the abnormal FLC concentration pre-autologous stem cell transplantation (ASCT) compared to diagnosis predicts response post-ASCT in myeloma patients.

#### Method

We performed a retrospective review of forty-one consecutive patients who received ASCT from Feb 2003 to April 2006. Forty-four per cent (18/41) had serial FLC measurements and were eligible for inclusion. Disease response was defined according to international criteria.

#### Results

Of the 18 patients, 61% (11/18) had intact immunoglobulin secreting myeloma (IIMM); there were equal numbers of light chain myelomas (LCMM) and plasmacytomas, three each and one patient had non-secretory myeloma (NSM). There were 11 males; median age 56.5 years, range 35-71 years; there were four, four and ten Stage I, II and III patients respectively. Seventy-eight per cent (14/18) had abnormal FLC measurements at diagnosis, with the remainder (4/18) excluded due to normal FLC measurements (2 of each, IIMM or plasmacytoma). The median follow-up was 18 months, range 8-89 months. Eight of 14 reduced their FLC concentration by fifty per cent with initial chemotherapy. Seven of 14 achieved complete remission (CR) or very good partial remission (vgPR) post-ASCT. There was no correlation between pre-ASCT FLC reduction and achievement of CR/vgPR post-ASCT. Of eight patients who reduced their FLC concentration by fifty per cent, half went on to achieve CR/vgPR; of the remaining six patients (with less than fifty per cent reduction) half achieved CR/vgPR as well. Amongst nine patients with IIMM, there was no trend to CR/vgPR if the FLC concentration reduced by fifty per cent.

#### Conclusion

This data suggests that a reduction in the abnormal serum FLC concentration pre-ASCT compared to measurements at diagnosis does not predict disease response post-ASCT in myeloma. Larger prospective studies are required to validate these results.

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### Impact of cyclin D1 and p53 expression on bortezomib response in myeloma.

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#### Background

Bortezomib, a potent reversible proteasome inhibitor can disrupt the activity of many important intracellular proteins including p53, cyclin-dependent kinases and NF- $\kappa$ B.

#### Aim

To evaluate the significance of cyclin D1 and p53 expression as predictors of response to bortezomib in patients with relapsed/refractory myeloma.

#### Methods

24 patients with relapsed and refractory multiple myeloma were treated with standard doses of Bortezomib at 1.3 mg/m<sup>2</sup> day 1,4,8 and 11 until progression or death. Immunohistochemistry was performed on B5 fixed trephines with anti-cyclin D1 antibody (Clone SP-4, Lab Vision, Fremont, CA) and anti-p53 (Clone DO7, Neomarkers, Newcastle on Tyne, UK).

#### Results

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The median age was 59.5 years (range 39-75 years) comprising 12 females and 12 males. Seventeen (70.8%) had stage 3A disease (Salmon Durie classification) at diagnosis. Thirteen (54.2%) had an elevated  $\beta$ 2 microglobulin prior to bortezomib. Median number of prior treatment regimens was 3 (range 2-5), 18 (75%) had received  $\geq 3$  regimens, 20 (83%) received high dose therapy with stem cell rescue. Following a median of 4 cycles of bortezomib (range 1-12) there were 2 complete response (CR), 2 near complete response (nCR), 7 partial response (PR), 3 minimal response (MR), 6 no change (NC), 2 progressive disease (PD), 2 not evaluable (NE). Responders (CR, nCR, PR) were observed in 46% patients overall. Cyclin D1 expression was positive in 5 cases (21%) with 3 responders (60%). Cyclin D1 negative cases showed 8 (42%) responders. P53 immunostaining was positive in 11 (46%) with 5 responders. Interestingly the 2 CR and 2 PD patients were in this group.

#### Conclusion

Despite biologic rationale, cyclin D1 and P53 immunostaining did not appear to predict response to bortezomib in this small heavily pretreated cohort. Other cyclin kinases that have not been assessed and P53 may be important in the activity of bortezomib and warrant further investigation.

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### Predicting response of multiple myelomas to proteasome inhibitors

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#### Aim

To understand the role of the unfolded protein response (UPR) in sensitivity to Proteasome inhibitors (PI) and evaluate the utility of UPR regulator XBP-1 for predicting responses.

#### Background

Bortezomib is remarkably efficacious in relapsed multiple myeloma (MM). One of its effects is disruption of the unfolded protein response (UPR), which prevents accumulation of misfolded proteins in the endoplasmic reticulum. Transcription factor XBP-1 regulates the UPR via an unconventional mechanism of splicing *XBP-1* mRNA. XBP-1 is highly expressed in myeloma and is essential for plasma cell development. MM is more susceptible to apoptosis when XBP-1 is knocked down by siRNA. Proteasome inhibitors downregulate spliced, active XBP-1 and upregulate the unspliced, inactive form. We hypothesize that MM's dependence on UPR/XBP-1 renders it sensitive to PI and that the level of spliced versus unspliced XBP-1 affects the sensitivity.

#### Method

Myeloma cell lines were assessed for their sensitivity to Bortezomib by proliferation inhibition assay. Total *XBP-1* mRNA was quantitated by QPCR, validated by Northern analysis. The ratio of spliced:unspliced XBP-1 was determined by densitometry of PCR products resolved on polyacrylamide gels, using a Kodak Image Station.

#### Results

Unspliced XBP-1 levels were inversely related to Bortezomib sensitivity (Pearson coefficient  $r = -0.8$ ) (Figure 1).

#### Conclusion

High levels of unspliced XBP-1 may predict sensitivity to PI. This tentative relationship is now being checked in human myeloma samples, correlating with clinical response to Bortezomib.

Figure 1: The IC50 of Bortezomib and the levels of unspliced xbp-1 in myeloma cell lines.

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### The focal adhesion kinase inhibitor (fak) tae226 exhibits in vitro and in vivo activity against multiple myeloma

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Multiple Myeloma (MM) is an incurable malignancy of terminally differentiated B-cells characterised by both de novo and acquired resistance to presently available cytotoxic therapeutic agents. TAE226 (Novartis) is a potent and specific inhibitor of focal adhesion kinase (FAK) phosphorylation that also has the capacity to inhibit IGF1-R at sub-micromolar concentrations. FAK inhibition has been shown to abrogate intra-cellular phosphorylation of both ERK and AKT and to have anti-tumour effects in murine solid-tumour xenografts. Based on these data we have undertaken a pre-clinical evaluation of TAE226 as a potential therapeutic for MM. Eight genetically heterogeneous human myeloma cell lines (HMCL) (KMS11, KMS12, JIM1, LP-1, U266, RPMI8226, NCI H929, OPM2) were evaluated by MTS assay following exposure to TAE226 at various concentrations for 24 to 72 hours. All HMCL displayed reductions in viability (45% to 100% at 72

hours with 10µM) that were dose and time-dependent, with broadly effective TAE226 concentrations of 1 - 10µM. Under standard culture conditions 5µM TAE226 was markedly cytostatic with inhibition of cellular proliferation observed over a 72 hour time period and with cell viability at 72 hours based on trypan blue exclusion in the range of 40 – 90%. Four HMCL with modest (KMS11/U266) or high (LP-8, OPM2) TAE226 sensitivity were evaluated further using 5µM TAE226 for 48 hours. AnnexinV expression confirmed apoptosis induction while PhosFlow measurement of phospho-p44/42 MAPK confirmed abrogation of intra-cellular ERK phosphorylation – relative reduction following TAE226 compared to untreated controls of 11% KMS-11, 13% U266, 35% OPM2 and 64% LP-1. The induction of pronounced supra-additive killing of NCI H929 was observed by combining TAE226 (1µM) with sub-lethal concentrations of either bortezomib (5nM) or adriamycin (10µg/ml) with the scheduling of TAE226 prior to drug partner clearly superior than the reverse order. Primary MM cells from patients with advanced relapsed disease were treated after appropriate informed consent. Cell killing as determined by propidium iodide expression varied widely but with a clear dose-dependency – 5µM (n = 9) median 15%, range 0% - 46%; 10µM (n = 8) median 17%, range 0% - 61%; and, 20µM (n = 7) median 51%, range 9% - 67%. Finally, we tested TAE226 in the 5T33 murine model of systemic myelomatosis. C57BL/KaLwRiJ hosts were treated with TAE226 30mg/kg (n = 9) or vehicle (n = 9) for 14 days from day +7. Median time to hind limb paralysis post-treatment was prolonged in the TAE226 group compared to vehicle treated animals, 16 days vs 9 days, respectively (p = 0.07, Log Rank). We conclude based on these preliminary data that TAE226 warrants further evaluation as a potential therapeutic for MM.

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### **CMRF-56 Immunoselected blood dendritic cells acquire a mature phenotype and function after brief exposure to maturation stimuli and prime efficient multiple myeloma specific cytotoxic T cell responses**

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#### AIM

Dendritic cell (DC) therapy is a promising treatment for the eradication of minimal residual disease in multiple myeloma patients who have undergone autologous stem cell transplantation and chemotherapy. Blood dendritic cells (BDC) may have advantages over monocyte-derived DC vaccines in terms of cost, logistics and some functions.

#### Methods and Results

CMRF-56 immunoselection generates a BDC enriched cell population, which contains predominantly CD11c<sup>+</sup> BDC and few CD123<sup>hi</sup> plasmacytoid BDC. The isolated CD11c<sup>+</sup> BDC include BDCA1<sup>+</sup>, BDCA3<sup>+</sup> and CD16<sup>+</sup> BDC. CMRF-56 BDC display an intermediate BDC phenotype upon isolation, and exposure to DC activators for as little as 2 hours was sufficient to induce a mature BDC phenotype and increase inflammatory cytokine secretion. The optimal conditions for cytotoxic T cell (CTL) priming by CMRF-56 immunoselected BDC was established, using the model tumour antigen MART-1. Brief exposure to GM-CSF proved optimal for the generation of CTL in the model system and also significantly increased CCL21-specific BDC migration with the addition of PGE<sub>2</sub>. Polyclonal whole myeloma T cell responses were induced by the CMRF-56 BDC preparation loaded with lysate from HLA\* A201<sup>+</sup> myeloma cell line U266, with and without GM-CSF activation. GM-CSF activation of the CMRF-56 BDC preparation increased specific CTL mediated lysis of U266 cells, regardless of the proportion of CD8 T cells in the culture (Nil 18.8±4.3% v GMCSF activation 40.9±7.3% U266 specific lysis, n=3, p=0.051). These conditions have been used to expand CTL specific to peptides derived from the myeloma associated antigen HM1.24 in normal HLA\* A201<sup>+</sup> donors.

#### Conclusions

These results indicate that the CMRF-56 BDC preparation, responds in a physiologically predictable manner and do not require prolonged exposure to maturation stimuli to produce functionally and phenotypically mature cells. Their ability to induce effective anti-myeloma CTL responses justifies proceeding with the planned Phase 1 study to test their safety and capacity to induce surrogate markers of an effective immune response.

## 97

### **The development of neuropathy in patients with myeloma treated with thalidomide – patterns of occurrence and the role of electrophysiologic monitoring**

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## Aim

Peripheral neuropathy frequently limits the duration of treatment with thalidomide for patients with multiple myeloma. We assessed the time course of occurrence, possible predictive factors, and the utility of serial nerve electrophysiological studies (NES) for detecting onset of neuropathy.

## Methods

Seventy-five patients with relapsed/refractory myeloma were enrolled in a multi-centre trial of dose-escalating thalidomide ± interferon. Patients underwent clinical assessment plus NES at baseline and every 3 months. Time to development of neuropathy according to clinical or NES criteria was compared. Patient and treatment-related factors were compared as predictors of neuropathy.

## Results

Thirty-nine percent had some NES abnormalities at baseline. Patients received thalidomide at a median dose intensity of 373 mg/day. Thirty-one of 75 patients (41%) developed neuropathy during thalidomide treatment, with 11 (15%) ceasing thalidomide due to neuropathy. The actuarial incidence of neuropathy increased from 38% at 6 months to 73% at 12 months with 81% of responding patients developing this complication. Serial NES did not reliably predict the imminent development of clinical neuropathy requiring thalidomide cessation. Nor were patient age, gender or prior therapy predictive. Patients who developed neuropathy had a longer duration of thalidomide exposure (Median 268 vs 89 days:  $p = 0.0001$ ). Cumulative dose or dose intensity received were not predictive.

## Conclusion

The majority of patients will develop peripheral neuropathy given sufficient length of thalidomide. To minimize the risk of neurotoxicity, therapy should be limited to less than six months. Electrophysiologic monitoring provides no clear benefit over careful clinical evaluation for the development of clinically significant neuropathy.

# Leukaemia Biology

98

## Analysis of human leukaemias and lymphomas using extensive immunophenotypes from an antibody microarray

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## Aim

Leukaemias are currently diagnosed using criteria involving morphology, a limited immunophenotype, cytochemistry and cytogenetics. We have developed a novel cluster of differentiation (CD) antibody microarray that provides an extensive immunophenotype of leukaemia cells. The aim of this project was to assess the diagnostic performance of the microarray against the established criteria for classification.

## Methods

HSANZ Oral Abstracts, HAA, 15-18 October, 2006

The assay uses a microarray of 82 CD antibodies immobilized on a nitrocellulose slide.

Leukocytes are captured on antibody dots (10 nL) that correspond to a particular surface molecule on the cell. A dot pattern is obtained that is the immunophenotype (expression profile, disease signature) for that population of cells.

#### Results

This microarray was used for analysis of samples from 733 patients with a variety of leukaemias and lymphomas from peripheral blood and bone marrow. Discriminant Function Analysis of the expression profiles from these 733 patients and 63 normal subjects were clustered and showed high levels of consistency with diagnoses obtained using conventional clinical and laboratory criteria. The overall levels of consensus for classification using the microarray compared with established criteria were 93.9% (495/527 patients) for peripheral blood and 97.6% (201/206 patients) for bone marrow aspirates showing that the extensive phenotype alone was frequently able to classify the disease when the leukaemic clone was the dominant cell population. Immunophenotypes for neoplastic cells were distinguishable from normal cells when the leukaemic count was at least  $5 \times 10^9$  cells/L in peripheral blood, or 20% of cells obtained from bone marrow aspirates.

#### Discussion

This technique will be a useful adjunct to flow cytometry and other methods when an extensive phenotype of leukaemia cells is desired for clinical trials, research and prognostic factor analysis. An extensive immunophenotype alone may be sufficient for diagnosis of most leukaemias and lymphomas.

## 99

### Unusual FISH patterns in APL lead to identification of a novel fusion gene

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A 66 yr-old-man presented clinical and morphological evidence of acute promyelocytic leukaemia (APL). He commenced ATRA, was enrolled onto the APL4 trial and now shows no evidence of leukaemia 12 months later. However, the karyotype was 47,XY,+22[5]/46,XY[30] with no t(15;17)(q22;q21). FISH with the Vysis LSI PML/RARA dual fusion translocation probe did not show any fusion signals but there was splitting of an *RARA* signal on one 17q. A second probe, the Vysis LSI *RARA* break apart probe, showed deletion of the 5' *RARA* probe and the 3' *RARA* probe appeared to localize more distally than normal. The Cytocell PML/RARA ES probe also showed no fusion signals but one *RARA* signal appeared smaller.

These FISH results were not consistent with any of the known variant translocations involving *RARA*: t(11;17)(q13;q21), causing a *PLZF/RARA* fusion; t(5;17)(q35;q21) involving *NPM* on 5q35 and t(11;17)(q23;q21) producing a *NUMA/RARA* fusion. Two cases of a *STAT5b/RARA* fusion, resulting from a rearrangement of 17q have been reported. It was possible that the FISH pattern in this case could represent a third case of *STAT5b/RARA* fusion. Molecular studies performed at the RPAH, however, identified a novel fusion gene: *PRKAR1A/RARA*<sup>1</sup>. The *PRKAR1A* gene is located distal to *RARA* at 17q24. FISH using a BAC probe (RP11-120M18) encompassing the *PRKAR1A* gene identified signals on both copies of 17q – a strong signal on the normal 17 and a weaker signal on der(17). We hypothesize that at least two genetic events must have occurred. The simplest series of events consistent with our FISH results would be an insertion of *RARA* distal to *PRKAR1A* followed by a deletion removing 3' *PRKAR1A*, 5' *RARA* and any intervening sequences. This case would be the first description to our knowledge of an insertion plus deletion being required to create a leukaemia fusion gene.

1

Catalano A *et al.* The *PRKAR1A* Gene, Encoding the Regulatory Subunit Type-I of Cyclic AMP Dependent Protein Kinase A, is fused to *RARA* in a New Variant Acute Promyelocytic Leukaemia (Abstract, this meeting)

## 100

### The *PRKAR1A* Gene, Encoding the Regulatory Subunit Type-I of Cyclic AMP Dependent Protein Kinase A, is Fused to *RARA* in a New Variant Acute Promyelocytic Leukaemia

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A 66 yr old male, diagnosed with acute promyelocytic leukaemia (APL), had a bone marrow karyotype of 47,XY,+22[5]/46,XY[30] with no t(15;17)(q22;q21). However, FISH indicated that the *RARA* gene was both disrupted and deleted at the 5' end<sup>1</sup>. He was treated with ATRA, idarubicin and arsenic trioxide according to the ALLG APL4 protocol until day 22 when arsenic was ceased due to toxicity.

Morphological and cytogenetic complete remission was documented on day 35.

Using *PML* and *RARA* specific primers, the diagnostic marrow was negative for *PML-RARA* transcripts by RT-PCR, but an atypical product was observed. Sequencing showed partial homology to the *PRKAR1A* gene, encoding the regulatory subunit type I-alpha (RI $\alpha$ ) of cyclic AMP-dependent protein kinase A. RT-PCR using *PRKAR1A* and *RARA* specific primers amplified two transcript splice variants of a *PRKAR1A-RARA* fusion gene.

The shorter out-of-frame fusion transcript lacks *PRKAR1A* exon 3 and encodes a carboxy-truncated RI $\alpha$  protein. The longer in-frame fusion transcript results from cryptic splicing of the first 100 bases of *PRKAR1A* exon 3 to *RARA* exon 3, and encodes a chimeric RI $\alpha$ -retinoic acid receptor  $\alpha$  (RAR $\alpha$ ) fusion protein that contains the dimerization domain RI $\alpha$  and the same carboxy terminal domains of the RAR $\alpha$  that are found in all other known *RARA* rearrangements in APL.

Bone marrow biopsy eleven months from original diagnosis showed no evidence of leukaemia and *PRKAR1A-RARA* RT-PCR was indicative of molecular remission.

The vast majority of APL cases are characterized by the formation of a *PML-RARA* fusion gene. Disruption of RAR $\alpha$  function has also been described in four types of variant APL in which an alternative partner gene (*PLZF*, *NPM*, *NUMA*, or *STAT5B*) is fused to *RARA*. This novel *PRKAR1A-RARA* gene rearrangement is the fifth variant APL in which the *RARA* partner gene has been identified and the second known rearrangement of *PRKAR1A* in a malignant disease.

1

Campbell LJ, Somana K, *et al.* Unusual FISH Patterns in APL Lead to Identification of a Novel Fusion Gene. (abstract, this meeting)

## 101

### Cryptic *PML-RARA* fusions in APL may be difficult to visualize by FISH

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Acute promyelocytic leukaemia (APL) is characterised by the t(15;17)(q22;q21) which is demonstrated by conventional cytogenetics (CC) in most cases. However, there are rare cases of cryptic rearrangements leading to the *PML-RARA* fusion. Cryptic *PML-RARA* fusions in cases where CC does not show the t(15;17) are generally identified by fluorescence in situ hybridization (FISH) and/or RT-PCR. We present 6 cases of APL in whom the diagnosis was made by RT-PCR detection of the *PML-RARA* transcript with both CC and initial FISH studies showing no evidence of t(15;17) or *PML-RARA* fusion. Four patients presented with classical morphology APL and two with variant morphology. All but one patient had a normal karyotype by CC; one had structural abnormalities of 4q and 5q but apparently normal chromosomes 15 and 17. Molecular studies showed 4/6 had breakpoints within *PML* intron 3 (*bcr3*) that results in the short isoform of *PML-RARA*. Initial FISH studies using a dual fusion *PML-RARA* probe in 4 cases and a single fusion probe in 2 cases showed no fusion signal. Subsequent FISH testing with a smaller extra signal probe (Cytocell) clearly showed a single fusion signal on the der(15) in all cases. Careful scrutiny of the dual fusion probe result identified a very small *RARA* signal co-localizing with a normal sized *PML* signal in 3/6 but these fusions were not easily seen. The findings suggest that all 6 had a *PML/RARA* fusion resulting from insertion of a very small segment of DNA containing *RARA* into *PML*. The fusion was difficult to visualize in 3 and not seen in the other 3 cases using standard Vysis FISH probes. The incidence of cryptic rearrangements was 2/25 (8%) and 3/53 (6%) in Auckland and Melbourne, respectively, both higher than previously reported. Thus, more than one FISH probe may be required to demonstrate cryptic *PML-RARA* fusions.

## 102

### Activating mutations in *JAK2* (V617F) and *c-KIT* (D816V) are not mutually exclusive in patients with systemic mastocytosis

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Aim

HSANZ Oral Abstracts, HAA, 15-18 October, 2006

An activating mutation in one type of receptor tyrosine kinase is hypothesised to confer proliferative advantage for a tumour in an individual. We investigated the frequency of the activating point mutations JAK2 V617F and c-KIT D816V in patients with systemic mastocytosis (SM).

#### Methodology

An allele-specific competitive blocker PCR (ACB-PCR) method was used for V617F and D816V mutation detection. The ACB-PCR utilises three primers: (1) targeting the normal allele tagged with a 3' phosphate to block amplification, (2) targeting the mutant allele with mismatches to ensure that the normal allele is not amplified and (3) a reverse primer. The ACB-PCR assays were sensitive to the level  $\leq 1\%$ . No false positives were obtained analysing 20 different normal DNA samples. Detection of V617F using ACB-PCR was performed in 28 WHO-classified SM patients with D816 mutations.

#### Results

Three of the 28 patients with SM and D816 mutations were also JAK2 V617F positive. On review of the clinical and morphological data, patient #1 showed concurrent essential thrombocytosis and SM and was formally classified as SM-with an associated clonal haematological non-mast cell lineage disease. Patient #2 and #3 had SM alone. No second haematologic disorders have been detected with clinical follow up of 60 and 6 months, respectively.

#### Conclusion

This is the first observation of JAK2 mutations occurring in SM patients, not associated with other haematological disorders. The presence of two activating mutations in two different oncogenic tyrosine kinases within the same individual raises the possibility of primary mutations in primitive haematopoietic stem cells with secondary mutations driving the haematological phenotype. This result may indicate pathophysiologically, the potential for a more aggressive clinical disease. Monitoring for development of additional haematological JAK2 positive disorders in these two patients is also appropriate. The presence of a secondary kinase mutation may also be a potential target for therapy in these patients.

## HSANZ Masterclasses

### 110

#### Waldenstrom's macroglobulinaemia

Donna M Weber, M.D.

Anderson Cancer Centre, Medical Oncology/Haematology, University of Texas, USA

### 111

#### New strategies for GVHD prophylaxis

John Wagner

Scientific Director of Clinical Research, Stem Cell Institute University of Minnesota, USA

### 112

#### Risk adapted therapy for AML

David Grimwade

Cancer Genetics Laboratory, Guy's Hospital, London, UK

This masterclass will focus particularly upon pre-treatment prognostic factors used to provide a framework for risk adapted therapy of AML. The mainstay has traditionally been conventional cytogenetics; however this approach has a number of limitations and it is clear that molecular diagnostics provides valuable additional information, not only serving to identify further subgroups of patients who could benefit from molecularly targeted therapies, but also enabling stem cell transplantation and more experimental therapeutic strategies to be more effectively deployed. The potential of minimal residual disease monitoring to provide an additional independent prognostic factor which can further enhance risk-stratified treatment approaches to AML will be discussed.

## Presidential Symposium – Joint HSANZ and ASTH

### 117

#### Real-time quantitative polymerase chain reaction (RQ-PCR) monitoring of minimal residual disease (mrd) in core binding factor acute myeloid leukaemia (cbf aML)

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#### Background

CBF AML, with t(8;21)(q22;q22), inv(16)(p13q22) or t(16;16)(p13;q22) and the associated fusion proteins AML1/ETO or CBF $\beta$ /MYH11, has a favourable clinical prognosis although significant numbers of patients still relapse. We examined the prognostic utility of MRD monitoring by RQ-PCR and propose a simple model for prediction of impending haematological relapse.

#### Methods

Patients with CBF AML had samples collected at diagnosis, after induction and consolidation chemotherapy and at routine regular intervals thereafter. RQ-PCR, using the Applied Biosystems 7700 Sequence Detection System, was performed in triplicate and the final result was calculated by averaging 3 values, expressed relative to PGK levels. Stratified Cox regression was used to assess the impact of predictor variables (diagnostic, post-induction, post-consolidation RQ-PCR levels and presence of a 1 log<sub>10</sub> increase in sequential RQ-PCR levels) on leukaemia-free survival (LFS).

#### Results

Of 46 patients identified with CBF AML, 29 had diagnostic, regular longitudinal samples and clinical follow up allowing further evaluation; 12 AML1-ETO and 17 CBF $\beta$ -MYH11. The median age was 39 years (range 7-68) with 52% male. Median follow up was 34 months (range 1-106) and median sample number was 6 (2-15). Twelve relapses occurred at a median of 11 months (range 4-17) from diagnosis. There were significant differences between transcript levels at diagnosis (median 1.9), post-induction ( $8.96 \times 10^{-04}$ ), post-consolidation ( $5.01 \times 10^{-05}$ ), in remission ( $1 \times 10^{-06}$ ) or relapse (0.15) ( $p=0.01$ ). Diagnostic, post-induction and post-consolidation RQ-PCR levels did not predict outcome. A log<sub>10</sub> rise in a remission bone marrow sample correlated with adverse LFS and imminent risk of haematological relapse (HR 8.6). Relapses occurred a median of 60 days (range 45-272) after a log<sub>10</sub> rise.

RQ-PCR levels	Hazard Ratio(HR) LFS	95% CI	P-value
Diagnosis	0.22	0.02-2.9	0.25
Post-induction	1.3	0.8-2.1	0.36
Post-consolidation	0.8	0.5-1.2	0.27
1 log <sub>10</sub> increase	8.6	1.8-42	0.008

#### Conclusions

A 1 log<sub>10</sub> rise in transcript levels was a highly significant predictor of haematological relapse. Transcript levels at early post-treatment time points did not predict long term outcome, cautioning against de-escalation protocols based on these results. Prospective identification of high risk patients will enable clinical trials to address the efficacy of treatment initiated at molecular progression.

## 118

### Identification of a large deletion in pros1 in a family with type i protein S deficiency

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#### Aim

Congenital protein S deficiency is associated with a 5-10 fold increased risk of venous thromboembolism. Standard mutation screening techniques fail to detect a genetic abnormality in approximately 50% of families with hereditary protein S deficiency. We aimed to identify a protein S mutation in one such family.

#### Method

The proband was a 29-year-old male who presented with a pulmonary embolism after travelling over 3,000 kilometres by car. The patient was found to have type I protein S deficiency. The patients' DNA and mRNA was examined using the previously described combined single stranded conformational polymorphism and heteroduplex analysis (CSHA), which failed to detect a causative mutation. The DNA was further analysed using multiplex ligation-dependent probe amplification (MLPA), revealing the presence of a large deletion. The exact location of the mutation was identified using sequencing. Additional family members were subsequently screened for the mutation.

#### Results

The proband and his paternal grandmother were found to be heterozygous for a 14,091 bp deletion spanning from intron G to intron K and encompassing exons 8-11. Exons 8-11 code for part of the sex-hormone binding globulin-like domain of protein S. This region is important for interactions with activated factor V and C4b-BP.

#### Conclusions

Detection of protein S mutations is complex requiring multiple screening techniques to obtain accurate results. MLPA is a valuable tool for detecting large deletions while traditional screening techniques such as CSHA are required to detect point mutations and small deletions and insertions.

## 119

### The BH3 mimetic, ABT-737, is effective against Bcl-2 overexpressing lymphoid tumors

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#### Aim

Lymphoid tumors often respond poorly to conventional cytotoxics, a common cause being their impaired sensitivity to apoptosis, such as that caused by Bcl-2 overexpression. A strategy to overcoming this is to utilize mimics of the natural antagonists of pro-survival Bcl-2, the BH3-only proteins. One promising BH3 mimetic is ABT-737, which targets Bcl-2 and closely related pro-survival proteins. We evaluated its potential utility by testing it on cell lines, clinical samples or on a mouse lymphoma model.

#### Methods

We assessed the sensitivity of B cell lymphoma cell lines as well as primary CLL samples to ABT-737, either alone or in combination. To ascertain its efficacy *in vivo*, we utilized a mouse model based on the E $\mu$ -myc tumor that is readily transplantable and amenable to genetic manipulation. When syngeneic recipient mice were inoculated with tumors, they develop widespread lymphoma, which is fatal unless treated by agents such as cyclophosphamide.

#### Results

We found that ABT-737, on its own, was cytotoxic only to a subset of cell lines and primary CLL samples. However, it can synergize potently with agents such as dexamethasone, suggesting that this agent might be useful in combination with currently used cytotoxics. In the mouse lymphoma model, we found that ABT-737 was partially effective as a single agent for treating E $\mu$ -myc tumors overexpressing Bcl-2. More strikingly, long-term remissions were achieved when combined with a low dose cyclophosphamide, which was otherwise ineffective.

#### Conclusions

ABT-737 appears to be a promising agent for the clinic. It potently sensitizes certain lymphoid tumors to conventional cytotoxics *in vitro*. Of note, the synergy observed between dexamethasone and ABT-737 suggests that it is attractive for clinical testing. Encouragingly, ABT-737 appeared efficacious *in vivo* against Bcl-2-overexpressing tumors when combined with a reduced dose of cyclophosphamide, suggesting that it will be useful for treating even those Bcl-2-overexpressing chemoresistant tumors.

## 120

### Mesenchymal stem cells for treatment of steroid-resistant graft-versus-host disease

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## Background

Allogeneic haematopoietic stem cell transplantation is a well established treatment for haematological malignancies but can be associated with significant morbidity and mortality, particularly with the development of severe acute graft-versus-host disease (GVHD). Severe steroid-refractory acute GVHD is associated with an 80-90% mortality despite many different immunosuppressive drugs being used.

Mesenchymal stem cells (MSC) are rare, non-haemopoietic cells found in the bone marrow, capable of differentiation into bone, cartilage, muscle and fat cells. In addition to their potential role in tissue repair, MSC have unique immunomodulatory properties. In vitro studies demonstrate they are both immunogenic-immunosuppressive. This property has been exploited in the treatment of severe aGVHD. In a recently reported EBMT study, an overall response rate of 69% was seen in 28 patients with steroid refractory aGVHD.

We report our experience in the use of MSC for GVHD in 3 patients over the last 7 months.

## Cases

MSCs were cultured using a standardised protocol and reagents in class 350 clean rooms.

Doses of MSC ranged from 0.92 to 1.34 x 10<sup>6</sup>/kg. All three recipients had received high dose steroids prior to MSC infusions. The 1<sup>st</sup> recipient (41 yo F) received MSCs on day+55 for grade II-III skin GVHD with improvement noted 7 days later and complete resolution by day+11. She is now 8 months post transplant with no confirmed GVHD on minimal immunosuppression. The 2<sup>nd</sup> recipient (31 yo M) received MSCs on day +39 and day +47 for progressive grade III-IV skin, gut and liver GVHD. ATG and etanercept were also given on day+42. He succumbed on day +54. The 3<sup>rd</sup> recipient (19 yo M) received MSCs on day+ 42 for grade II-III skin GVHD with some encouraging initial response.

## Conclusion

Preliminary studies demonstrate MSC infusion is feasible in the treatment of steroid refractory aGVHD with initial encouraging results. More studies are needed to determine optimal dosing and timing.

## 121 Analysis of the sensitivity of the overall haemostatic potential (OHP) assay to components of the coagulation system

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## Aim

To determine the sensitivity of the OHP assay parameters to changes in components of the coagulation system. To assess changes seen with a modified OHP using tissue factor as the coagulation trigger.

## Methods

In the standard OHP we use a small amount of thrombin to trigger fibrin generation in platelet poor plasma (PPP) with rt-PA added to initiate fibrinolysis. In order to model in vivo events more closely, we developed a modified version of the assay using tissue factor (TF) as the coagulation trigger. We then analysed changes seen in assay parameters after spiking PPP, to alter levels of components of the coagulation system including: antithrombin, fibrinogen, prothrombin and factors V, VII, VIII and X. Results were compared with reference intervals we have established in a healthy Australian population. Method comparisons were analysed using a repeated measures ANOVA.

## Results

Fibrinogen levels (0 to 10g/L) showed a direct correlation with all OHP parameters: OCP (overall coagulation potential), OHP, OFP (overall fibrinolysis potential), maximum OD (Max OD), maximum slope of the OCP curve (Max slope) and delay in onset of fibrin generation. Factors II, VIII and X showed similar correlations for fibrin generation parameters but fibrinolysis was not altered until the individual factor levels were ≤ 5% and therefore clot formation was markedly reduced. Samples from individuals with elevated FVIII levels were associated with increased fibrin generation and reduced fibrinolysis. Lowering of the high FVIII levels reduced fibrin generation into the normal range but fibrinolysis remained reduced in these hypercoagulable individuals. Standard OHP assay parameters were not influenced by factor V and VII until levels were ≤5%, however the TF triggered assay showed correlation for all assay parameters with FV and FVII levels. Reduced antithrombin levels showed more rapid fibrin generation, consistent with a hypercoagulable state.

## Conclusions

We have shown that the OHP is influenced by various components of the coagulation system with both hypocoagulable and hypercoagulable states demonstrated. Fibrin generation and fibrinolysis parameters show different responses to alterations in coagulation factor levels. The modified OHP, triggered by TF, may be more sensitive than the standard assay to abnormalities of Factor V and VII.

## 122 The dominant effect of initial treatment regimen on the survival of patients with chronic lymphocytic leukemia (CLL): an analysis of 616 patients treated at the MD Anderson Cancer Center.

Constantine S Tam<sup>1\*</sup>, Sijen Wen<sup>2</sup>, Kim-Anh Do<sup>2</sup>, Susan Lerner<sup>1</sup>, William G Wierda<sup>1</sup>, Susan O'Brien<sup>1</sup>, Michael J

## Keating<sup>1</sup>

<sup>1</sup>Leukemia and <sup>2</sup>Biostatistics Department, The University of Texas MD Anderson Cancer Center.

### Aims

Successive advances in CLL therapy have led to significant gains in response quality and remission duration but have not improved survival, leading some to question the benefit of an intensive approach to initial treatment. An inherent limitation of randomised trials is the long followup required to demonstrate survival benefit in indolent diseases. In order to examine the effect of initial therapy on survival, we retrospectively analyzed the outcomes of patients treated in frontline protocols at the MD Anderson Cancer Center.

### Methods

Between 10/86 to 01/04, 616 patients received initial therapy with one of three fludarabine-based chemotherapy regimens. Patients in Cohort 1 (F; n=190) received fludarabine±prednisone, Cohort 2 (FC/M; n=140) fludarabine & cyclophosphamide or mitoxantrone, and Cohort 3 (FCR; n=286) FC & rituximab. Patient characteristics: median age 57 years; Rai intermediate/high risk, 60%/36%; abnormal cytogenetics 27%. Median followup was 176, 108 and 56 months for F, FC/M and FCR respectively.

### Results

Median survival from initial therapy was 91 months for the entire cohort. On univariate analysis, significant predictors of survival were (p-value all <0.05): age, hemoglobin, B2-microglobulin(B2m), abnormal cytogenetics, performance status, regimen, Rai stage, albumin and absolute lymphocyte count. In a multivariate model adjusting for all factors, significant predictors for survival were: age (RR 1.05 per year; p<0.0001), FCR regimen (RR 0.42 vs F, 0.41 vs FC/M; p<0.001), high B2m (RR 1.13 per mg/L, p=0.001) and abnormal cytogenetics (RR 1.48, p=0.05). FCR significantly improved survival in the following adverse prognostic groups: age≥60, high-risk Rai stage, B2m ≥4mg/L, performance status ≥2 and abnormal cytogenetics not involving chromosome 17p13. Patients with deletion or abnormality of the 17p13 locus had median survival of <24 months irrespective of treatment regimen.

### Conclusion

The choice of initial therapy significantly determines survival in patients with CLL.

## HSANZ/BMTSANZ Symposium Transplantation

### 125

#### **Multi-unit cord blood transplants – impact on trm and relapse**

John Wagner

Scientific Director of Clinical Research, Stem Cell Institute University of Minnesota, USA

### 126

#### **Severe non haematological toxicity of fractionated total body irradiation and high dose etoposide (FTBI/VP16) as allograft conditioning is limited to mucositis**

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Bone Marrow Transplant Service, The Royal Melbourne Hospital

### Aim

FTBI/VP16 (1320cGY in 11 fractions of 120cGY over 4 days; etoposide 60mg/kg) is a widely used conditioning for allografting, but detailed assessment of the non-haematological toxicities, particularly mucositis, has not been published. This retrospective audit aimed to address the non-haematological toxicities in patients receiving cyclosporin (CSA) and methotrexate (MTX) as GVHD prophylaxis.

### Methods

A retrospective review of patients undergoing FTBI-VP16 allografts at RMH between 1992-2006 who received CSA/MTX.

### Results

Toxicity data were available in 38 of 42 pts; adequate data could not be obtained for the remaining 4 patients. The majority underwent sibling peripheral stem cell allografts for advanced lymphoid malignancies. All except two patients experienced grade 3-4 mucositis and 50% required MTX dose reduction or omission. All except one patient required total parenteral nutrition (TPN) and IV narcotic analgesia for a median of 18 and 21.5 days respectively. Significant narcotic side effects occurred in 55% of patients. Platelet transfusion support above normal and/or anti-fibrinolytic therapy to reduce mucosal bleeding was required in 63% of patients. In contrast, other grade 3-4 non-haematological toxicities were uncommon; 2 patients developed steroid-responsive early onset pericarditis. The average inpatient stay was 35 days (range 25-84). There were no transplant-related deaths in the first 100 days. Analyses of the relationship between severity of mucositis and age, previous chemotherapy, MTX deliverability, duration of TPN and MTHFR C677T genotype will be presented.

### Conclusion

FTBI-VP16 is intensely mucositic and therefore, an ideal regimen to assess the efficacy of novel mucoprotective agents. A pilot protocol HSANZ Oral Abstracts, HAA, 15-18 October, 2006

evaluating the mucosal protective effect of keratinocyte growth-factor (palifermin) in these patients is underway.

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### **Bisphosphonate associated osteonecrosis of the jaws**

Ian Hewson

Osteonecrosis of the jaw (ONJ) is an increasingly common clinical problem in patients with multiple myeloma and other conditions requiring longer term use of bisphosphonates<sup>1</sup>. This overview will summarise recent data about the condition, including diagnosis and recommendations for treatment. Awareness of this condition among patients, haematologists and dentists is essential for prevention and to avoid poor outcomes in established cases.

Reference: <sup>1</sup> MJA 182:17-18, 2005

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### **Quality Assurance Program and Morphology**

Surender Juneja<sup>1</sup> Katherine Marsden<sup>2</sup>

QAP slide discussants: Merrole Cole-Sinclair<sup>3</sup>, David Westerman<sup>4</sup>

<sup>1</sup> Department of Diagnostic Haematology, Royal Melbourne Hospital.

<sup>2</sup> RCPA Quality Assurance Program for Haematology

<sup>3</sup> Department of Diagnostic Haematology, The Alfred Hospital

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The first part of this session will be a discussion of the slides sent to laboratories in the most recent RCPA Haematology QAP Morphology Survey and will include the comments and opinions of two survey participants.

The second part is a presentation of selected unusual and interesting haematology morphology cases. Registrants are encouraged to examine the slides and consider the diagnoses of these cases before this morphology session.

Microscopes and the slides with details of the cases will be available in the Media Room which can be entered from the top exhibition foyer near the Plenary Hall.

## **HSANZ – Symposium Lymphoma**

**133**

### **Novel pathogenetic mechanisms and therapeutic targets in B cell lymphomas**

Laura Pasqualucci, M.D.

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B cell non-Hodgkin's lymphoma (B-NHL) represents a heterogeneous group of cancers arising from various stages of normal B cell differentiation. In most cases, these tumors derive from the malignant transformation of B cells in the germinal center (GC), a unique microenvironment where antigen-stimulated B cells undergo intense proliferation and remodeling of their immunoglobulin (Ig) genes by the process of somatic hypermutation (SHM) and class switch recombination (CSR). The molecular pathogenesis of B-NHL is associated with distinct genetic lesions affecting proto-oncogenes and tumor suppressor genes; these include alterations common to other cancers, such as gene amplification and deletion, but are mostly represented by specific lesions involving mistakes in these two Ig-associated DNA modification events: i) chromosomal translocations, that lead to deregulated expression of oncogenes (BCL1, BCL2, BCL6, cMYC) and are thought to be favored by DNA breaks accompanying VDJ recombination, CSR and SHM; and ii) aberrant somatic hypermutation (ASHM), a novel mechanism of genomic instability by which multiple mutations are introduced in the 5' sequences of a number of genes, likely as the result of SHM misfiring on non-physiologic targets<sup>1</sup>. Recent experimental evidence has led to further understanding of the pathogenetic role of some of these alterations and to the identification of molecular targets with potential therapeutic interest.

Role of BCL6 in lymphomagenesis. A common pathogenetic target of both translocations and SHM and an essential regulator of GC development is the BCL6 proto-oncogene, which encodes a transcriptional repressor necessary for GC formation. Deregulated expression of BCL6 by chromosomal translocation or SHM of its 5' regulatory region<sup>2</sup> is common in DLBCL (~40% of cases) and promotes DLBCL development in transgenic mice<sup>3</sup>. One major function of BCL6 is to repress GC B cell responses to genotoxic stress via direct suppression of p53 transcription or via MIZ1-mediated suppression of the cell-cycle regulator p21<sup>4,5</sup>; these activities are thought to allow the rapid proliferative expansion of GC as well as the execution of the physiologic genomic break/recombination events required for CSR and SHM. Notably, recent studies have identified a signaling pathway that down-regulates BCL6 expression in response to increasing levels of DNA damage and suggest that the ability of GC B cells to sustain genotoxic-stress is regulated, via BCL6, by the level of DNA damage itself. HSANZ Oral Abstracts, HAA, 15-18 October, 2006

These results imply that B-NHLs constitutively expressing BCL6 may be functionally impaired in apoptotic and DNA-damage responses, and that therapeutic targeting of BCL6 may represent an attractive strategy to inactivate the oncogene as well as to restore normal genotoxic responses.

Illegitimate interaction between BCL6 and cMYC in lymphomagenesis. The cMYC proto-oncogene encodes a transcription factor expressed in most proliferating cells, where it controls the expression of a large number of target genes involved in the control of cell growth. Surprisingly, highly proliferating GC B cells do not express cMYC, suggesting that the expression of this oncogene in BL and DLBCL (20% of cases) is ectopic<sup>6</sup>. Recent findings indicate that cMYC is absent in proliferating GC B cells because it is transcriptionally suppressed by BCL6 via specific BCL6 binding sites in the cMYC promoter region. Thus, cMYC escapes BCL6-mediated suppression in lymphoma leading to the co-expression of the two transcription factors, an event never observed in immunohistochemical and gene expression profile analysis of normal GC B cells. Surprisingly, when co-expressed in BL and DLBCL, BCL6 and cMYC are physically bound in a novel complex detectable in DLBCL and BL cell lines as well as in primary lymphoma cases, with significant consequences on the function of both cMYC and BCL6. Notably, the co-expression of cMYC and BCL6 has relevant pathologic effects in vivo, since double transgenic BCL6/cMYC mice display accelerated lymphoma development and the appearance of a novel GC-derived tumor phenotype containing the illegitimate cMYC/BCL6 complex. These results identify a novel mechanism of oncogenic function for BCL6 and cMYC and a tumor-specific protein complex with potential therapeutic interest.

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## 134

### **Bcl-2 family proteins – from biology to cancer therapeutics**

D.C.S. Huang \*, M. F. van Delft, K. D. Mason, A. Wei, C. Vandenberg, L. Chen, S. N. Willis, E. Lee, D. Fairlie, M. G. Hinds, C.L. Scott, C. L. Day, B. J. Smith, P. M. Colman, S. Cory, A. W. Roberts, J. M. Adams  
Walter & Eliza Hall Institute of Medical Research, Australia.

Overactivity of pro-survival Bcl-2 proteins promotes neoplasia and often impairs the response of malignant cells to conventional cytotoxic therapies, a common cause of treatment failure. Consequently, there is great interest in directly targeting the pro-survival proteins to promote apoptosis of tumor cells and to overcome chemoresistance. One promising approach is to mimic the action of their physiological antagonists, the BH3-only proteins.

Apoptosis is initiated when Bcl-2 and its pro-survival relatives are engaged by pro-apoptotic BH3-only proteins, via interaction of its BH3 domain with a groove on the Bcl-2-like proteins. These interactions have been considered promiscuous, but our analysis of the affinity of eight BH3 peptides for five Bcl2-like proteins has revealed that the interactions vary over 10,000-fold in affinity and, accordingly, only certain protein pairs associate inside cells. Bim and Puma potently engaged all the pro-survival proteins comparably. Bad, however, bound tightly to Bcl2, Bcl-x<sub>L</sub> and Bcl-w but only weakly to A1 and not to Mcl-1. Strikingly, Noxa bound only Mcl-1 and A1. In accord with their complementary binding, Bad and Noxa co-operated to induce potent killing. The results suggest that apoptosis relies on selective interactions between particular subsets of these proteins and that it should be feasible to discover BH3-mimetic drugs that inactivate specific pro-survival targets.

Unlike most other putative BH3 mimetics, ABT-737 (Abbott Laboratories) acts in a highly specific manner akin to the BH3-only proteins. ABT-737 triggers highly specific killing mediated by the multi-domain pro-apoptotic proteins Bax and Bak. However, on its own, the biological activity of ABT-737 is modest as it only targets selected pro-survival proteins (Bcl-2, Bcl-x<sub>L</sub>, Bcl-w). Because cell killing usually also requires neutralization of Mcl-1, concomitant targeting of Mcl-1 potently sensitizes a diverse range of cell types to ABT-737 (Bad-like BH3 mimetic), which can then induce efficient killing even of cells over-expressing Bcl-2.

## HSANZ Free Communications 1 – CLL

### 152 **ZAP-70 - the attack of the clones: differences between 2F3.2 and SB70 anti-ZAP-70 FITC conjugates, emphasizing the need for harmonisation for chronic lymphocytic leukaemia prognostication using flow cytometry**

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#### Aims

Zeta-associated protein (ZAP-70) is T-cell receptor signalling molecule. Gene-microarray analysis and recently, flow cytometry have identified the level of ZAP-70 expression in chronic lymphocytic leukaemia (CLL) as a surrogate marker for non-mutational status of the immunoglobulin heavy chain (IgHC) gene, and a significant clinical prognostic indicator. The power of ZAP-70 as a predictive marker in CLL, however, is affected by the current lack of harmonization of flow-cytometric strategies, including the selection of clones and fluorochrome conjugates.

We studied the impact of using two different FITC-conjugated clones for ZAP-70 quantitation in patients with CLL.

#### Methods

20 samples from patients diagnosed with CLL with the characteristic immunophenotype CD5+CD19+CD23+CD20(dim) and surface-immunoglobulin(dim) were analysed following paraformaldehyde fixation (2% final concentration) and saponin permeabilisation (1% final concentration). The two anti-ZAP-70 clones used were 2F3.2-FITC (Upstate) and SB70-FITC (Dako Cytomation). Samples were analysed on a Beckman-Coulter EPICS-XL MCL instrument according to the strategy described by Crespo et. al. to quantify ZAP-70 expression of the CD2-CD56- non-T-cell, non-NK-cell population. Contemporaneous CD38-expression was determined with the anti-CD38-PE clone, HB7 (Becton Dickinson) in 17 evaluable samples.

#### Results

ZAP-70 quantitation using clone SB70-FITC was significantly less compared to clone 2F3.2-FITC (mean difference 24.6%, 95% C.I. 16.8-32.3,  $p < 0.0001$ , two-tailed paired t-test). Although there was weak correlation for ZAP-70 quantitation between the two anti-ZAP-70 clones ( $r = 0.4522$ , 95% C.I. 0.012-0.74,  $R^2 = 0.20$ , Pearson Correlation), there was no relationship for ZAP-70 expression  $> 20\%$  ( $p = 1.00$ , Fisher's exact test). There was also no relationship between CD38 expression  $> 20\%$  and ZAP-70 expression  $> 20\%$  using clone 2F3.2-FITC ( $p = 1.00$ , Fisher's exact test), although a relationship was demonstrated using clone SB70-FITC ( $p = 0.044$ , Fisher's exact test).

#### Conclusion

Selection of the anti-ZAP-70 clone appears to be a critical determinant of ZAP-70 quantitation by flow cytometric assay. Clone selection should be standardised, along with the time to sample analysis, fixation and permeabilisation methodologies, and quantitative flow cytometric strategies to optimise and validate the prognostic and threshold values of ZAP-70 expression against IgHC mutation status and other clinical parameters in CLL.

### 153 **Correlated six-colour flow cytometry to determine prognosticators in CLL: examination of surface antigen expression, intracellular antigen expression, telomere length and P2X7 channel function**

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#### Aim

To establish an algorithm using the expression of a range of membrane and cytoplasmic antigens in Chronic Lymphocytic Leukaemia, correlated with functional assays, telomere length and the 'established' prognosticators of Zap70, IgVH mutation frequencies and CD38 expression. The use of this panel should allow more accurate prediction of cellular behaviour and reduce the reliance on single tests, which have not provided gold standards in CLL prognostication. Our methodologies in ZAP70 analysis together with other markers is aimed at improving reproducibility and interpretation of positive results.

#### Methods

Using six-colour flow cytometry the CD19/CD5 positive CLL tumour cells are examined for surface expression of adhesion and migration molecules together with CD38 expression and intracellular Zap70 and Bcl-2. We determine telomere length using a commercial kit (DakoCytomation), functional activity of P2X7 ATP-dependent channels using a flow cytometric assay and IgVH mutation rates using a PCR based method (A. Morley). Data are examined by supervised and unsupervised hierarchical clustering algorithms.

## Results

A cohort of CLL patients has been investigated. Preliminary analysis reveals the expected correlation between CD38 and Zap70 expression but there are also unexpected relationships including differential expression of CD54 on CD38 low populations, diversity in telomere length and in P2X7 channel activity for each of the defined subpopulations.

## Conclusion

With the availability of new therapies the ability to prognosticate between clinically significant groups of CLL patients is of increasing importance. Six-colour flow cytometry enables the accurate identification of tumour cells with correlated analysis of membrane and cytoplasmic antigen expression, together with functional assays. We propose that this may provide an opportunity for a new prognostic approach which can be standardised to facilitate direct comparison of individual CLL patients analysed at different institutions, whether on or off study protocols.

## 154 Dipeptidyl peptidase (DP) expression in primary B cell populations and cell lines, a basis for the use of DP inhibitors in chronic lymphocytic leukaemia

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## Aims

The Dipeptidyl peptidases (DPs) DP1V/CD26, DP8, DP9 and fibroblast activation protein (FAP) are serine proteases belonging to the S9b family. CD26 is widely expressed on T, B and natural killer (NK) cells and has a number of key immune substrates, including several chemokines such as RANTES and SDF-1. Recent studies have shown that DP inhibition by the inhibitor ValboroPro/PT-100 causes apoptosis of normal PBMC and a certain subset of B-CLL cells. This is suggested to occur primarily through CD26. To this end, ValboroPro is currently in Phase II trials with the anti-CD20 MAb MabThera for treatment of B-CLL. However, this inhibitor has been shown to be non-specific for CD26 and may be inhibiting other members of the DP family, including DP8 and DP9. The aims of this study were to 1) characterize the expression of DP family members on a range of cell lines and primary lymphocyte populations, and 2) investigate the response of these cells to 3 different DP inhibitors in terms of apoptosis induction and cell cycle effects, relative to the expression of DP family members.

## Methods

Peripheral blood lymphocytes (PBL) were isolated from normal human blood using a Ficoll gradient. PBL were seeded in 24-well plates at a concentration of  $1 \times 10^6$  cells/well. Cell lines were cultured in standard conditions. CD26 expression was assessed by flow cytometry using an anti-human CD26 monoclonal antibody. mRNA expression of other DP family members was assessed by quantitative, RT-PCR using TaqMan probes. One of three DP inhibitors, staurosporine (positive control) or vehicle (DMSO or H<sub>2</sub>O) were added to each well at a final concentration of 100µm. Apoptosis of lymphocytes was assessed by flow cytometry using AnnexinV (AV)-FITC and propidium iodide (PI) staining after 8 and 16 hours.

## Results

mRNA expression of DP8 and DP9 was up-regulated in comparison to CD26 in all cell lines. Differences were observed in mRNA expression of DP family members in primary lymphocyte populations. All three inhibitors induced cell death of PBLs after 16 hours (inhibitor 1: 57.3% compared to 10.6% vehicle control; inhibitor 2: 37.3% and inhibitor 3: 46.8% compared to 9.8% vehicle control).

## Conclusion

DPs other than CD26 are expressed on human primary lymphocyte populations and cell lines. DP8 and DP9 are up-regulated compared to CD26 on a number of lymphocytic cell lines. It is possible that DP inhibitors exert their effects through a DP other than CD26. More specific inhibition of DP family members may be achievable through an RNA interference approach.

## 155 Outcome after allogeneic stem cell transplantation in fludarabine-refractory chronic lymphocytic leukaemia

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## Aim

To determine the outcome of myeloablative and reduced-intensity allogeneic transplantation in patients with advanced B-cell chronic lymphocytic leukaemia (CLL) and CLL with increased prolymphocytes (CLL/PL).

## Methods and Patients

Retrospective chart review of thirteen patients with refractory chronic lymphocytic leukaemia (CLL,  $n = 9$ ; CLL/PL,  $n = 4$ ) who underwent HSANZ Oral Abstracts, HAA, 15-18 October, 2006

myeloablative ( $n = 5$ ) or reduced-intensity ( $n = 8$ ) allogeneic transplantation from HLA-identical ( $n = 11$ ) or matched unrelated donors ( $n = 2$ ). Median age at transplant was 52 years (range, 47 – 60) and time from diagnosis was 5.4 years (range, 1.3 – 10.3). All patients had been previously treated with purine analogues, and at transplant all were refractory to these agents, having either achieved less than a PR or progressed within less than 6 months from purine analogue therapy. The median number of prior regimens was 4 (range, 2 – 6), and the median number of prior purine analogue-containing regimens was 2 (range, 1 - 4). Conditioning therapy was cyclophosphamide and total body irradiation in the 5 myeloablative transplants, and fludarabine and melphalan ( $n = 6$ ) or fludarabine and cyclophosphamide ( $n = 2$ ) in the 8 reduced-intensity transplants. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin (CSA) and methotrexate in all patients.

#### Results

The median follow-up was 1852 days (range, 145 – 2325). Seven patients achieved a CR by morphology and flow cytometry, four patients achieved a partial response, and two had stable disease. Six of 7 patients achieving CR did so within 30 – 340 days, and the response of 1 further patient improved from a nodular PR with minimal residual disease (MRD) by flow cytometry to a CR at 1076 days without further therapy. All patients achieving a CR had acute and chronic GVHD. The 2 patients achieving CR who relapsed did so at 609 and 1563 days. The 2 patients achieving a PR who progressed required further treatment at 74 and 194 days. Seven patients have died, of progressive disease ( $n = 2$ ), GVHD with infection ( $n = 4$ ), and fungal infection in the context of progressive disease ( $n = 1$ ). All 4 patients with CLL/PL have died (progressive disease,  $n = 2$ ; GVHD,  $n = 2$ ). Treatment-related mortality was 0% at day 100, 27% at 1 year, and 45% at 2 years. The cumulative incidence of grade II-IV acute GVHD was 91%, whereas limited and extensive chronic GVHD occurred in 42% and 50% of evaluable patients, respectively. At last follow-up, four patients are alive in CR and two patients are alive with disease. Hence, event-free survival is 31% and overall survival is 46%.

#### Conclusions

Among this group of patients we have found inferior progression-free survival, as well as higher incidences of acute and chronic GVHD compared to other studies reporting on either myeloablative or reduced-intensity transplants. We speculate this may be due to older age at transplant or a higher proportion of patients who are truly refractory to purine analogues. Further study is required to evaluate what biological aspects of this patient population render them more prone to transplant-related complications including GVHD.

## HSANZ Free Communications 2 Infection/Supportive Care

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### **A loss of function polymorphism in the macrophage P2X7 receptor increases susceptibility to extrapulmonary tuberculosis.**

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Tuberculosis (TB) is a leading global health problem with over 2 million deaths each year. Most cases of active TB in adults result from reactivation of dormant (primary) infection due to use of steroids or anti-TNF agents, coinfection with HIV, alcohol abuse or advanced age. Epidemiological studies show a genetic predisposition to reactivation of TB. Polymorphisms in the genes for the vitamin D3 receptor, NRAMP, interferon  $\gamma$  promoter and mannose binding lectin have been associated with susceptibility to TB although collectively these account for only a modest part of the genetic risk. Previous studies have shown that the purinergic receptor, P2X<sub>7</sub> is involved in the killing of engulfed mycobacteria by macrophages.

Our previous studies have identified four loss-of-function polymorphisms in the P2X7 gene, the most prevalent being 1513A>C which changes Glu-496 to Ala in the carboxyl tail of the receptor. This polymorphism shows a gene dosage effect such that monocytes from heterozygote subjects have 50% P2X7 function of wildtype subjects. Subjects homozygous for the 1513C allele have complete loss of P2X7 function. Three other severe polymorphisms present in Caucasian subjects at 1-3% prevalence were not found in individuals of Southeast Asian origin. We determined the prevalence of the 1513A>C polymorphism and its association with reactivation of TB in two separate case-control cohorts.

The first cohort were refugees ( $n=86$ ) who had clear CXR and positive tuberculin skin tests on arrival. Subsequent development of TB was identified from the NSW Dept. of Health database. The second cohort comprised patients with active TB ( $n=99$ ) attending clinics. All subjects with positive HIV serology were excluded. Control subjects were ethnically matched ( $n=157$  and 102 for each cohort). The 1513A>C polymorphism was strongly associated with extrapulmonary, but not pulmonary, TB both in the first cohort (OR 3.8,  $p=0.002$ ) and

the second cohort (OR 2.9, p=0-003). The 1513C allele frequencies in the pulmonary TB patients did not differ from the control subjects.

ATP-mediated killing of mycobacteria in vitro was ablated in macrophages from subjects homozygous for the 1513C allele and significantly impaired in macrophages from heterozygous subjects. There was strong correlation between the degree of mycobacterial killing and ATP-induced apoptosis of infected cells.

Thus the 1513C allele increases susceptibility to extrapulmonary TB. This defect is associated with the inability of macrophages to kill phagocytosed mycobacteria via ATP-induced activation of the P2X<sub>7</sub> receptor.

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### A role for the P2X<sub>7</sub>R receptor in the host response to *Toxoplasma gondii* infection

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Infection with *Toxoplasma gondii* represents an important worldwide health problem with over one third of the world population seropositive for past infection. *T. gondii* is an obligate intracellular protozoan that is an important cause of morbidity and mortality in immunocompromised subjects and causes severe congenital defects and foetal loss if infection occurs in pregnancy. We have identified three subjects with severe, symptomatic toxoplasmosis, two with cervical lymphadenopathy and one with intrauterine foetal hydrocephalus requiring termination at 18 weeks gestation.

The P2X<sub>7</sub>R receptor is an ATP-gated cation selective channel that is highly expressed on monocyte/macrophages and that mediates ATP-induced apoptosis and killing of intracellular pathogens including mycobacterial spp. and chlamydial spp. Our previous studies have identified four loss-of-function polymorphisms in the P2X<sub>7</sub>R gene, the most common being 1513A@C which changes Glu<sup>496</sup> to Ala in the carboxyl tail of the receptor. This polymorphism shows a gene dosage effect with heterozygote subjects having 50% normal P2X<sub>7</sub>R function and homozygotes complete loss of function. Three other rare polymorphisms, with prevalence of 1-3% in Caucasian subjects, cause severe loss of function in heterozygote subjects. We examined for the presence of loss of function polymorphisms in P2X<sub>7</sub>R in our three subjects with Toxoplasmosis. We then studied the role of P2X<sub>7</sub>R in the host immune response to *T. gondii* in *in vitro* and *in vivo* experiments.

All three subjects had low or absent P2X<sub>7</sub>R function as measured by ATP induced ethidium influx in monocyte-derived macrophages. Sequencing of the P2X<sub>7</sub>R gene in all three subjects showed the presence of loss of function single nucleotide polymorphisms. The first subject was heterozygote for the Glu<sup>496</sup> to Ala polymorphism, the second subject was double heterozygote for the Arg<sup>307</sup> to Gln and the Thr<sup>357</sup> to Ser polymorphisms and the third subject was double heterozygote for the Arg<sup>307</sup> to Gln and Glu<sup>496</sup> to Ala polymorphisms.

We performed *in vitro* studies using RAW 264.7 murine macrophage cells to show that activation of the P2X<sub>7</sub>R receptor results in significantly increased death of intracellular *T. gondii*. We confirmed that in *in vivo* studies, P2X<sub>7</sub>R knockout mice infected with both the RH and CTG strains of *T. gondii* have increased mortality and reduced time to death.

Therefore, our *in vitro* data suggest that P2X<sub>7</sub>R is required in the host response to infection by *T. gondii*. The clinical study supports the conclusion that low or absent function of the receptor results in severe clinical infection.

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### Identifying Varicella-Zoster virus and *Aspergillus fumigatus* antigens as targets for adoptive immunotherapy

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#### Aims

Infectious agents are responsible for significant morbidity and mortality in patients following hematopoietic stem cell transplantation. The adoptive transfer of pathogen specific T lymphocytes is a promising approach to prevent or treat infection in transplant recipients. The aims of this study were to identify Varicella-Zoster Virus (VZV) and *Aspergillus fumigatus* antigens suitable for the generation of pathogen specific T cells for adoptive immunotherapy.

#### Methods

There were two different approaches to generate pathogen specific T lymphocytes. The first utilised peripheral blood mononuclear cells (PBMCs) transfected with recombinant plasmids encoding putative fungal or viral antigens to stimulate autologous T lymphocytes. After 7 days, cultures were restimulated with transfected PBMCs and expanded from day 14 with OKT3, anti CD28, IL-7 and IL-15 for a further 14 days. The second approach used PBMCs stimulated with heat killed *Aspergillus* spores or a cell lysate of VZV infected human fibroblasts. Cultures were restimulated with irradiated PBMCs pulsed with antigen after 7 day and expanded in the presence of IL-2 (VZV) or IL-7/IL-15

(*Aspergillus*) for up to 14 days.

#### Results

*Aspergillus fumigatus* candidate antigens selected for these experiments included known allergens and homologues to other pathogenic fungal antigens. Sequences encoding AspF4 and AspF16 (allergens), outer membrane protein A (OMPA), galactomannan protein 1 (MP1), heat shock protein 30 (HSP30) and cell wall glycoprotein 1 (gp1) were amplified by polymerase chain reaction and cloned into the mammalian expression vector pEGFPN1. Three known VZV antigens, immediate early transactivator protein 62 (IE62), glycoprotein E (gE) and glycoprotein I (gI) were also cloned. Freshly isolated PBMCs were transfected using nucleofector technology (AMAXA biosystems) with pooled VZV or *A. fumigatus* plasmids to stimulate autologous PBMCs. Total cell number increased 23 and 60 fold for *A. fumigatus* and VZV cultures resulting in a total of  $1.68 \times 10^7$  and  $4.28 \times 10^7$  cells respectively. The phenotype of cells from both cultures were predominantly CD3<sup>+</sup> T cells (94-96%) with CD3<sup>+</sup>CD4<sup>+</sup> (4.6-10%), CD3<sup>+</sup>CD8<sup>+</sup> (68-72%) and CD3<sup>+</sup>CD56<sup>+</sup> (19-24%) subsets. Using the second approach for generating pathogen specific T cells, proliferation was observed in response to stimulation with *Aspergillus* spores or the lysate of VZV infected human fibroblasts. After 21 days  $4.2 \times 10^7$  cells were obtained from the VZV stimulated culture and  $3.2 \times 10^7$  cells were harvested after 28 days from the *Aspergillus fumigatus* culture.

#### Conclusions

Identification of suitable antigen preparations or individual antigens for generating pathogen specific T cells will be important for developing adoptive cell therapy strategies to prevent infectious disease following stem cell transplantation.

## 159

### **Immunosuppression can be safely ceased during chemotherapy for post-transplantation lymphoproliferative disorders (PTLD) in renal transplant patients**

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#### Aim

In the treatment of PTLT, current guidelines for immunosuppression therapy suggest 50% reduction in calcineurin inhibitor drugs with cessation of other immunosuppression. The objective of this study was to assess whether immunosuppression can be *completely ceased* during chemotherapy and recommenced at reduced doses after chemotherapy without deleterious effects on renal graft function.

#### Methods

We performed a retrospective audit of 41 consecutive adult renal transplantation patients who developed PTLT at our institution from. Data was analysed on 20 patients (between 1984 and 2006) who had immunosuppression ceased during chemotherapy. Reduced dose immunosuppression was recommenced 4-6 weeks after chemotherapy. Outcomes were compared against a matched cohort of 40 renal transplant patients *without* PTLT.

#### Results

Median age at diagnosis was 36 years (range, 17-57); with male predominance (85%). Median time to onset of PTLT after transplantation was 114 months (range, 4-276). A majority (18 patients) received prior triple combination immunosuppression (cyclosporine/tacrolimus plus azathioprine/mycophenolate mofetil plus prednisone). All patients received anthracycline-based chemotherapy with additional therapies of rituximab in 6 patients (30%) and radiation therapy in 4 patients (20%). Seventeen patients (85%) attained complete remission. Four patients relapsed in CR. The actuarial five-year survival was 75% and median follow up was 67 months. Six patients (30%) had died at time of analysis (3 due to progressive disease, 2 relapsed disease, 1 unknown). Of the surviving 14 patients, 2 patients had failed renal allografts (1 acute vascular rejection, 1 chronic allograft nephropathy (CAN)) and recommenced haemodialysis; while 2 patients had >25% increment in serum creatinine, assumed secondary to CAN. 10 patients had normal functional allografts with no significant decrement in renal function. There were no significant differences between the study group and the matched cohort with respect to rates of renal allograft loss and chronic allograft nephropathy.

#### Conclusions

Our data suggests that immunosuppression can be safely ceased during chemotherapy for PTLT in renal transplant patients and recommenced at reduced doses (calcineurin inhibitor at 50%, prednisolone  $\leq 6$ mg daily and no third agent) after chemotherapy, without deleterious effects on renal graft function.

## **HSANZ – Free Communications 3 Stem Cell Transplants - Autografts**

## 160

### **Consensus for rainy day autologous stem cell harvests**

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In NSW more than 1800 stem cell harvests are currently in long-term storage, creating a strain on storage capacity. A survey of Haematologists in NSW has shown that for several disease/patient scenarios many clinicians would perform a Rainy Day autologous stem cell Harvest (RDH), for possible future use in transplant eligible patients lacking an HLA matched sibling donor. This practice has implications for stem cell storage in NSW and there is the potential for limited storage capacity to influence clinical decision making. To address this a consent form for limited stem cell storage was drafted and consensus sought on the appropriate indications for long-term storage.

#### Aim

To determine if consensus could be obtained on indications for autologous RDH in NSW.

#### Methods

The RDH Working Party reviewed the literature on the role of autologous transplantation, the relative merits of harvest in first complete or molecular remission, and calculated the likely duration of storage and utilisation rate of autologous stem cells, for several diseases and patient groups. Each disease-specific review was sent to local experts for comment, and then circulated within the BMT Network for further review.

Finally, individual agreement/disagreement with the proposed indications for RDH contained in the final document was surveyed.

#### Results

Responses were received from 46 physicians: 36/53 (68%) member physicians of BMT Network NSW and 10 inter-state respondents. Overall agreement varied between 52 and 93%.

INDICATIONS	% AGREE
CML in CC/CMR (no sibling donor)	63
Follicular Lymphoma in CR1	61
Multiple Myeloma (Sufficient for two ASCTs)	89
Acute Myeloid Leukaemia (Good/Int risk in CR1, no sibling donor)	57
Mantle Cell Lymphoma (CR1)	72
Waldenstroms (Young, CR1 prior to purine analogues)	52
Aggressive Lymphoma (CR1) - 1° mediastinal DLBCL(poor risk) - Low IPI PTCL	67
Paediatric - CBT	93 (14/15)

#### Conclusion

This survey identifies a reasonable consensus amongst NSW Transplant Physicians and will help structure a costing model for the various options for storage of RDH.

## 161 High dose chemotherapy with autologous stem cell transplant for relapsed and refractory germ cell tumours: outcome data from a single institution

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#### Aim

The optimal treatment for relapsed germ cell tumours (GCT) is controversial. High dose chemotherapy (HDT) with autologous stem cell transplant (ASCT) can salvage a proportion of patients with relapsed or primary refractory disease. The optimal selection of patients and timing for HDT remains unknown. We reviewed the outcomes of patients treated at our institution with HDT and ASCT to determine the safety and efficacy of this approach.

#### Methods

Chart review of all patients treated with HDT and ASCT at Sir Charles Gairdner Hospital between September 1996 and February 2006.

#### Results

Fourteen patients (two female) were treated. Six had double transplants.

At diagnosis, five patients had poor, five intermediate, two good and two unknown prognostic markers (International Germ Cell Cancer Collaborative Group prognostic model)<sup>1</sup>. Seven patients were refractory to initial cisplatin containing chemotherapy (four poor, two intermediate and one good prognostic features).

Relapsed or refractory disease was treated with VIP or modified VIP salvage chemotherapy in 13/14 patients. Two patients had no response to salvage chemotherapy, five had improvement without normalisation in markers and four had normalisation of tumour markers post salvage therapy. One patient had reduced disease by radiological appearance although the marker response was unknown. The two remaining patients had normal markers at the time of relapse.

Six of seven patients treated prior to 2004 had ifosfamide, mesna, etoposide and cisplatin (IMEC) conditioning. One received CMEC and this was the only patient to have a double transplant during this period. One patient developed renal impairment and peripheral neuropathy as a complication of IMEC conditioning therapy. All seven patients transplanted from 2004 onwards had a conditioning regimen of carboplatin and etoposide and five had double transplants. Five sustained hearing loss and four also developed peripheral neuropathy. Bone marrow recovery and immediate complications were as expected for this therapy. Most patients suffered moderate to severe mucositis.

Patients were followed up for a median of 22 months (range 3 months to 9 years) post SCT. Four patients (29%) died from relapse at 7, 12, 21 and 47 months post transplant. Two had poor prognostic features at diagnosis, one intermediate and one unknown. Three had progressive disease with initial chemotherapy. One had no response to salvage chemotherapy, the others had reduced tumour markers, but they did not normalise. Two had marker evidence of relapse by three months post transplant (all by 1 year). Ten patients (71%) remain alive. Four have less than one year of follow up (one relapsed at three months, was treated with surgical resection and has no evidence of disease at nine months). The other six patients (43%) have no evidence of disease recurrence after two to nine years follow up.

#### Conclusion

High dose therapy and autologous stem cell transplantation is an effective treatment that can result in long term survival in some patients with relapsed germ cell tumours. The major long-term side effects are peripheral neuropathy and sensorineural hearing loss.

## 162 An audit of outcomes with Stemgen mobilisation in patients who have failed previously with G-CSF

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#### Aim

To examine the efficacy of Stemgen as a second-line mobilisation strategy in patients with haematological malignancy, in whom a prior

attempt at stem cell mobilisation was unsuccessful with chemotherapy and G-CSF.

#### Methods

Chart reviews of patients who received Stemgen were identified from departmental and pharmacy records in Sir Charles Gairdner Hospital from 2000 to 2006.

#### Results

Twenty-one patients were identified, 13 male (62%) and 8 female (38%), with a median age of 56 (range: 27-70). Their diseases were NHL (n=6), myeloma (MM, n=11), AML (n=2) and germ cell tumour (n=2). In NHL patients, the median number of prior therapies was 3 (range 2- 4). Prior therapies included CHOP/R-CHOP (n=5), hyper-CVAD (n=2), various second line salvage regimens (n= 10), FMD (n=1) and other (n= 5). The median number of prior therapies for the MM patients was 2 (range 1-7). Prior systemic therapies for MM included VAD (n=8), melphalan with prednisolone (n=5), thalidomide (n=5), DCEP (n=3), cyclophosphamide with prednisolone (n=2), CTD (n=2), PCAB (n=2), DTPACE (n=1) and prior autologous transplant (n=3). One patient with AML was harvested electively in CR1; the other was harvested in CR2 after a second cycle of Ida-FLAG. Prior therapies for germ cell tumour included BEP as primary therapy and salvage with VIP (n=2). The initial mobilisation regimen consisted of chemotherapy specific for their disease and G-CSF or cyclophosphamide with G-CSF. Second line mobilisation was performed with chemotherapy, G-CSF 5mcg /kg bd and Stemgen 20mcg/kg sc daily.

Mobilisation was successful in 16 of the 21 patients (76%). In MM, this included 9 of 11 patients; in NHL only 2/ 6 were successful; in AML 2/2 and in germ cell tumours 2/2. The time to collection ranged from 5 to 20 days with a median of 10 days. The number of phereses required were 2 (n=9), 3 (n=5) or 4 (n=2). Cell doses collected ranged from 1.6 to 12.5x10<sup>6</sup>/kg with a mean of 3.8 x10<sup>6</sup> CD34+ cells/kg. Fourteen of the 16 (88%) successful collections subsequently went onto autologous transplant. Reasons for not proceeding to transplant were ongoing remission (n=1, AML) or concurrent septicaemia and pancytopenia (n=1, MM). Absolute neutrophil count (ANC) >1x10<sup>9</sup>/L was achieved at a median 15 days (mean 21, range 10-75). Platelet count of >20x10<sup>9</sup>/L was achieved at a median 12 days (mean 13, range 7-20). In the two NHL patients who underwent PBSCT, both relapsed and died within 4 months of transplant.

#### Conclusion

Second mobilisation can be achieved with the addition of Stemgen in most patients who have failed prior mobilisation with chemo/G-CSF. Subsequent post-transplant engraftment is satisfactory.

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### **Absolute number of transplanted CD34<sup>+</sup> cells expressing c-mpl (CD110) correlates with speed of platelet engraftment following autologous stem cell transplantation**

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Recovery of neutrophil numbers after peripheral blood stem cell transplantation (PBSCT) is closely associated with graft CD34<sup>+</sup> cell dose. Predicting the speed of platelet recovery is more difficult but would be of value given that a significant minority of patients experience delayed platelet recovery and bleeding complications after transplantation. In this study we retrospectively analysed the graft composition of 41 patients who underwent autologous transplantation, using peripheral blood stem cells to assess the utility of CD110 (c-mpl) expression on CD34<sup>+</sup> cells as a predictor of platelet engraftment (ie. time to platelet count greater than 20 x 10<sup>9</sup>/L for seven consecutive days without the need for platelet transfusion).

Absolute CD34<sup>+</sup> cells and CD34 subsets expressing CD110 were enumerated using an in-house single platform viable CD34 flow cytometry assay (BMT, 2005).

Of the 41 patients 9 required at least 21 days for platelet engraftment. These patients received a median graft dose of 5.5 x 10<sup>4</sup> CD34<sup>+</sup>CD110+ cells/kg compared with a median dose of 16.0 x 10<sup>4</sup> cells/kg received by patients who experienced platelet engraftment within 21 days of transplant (p=0.002). In contrast, there was no difference in the number of CD34<sup>+</sup> cells/kg infused (4.6 v 3.0 x 10<sup>6</sup>/kg for < versus >21 days to platelet engraftment respectively, p=0.07). There was a poor correlation between the absolute number of CD34+ cells and the number of CD34+CD110+ cells in the graft (r2 = 0.48). Similarly there was no correlation between the percentage of CD34+ cells expressing c-mpl and the speed of platelet engraftment (8.1 v 5.8%) for > or < 21 days for platelet engraftment respectively, p=0.39). Patients with >21 days platelet engraftment received platelet transfusions more often than those with <21 days for platelet engraftment (median 9 v 2 transfusions, p <0.001). Statistical analysis was performed using two-tailed, non parametric Mann Whitney U-test.

The absolute number of CD34+/CD110+ cells /kg infused at time of transplantation appears to be an important factor identifying patients at risk of delayed (>21 days) platelet engraftment. Those with <6 x 10<sup>4</sup> are at particularly high risk of delayed (>21 days) platelet engraftment requiring multiple transfusion after transplantation.

## **HSANZ – Free Communications 1 – Acute Leukaemia**

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HSANZ Oral Abstracts, HAA, 15-18 October, 2006

## **The use of Hyper-CVAD for acute lymphoblastic leukaemia outside of the M.D. Anderson**

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### **Aim**

The Hyper-CVAD regimen is widely used locally as first-line therapy for patients with acute lymphoblastic leukaemia (ALL), however the published evidence regarding its use is based on the single-centre experience of the M.D. Anderson Cancer Center. We aim to review the use of this regimen in our local setting to determine if the published outcomes can be reproduced.

### **Methods**

All patients treated with Hyper-CVAD for precursor B- or T-cell ALL at the Princess Alexandra Hospital from 1996-2006 and at the Royal Brisbane and Women's Hospital from 1999-2006 were retrospectively identified and hospital records reviewed.

### **Results**

48 patients were identified, with a median age of 30 years (range 16-76 including 6 patients (12.5%) over the age of 60). 40/48 (83%) of subjects had a B-cell phenotype. A t(9;22) translocation was found in 3 of 47 (6.4%) patients with cytogenetic analysis available. Complete response was achieved in 41/48 (85%) of patients. Three patients (6.2%), all under the age of 60, died during the first cycle of therapy from septic complications. Two patients were deemed refractory and proceeded to alternative therapies, and two patients (aged 55 and 76) refused further intensive therapy after the first cycle. With a median follow up of 18 months (range 0-119) median event free and overall survival is 18 and 39 months respectively. The predicted EFS and OS at 3 years is 35% and 53% respectively. 19 of the 41 patients attaining remission have relapsed at a median of 15 months from diagnosis (range 5-62 months), and survival in this group was poor. Age (> 30 years) and presence of Philadelphia chromosome predicted for worse event free survival, whereas no statistically significant survival correlation could be demonstrated according to presenting WCC, other cytogenetic abnormalities, immunophenotype, or time to remission. 8 patients received allogeneic stem cell transplantation in first remission for poor risk features of which 7 remain alive at a median follow up of 21 months.

### **Conclusions**

The outcomes of our patients with ALL treated using the Hyper-CVAD regimen are comparable to the published data. The use of allogeneic transplantation in first remission for high risk patients can produce excellent long term results.

## **170**

### **Allogeneic stem cell transplantation for adult acute lymphoblastic leukaemia: Local results of modern transplant practice**

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### **Aims**

To report the single institution outcomes for recent allogeneic transplantation for acute lymphoblastic leukaemia (ALL), and explore variables predictive of successful outcome.

### **Methods**

All patients with ALL transplanted at the Royal Brisbane Hospital between 1<sup>st</sup> January, 2000 and 31<sup>st</sup> December, 2005 were included in the study sample. Survival analysis was performed using the Kaplan-Meier product-limit method. Follow-up was censored at 30<sup>th</sup> June, 2006

### **Results**

A total of 41 patients were included in the study cohort, including 28 males and 13 females. Median age at transplantation was 27.6 years (range 15.2 to 64.5). 34 patients had precursor-B phenotypes; the remaining 7 had T-lineage ALL. Based on cytogenetics, patients were high-risk (n=19), intermediate risk (n=12), good risk (n=2), or indeterminate (n=8). Patients were transplanted in CR1 (n=23, 2 patients demonstrating delayed CR), 1<sup>st</sup> relapse (n=5, 2 with early relapse, 1 with untested frank relapse, and 2 with refractory disease), CR2 (n=9, 2 with treated isolated CNS relapse), CR3 (n=1) and primary refractory disease (n=3). Conditioning regimen was CyTBI, with the exception of 1 patient who received fludarabine (125mg/m<sup>2</sup>) and cyclophosphamide (120mg/kg). All patients received cyclosporine and full-course methotrexate for GVHD prophylaxis. Donor source was matched sibling (n=17) and matched unrelated donor (n=24). 5 patients received unprimed bone marrow grafts; the remaining patients received peripheral blood stem cells. At a median follow-up for survivors of 1.8 years (range 0.5 to 6.4), overall and progression-free survival at 2 years is 61% (95% CI 43 to 75%) and 74% (95% CI 55 to 85%) respectively. 32 patients developed acute GVHD, of whom 27 (66%) developed Grades II-IV. 21/40 evaluable patients developed chronic GVHD; all were extensive stage. 9 patients relapsed during follow-up at a median of 0.47 years post transplant (range 0.02 to 1.46); 1 remains alive in remission following reinduction therapy.

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6 patients died from therapy-related causes – 3 from pulmonary toxicity, and 1 each from fungal sepsis, leukoencephalopathy, and hepatic GVHD. On both univariate and multivariate analyses of overall survival, younger age, transplantation in CR1, and development of chronic GVHD were predictors for an improved outcome.

#### Conclusion

Allogeneic stem cell transplantation for ALL is associated with excellent long-term outcome with respect to both disease control and treatment related toxicity. From these data, transplantation in CR1, younger age, and the development of chronic GVHD are associated with prolongation of overall survival.

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### **The incidence of myelodysplasia (MDS) and secondary acute myeloid leukemia (sAML) among patients treated with fludarabine combination chemotherapy at a single institution**

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#### Aims

MDS and sAML are rare complications of fludarabine monotherapy, with reported rates <1% among patients treated for chronic lymphocytic leukaemia (CLL) or follicular lymphoma (FL). The addition of DNA damaging agents to fludarabine in combination has greater efficacy, but may also increase the risk of MDS/sAML. We describe our experience with MDS/sAML among patients receiving fludarabine-combination regimens.

#### Methods

From 10/96-06/05, 137 patients received fludarabine-cyclophosphamide (n=65), FC-Rituximab (n=66), or FC-mitoxantrone-rituximab (n=6) either as initial (n=40) or salvage therapy (n=97) for CLL or indolent NHL. Baseline characteristics [median(range)]: age, 59 years (30–89); male, 66%; time from diagnosis, 36 months (1–324); CLL/FL/other indolent lymphoproliferative, 38%/35%/27%. Pretreated patients had received a median of 2 (range 1-10) prior therapies.

#### Results

Median follow-up from the completion of treatment was 40 months (5-86). Ten patients developed MDS/sAML (crude rate 7.3%). Within this limited sample, there was no significant difference in MDS/sAML crude rate between previously untreated and pretreated patients (2.5% vs 9.3%, p=0.28), although a trend to higher MDS/sAML rate with increasing number of previous therapies was evident (chi-square test for trend, p=0.04). On univariate analysis, both FL and mitoxantrone were associated with increased risk of MDS/sAML (p=0.03 and 0.005, respectively); this association was however confounded by a correlation between FL and mitoxantrone (p=0.02). Eight patients had evaluable cytogenetics, and abnormalities of chromosomes 5 and/or 7 were present in six. At median 16 months (range 3–30) of follow-up, five patients had died of MDS or progression to sAML, two patients had died of lymphoma progression, and three patients remain alive with stable MDS/sAML.

#### Conclusions

MDS/sAML may develop in up to 10% of patients treated with fludarabine combination regimens as salvage, similar to that expected for alkylating agents or autologous stem cell transplantation. Clinicians should consider the risk of MDS/sAML when using fludarabine combination regimens.

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### **Salvage therapy for AML relapsing after allogeneic SCT is associated with a dismal prognosis, high rates of extramedullary progression and responses are reliant on the induction of GVHD**

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#### Introduction

AML relapse after allogeneic SCT has an extremely poor prognosis. Salvage regimens include (FLAG +/- Ida) and the anti-CD33 monoclonal antibody gemtuzamab or gatazoicin (GO). We have analysed the outcome of 19 patients with non-promyelocytic AML treated with a range of salvage following relapse post-allogeneic SCT.

#### Results

Twenty five (30%) of 83 patients allografted for AML at RMH since 2000 have relapsed. Of these, 19 (9 male), median age 32 (range 17-60) have undergone salvage therapy. The median time from initial SCT to relapse was 120 days (range 30 to 750). In addition to immunosuppression (IS) withdrawal in all patients, three main regimens were utilized; chemotherapy (CT) alone (n=5), CT + DLI (n=4), or HSANZ Oral Abstracts, HAA, 15-18 October, 2006

GO+/-DLI (n=6). Two patients, initially treated with reduced intensity allograft, underwent myeloablative transplants. Four patients (two treated with DLI, one following a second transplant and one with IS withdrawal only), had interferon- $\alpha$  added to induce GVHD. One patient with Ph+ AML received the tyrosine kinase inhibitor dasatinib. Nine patients attained a CR as a result of salvage therapy. Of these, 3 were treated with FLAG+DLI+/- IFN- $\alpha$ , two with GO, two with a myeloablative sibling allograft, one with IFN- $\alpha$  alone and one with dasatinib. CT alone without DLI did not result in CR and 4 of 6 treated with GO showed no response.

Median overall survival (OS) post salvage was 144 days (range 50-623). Patients attaining a further CR had a median CR duration of 200 days and median OS of 330 days. These findings were unchanged if the single dasatinib treated patient was excluded. Median survival was 84 days (range 11-204) for those who did not attain a second CR. Seven developed grade II-III GVHD following salvage therapy, three with CT + DLI (+IFN- $\alpha$  in two), two with of GO without DLI and two with myeloablative SCT (+IFN- $\alpha$  in one). Median survival = 370 days (range 60-623) in those with GVHD compared to 96 days (range 11-204) in those without GVHD (p<0.01). Other than with dasatinib, CR was not achieved in the absence of GVHD. Sixteen have progressed post salvage. Two remain in CR and one developed fatal marrow fibrosis without leukaemic relapse. Medullary relapse occurred in 12. Extramedullary relapse occurred in 4 including leukemia cutis, pleural effusion, breast/CNS/pericardial relapse, and isolated CNS relapse. Overall, we reiterate the very poor prognosis of this group of patients and the close relationship between GVHD and duration of AML remission.

## 173 Treatment outcomes of *de novo* acute myeloid leukaemia in the elderly – a Queensland experience

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### Aims

To review the outcome of elderly patients with *de novo* acute myeloid leukaemia (AML) treated at our institution.

### Methods

Outcomes of all patients  $\geq 60$  yrs who received chemotherapy for *de novo* AML between January 2001 and December 2005 were retrospectively reviewed. Patients treated for secondary AML (defined as occurring after previously documented MDS or MPD, or having received prior chemotherapy and / or radiotherapy for solid cancers) were excluded. Selection of patients for chemotherapy was based on individual patient and physician discretion. Survival analysis was performed using the Kaplan-Meier product-limit.

### Results

32 patients  $\geq 60$  yrs received chemotherapy for *de novo* AML during the time period under review, including 19 males (59%) and 13 females (41%). Median age at AML diagnosis was 69 yrs (range 60-83 yrs), with 18 patients (56%) aged 60-69 yrs and 14 (44%)  $\geq 70$  yrs. Using Intergroup criteria, 3 (9%) of patients had good risk (including 2 patients with APL), 21 (66%) intermediate risk and 8 (25%) poor risk cytogenetics. The predominant protocol used for induction was '7'3' (cytarabine + idarubicin); other induction protocols used included Mylotarg (9% of cases), FLAG (6%) and MIDAC (6%). APL patients were treated with ATRA + idarubicin induction followed by arsenic trioxide (1 case) or AIDA consolidation (1 case). Overall 16 patients (50%) achieved morphological + cytogenetic remission post-initial induction therapy; 5 patients (16%) suffered fatal complications during induction / consolidation therapy. At a median follow-up post-initial diagnosis of 37 mths (range 8-67 mths), median PFS, EFS and OS are 14 mths, 13 mths and 14 mths respectively. On univariate analysis, neither cytogenetic risk nor age (<70 vs  $\geq 70$  yrs) were associated with improved PFS or OS.

### Conclusion

Even in this highly selected patient group, chemotherapy for elderly patients with *de novo* AML was associated with high rates of relapse and poor overall survival. New therapies for treatment of AML in the elderly are needed.

## 174 Improved Measurement of MRD

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A new and improved method for measurement of minimal residual disease (MRD) was developed

### Method

(a) the total repertoire of leukaemic rearrangements of the immunoglobulin heavy chain (IgH) gene was determined by performing multiple parallel Q-PCRs to determine usage of individual V and J segments

(b) MRD measurement involved nested Q-PCR and primers based on the sequence of the rearrangement of interest.

23 patients with childhood B-ALL were studied at the end of induction therapy. Four aspirations, 2 from each iliac spine, were performed and MRD measurement was performed on each sample on 2 different days. This enabled definition of the laboratory and clinical factors important in measurement of MRD.

## Results

The median MRD level at the end of induction was  $2.1 \times 10^{-5}$ . A level of  $> 10^{-3}$  was seen in 4 patients and  $< 10^{-7}$  in 4.

Sensitivity of detection in a single sample was approximately  $2 \times 10^{-6}$

The SD of measurement depended on the number of rearrangements present in the sample. For  $> 50$  rearrangements SD was 0.23 log units, but below this level the SD rose steeply. 50 targets in  $1 \mu\text{g}$  of DNA corresponds to approximately an MRD of  $3 \times 10^{-4}$ .

There was significant sampling error. In 1 patient there was 1000-fold MRD difference between the 2 iliac spines.

## Conclusions

We recommend that

- an aspiration should be performed from each iliac spine, with each sample being quantified separately and an average obtained
- each measurement should involve at least  $10 \mu\text{g}$  of good-quality DNA

The MRD value so obtained should have sufficient accuracy, sensitivity and precision for clinical decisions based upon the value to be made with confidence.

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#### **A study of alpha thalassaemia due to mutations of the alpha2 globin gene in a West Australian cohort**

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#### Aims

During the course of routine reporting in the haemoglobinopathy laboratory at the PathWest QEII laboratory a number of cases were noted to have microcytosis which could not be explained by iron deficiency, beta thalassaemia trait, haemoglobin variants or the common alpha thalassaemia deletions. The aim of this study was to investigate the frequency of this “unexplained microcytosis”, and to investigate the proportion of cases of alpha thalassaemia due to mutations in the alpha2 globin gene within this group.

#### Methods

1. An initial retrospective audit was undertaken of cases submitted for investigation of haemoglobinopathies over the period January – June 2005.
2. A prospective study of alpha2 globin gene sequencing was initiated over a subsequent 6 month period: October 2005 – April 2006.

#### Results

A total of 1292 specimens were processed in the six month period, January – June 2005. There were 306 (24%) cases of deletional alpha thalassaemia, 56 (4%) cases of beta thalassaemia trait, 102 (8%) Hb variants, 232 (18%) cases with isolated iron deficiency, and 100 cases (8%) with “unexplained microcytosis”.

In a subsequent 6 month period 97 cases were identified for alpha2 globin sequencing. 11 were positive for mutations associated with a thalassaemic phenotype. These included 1 poly(A) site mutation, 7 splice junction mutations, 1 initiation codon mutation and 2 termination codon mutations. Three novel alpha globin mutations were identified – these include 1 splice junction mutation, 1 initiation codon mutation and 1 frameshift mutation which results in a downstream premature termination codon. The mechanisms underlying the thalassaemic phenotype will be discussed.

Thus, in a select group of patients where the more common causes of microcytosis have been excluded, the incidence of non-deletional alpha thalassaemia is at least 10% and alpha globin gene sequencing should be considered in these patients.

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#### **Study of systolic & diastolic functions by echocardiogram as a predictor of cardiac morbidity in multitransfused thalassaemic children**

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#### Background

The aim of this study was to investigate the effect of iron overload on left ventricular (LV) remodeling and function in 75 children affected by [beta]-thalassaemia major (TM)].

#### Introduction

Thalassaemia syndromes often are complicated by cardiac involvement that is related mainly to iron tissue overload as a result of  
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hemolysis, increased intestinal absorption, and multiple transfusions. Moreover, iron-induced cardiac disease is considered to be the primary cause of death in patients who have transfusion-dependent [beta]-TM. Although left ventricular systolic function in beta -TM has been considerably studied, <sup>[1]</sup> left ventricular diastolic function has not been assessed adequately. Previous studies on left ventricular diastolic function are rather conflicting. <sup>2</sup> Recently, Spirito et al <sup>3</sup> using Doppler echocardiography, detected irreversible diastolic left ventricular filling abnormalities which developed even in the early phase of the disease in young beta-TM patients. However, age and body size strongly affect the isovolumic relaxation time and deceleration time in childhood and early adulthood. The purpose of this study was to investigate the characteristics of left ventricular diastolic function, LV remodeling by two dimensional and doppler echocardiography in a relatively large number of beta-TM patients to look at the effect of iron overload as well as that of chelation.

#### Methods

LV volumes and shapes, mass index, mass/volume ratio, systolic and diastolic function, and stroke volume, were determined by two-dimensional and M-mode echocardiography

#### Results

LV systolic function : LV volumes both systolic and diastolic were reduced in the chelator group however this did not approach statistical significance. FS, and EF were analysed and though showed a better trend in the chelator group this failed to approach statistical significance. The group on chelator therapy showed a decrease in LV mass however this did not approach statistical significance.

LV diastolic function: It was assessed at mitral inflow and at pulmonary vein inflow. At the mitral valve level it was observed that there was a decrease in peak early filling velocities which was statistically significant and a decrease in late filling velocities, an increase in the E/A ratio and deceleration time showing a more normalised trend in the chelator group compared to the non chelator group, however this did not approach statistical significance. At the pulmonary vein level a similar pattern was observed showing an improved trend in the chelator group without any statistical significance. Myocardial performance index using the aortic ejection time and LV filling time also predicted a better trend for the chelator group.

#### Conclusions

Asymptomatic young adults with TM on regular blood transfusions show insignificant changes in LV systolic and diastolic properties, compared to those on chelator therapy.

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### Laboratory tumor lysis syndrome complicating LBH589 therapy in a patient with acute myeloid leukaemia

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LBH589 (Novartis) is a potent histone deacetylase inhibitor (HDACi) currently in early phase clinical development. Preliminary data suggests biological activity in a range of malignant haematological disorders including acute myeloid leukaemia (AML). To-date, laboratory tumor lysis syndrome (LTLS) has not been described as a complication of LBH589 therapy.

We report a case of a 60 year-old man who developed LTLS following treatment for AML with oral LBH589. The patient was diagnosed with refractory anaemia with excess blasts (RAEB) in July 2005. Cytogenetic analysis demonstrated trisomy 8 and del(20q). He was initially treated with standard dose cytarabine and idarubicin (7 + 3). Post-induction bone marrow examination revealed ongoing dysplasia but no excess of myeloblasts. He subsequently received further cytarabine and idarubicin as consolidation. This was complicated by prolonged pancytopenia and subsequently by rapid progression to overt AML. Salvage therapy with fludarabine/cytarabine/G-CSF (FLAG) failed and he was referred to our institution for investigational therapy with LBH589.

The patient was enrolled in a Phase IA/II trial of oral LBH589 for patients with advanced haematological malignancies and commenced intermittent oral LBH589 (30mg orally Monday, Wednesday and Friday on alternate weeks). He re-presented on day 14 with deteriorating renal function – creatinine 0.28mmol/L (baseline 0.12mmol/L) and hyperleukocytosis (WBC 68 x 10<sup>9</sup>/L). He recommenced LBH589 and was also commenced on hydroxyurea 1g bd, allopurinol and hydration. Within 24 hours, associated with a fall in his WBC to 9 x 10<sup>9</sup>/L, he developed LTLS - corrected calcium 1.91mmol/L (2.23 - 2.50), phosphate 2.99mmol/L (0.70 – 1.30) and uric acid 0.8mmol/L (0.15 – 0.50). He recovered uneventfully with the administration of rasburicase, intravenous fluid and electrolytes. Hydroxyurea was ceased and he continued LBH589 as per schedule. On day 28 with a WBC of 60 x 10<sup>9</sup>/L, LTLS again recurred within 24 hours of LBH589 administration despite prophylactic rasburicase and hyper-hydration - WBC fell to 6 x 10<sup>9</sup>/L, corrected calcium 2.1 mmol/L, phosphate 1.91 mmol/L and uric acid <0.0.2mmol/L (from 0.66 mmol/L). The patient again recovered uneventfully.

This first reported case of LTLS with LBH589 therapy clearly demonstrates the potent anti-leukaemic potential of LBH589 and mandates that caution be taken with LBH589 treatment of AML patients exhibiting highly proliferative and/or a high tumour burden disease.

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### Hypophosphataemia in patients receiving imatinib mesylate therapy

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#### Background

A recent report found a high incidence of hypophosphataemia and changes in bone metabolism in patients receiving imatinib mesylate therapy<sup>1</sup>.

#### Aim

To determine whether there is a similar significant incidence of hypophosphataemia in patients receiving long-term imatinib mesylate therapy for haematological disorders within our institution and identify any factors that may be linked to development of hypophosphataemia.

#### Methods

The records and biochemistry results of patients receiving imatinib mesylate therapy for haematological disorders for >1 month were reviewed to identify development of hypophosphataemia (phosphate <0.80mmol/L) and whether there were any differences with respect to age, sex, dose of imatinib mesylate and duration of therapy between the hypophosphataemic and normophosphataemic patients.

#### Results

Eighteen out of forty-six (39%) patients receiving imatinib mesylate, predominantly for chronic myeloid leukaemia but also Philadelphia positive acute lymphoblastic leukaemia - 2 patients and hypereosinophilic syndrome -1 patient, were identified as having at least one low serum phosphate level (mean 0.70mmol/L; range 0.50-0.79mmol/L). Compared to the normophosphataemic group, the hypophosphataemic group had a higher proportion of males (16 males:2 females versus 13 males:15 females in normophosphataemic group, p<0.005) and slightly longer duration of therapy (31 months versus 27.5 months). There were no differences with respect to dose of imatinib mesylate (median 400mg/day both groups; range 100-800mg/day) or age (median 49 versus 51 years; range 16-81 years) between the groups.

#### Conclusion

Hypophosphataemia is a relatively common finding in patients, particularly males, taking imatinib mesylate for haematological disorders and may warrant treatment and surveillance for effects on bone metabolism when imatinib mesylate is used long-term.

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### **Implementation of a cost-effective, consolidated and improved haematology & telediagnostic Service at the three university hospitals of Charité Medical School in Berlin**

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#### Aim

The merger of the three university hospitals (CVK, CCM and CBF) of the Charité medical school located in three different districts of Berlin and thereby creating the biggest European hospital (approx. 3500 in-house patients) went along with major challenges for the fusion of the three single laboratories at each location. As the need to economize was enormous an innovative telediagnostic setup between all three locations was chosen to reduce spendings on lab expenses and save on labor intensive work places. Albeit the overall laboratory costs were reduced, the haematologic service for clinicians improved because of the intensive deployment of telehaematological systems.

#### Methods

A central server based in the main laboratory (CVK) gathers all the numeric data from the rule-based SYSMEX-SIS middleware system and compiles all the visual data from the Cellavision DM96 and a K-Xpert microscopy system. Via the browser-based EXPERTviewer software the laboratory medical staff is able to televalidate all the numeric data, histoplots and scattergrams and the electronic morphological pictures at the three single hospital locations. Additionally, the EXPERTviewer enables clinicians to view the digital images from the blood smears of their patients directly at their wards.

#### Results

Four SYSMEX XE-2100 and two SP100 automated slide makers deal with the main routine workload from all three hospitals at the central laboratory (CVK). The peripheral laboratories (CCM and CBF) are equipped with a single XE-2100 and a KX-21 and process urgent samples predominantly.

The DM96 from Cellavision with its capability of fully automated image processing and the KXpert system for special haematological questions enable the laboratory staff to televalidate all diagnostic findings online via the EXPERTviewer IT-system independently of their physical location. Clinicians have access to the virtual blood smears from any of their patients at any time of the day at their wards and may use them for ward round presentations and teaching purposes. The morphological database is stored on a central server providing the possibility of comparing different virtual blood smears from patients over a long period of time.

#### Conclusion

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The investment in a telehaematological setup linking three big teaching hospitals provides a better diagnostic service for clinicians at the Charité medical school and saves laboratory expenses at the same time. Thus, teleconsultation from remote sites is feasible and a standardization and consistency of the haematological analytical processes can be acquired.

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### **The utility of holotranscobalamin in diagnosing cobalamin deficiency**

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#### **Aim**

Low serum cobalamin has high sensitivity in patients with symptoms of cobalamin deficiency. As a screening test in patients with few or no clinical manifestations, its diagnostic utility is significantly reduced due to low specificity.

The loss of the Schilling test, traditionally regarded as the gold standard in determining true cobalamin deficiency, has exacerbated the problem of determining true cobalamin deficiency. Transcobalamin binds the biologically active fraction of cobalamin to form holotranscobalamin (HTC). We aimed to explore the utility of HTC in diagnosing true cobalamin deficiency in patients with low cobalamin levels.

#### **Method**

The study was performed at the Alfred Pathology Service, Melbourne. We prospectively identified and collected blood samples from patients (n=210) in whom serum cobalamin < 200 pmol/l. The AXIS-SHIELD automated HTC assay has been adapted for routine use on the Abbott AxSYM platform. Additional data collected for each patient included Hb, MCV, serum homocysteine, creatinine, methyl malonic acid and intrinsic factor antibodies together with blood film and clinical history examination. Using these data, likely cobalamin deficiency was assessed by two independent haematologists.

Univariate and multivariate statistical analysis was performed using SAS software.

#### **Results**

On clinical review, 34/210 patients were considered likely to be cobalamin deficient. On univariate analysis, HTC levels were lower in the likely cobalamin deficient patients than in remaining patients with low cobalamin levels (18.9-31.8 pmol/L,  $p < 0.001$ ). In a multivariate analysis, HTC values provided additional independent information to the diagnosis of clinical cobalamin deficiency. Odds ratio estimates showed that each unit of HTC decrease is associated with a 5.0% increase in risk of cobalamin deficiency

(OR 0.950, 95% CI 0.917-0.985).

#### **Conclusion**

In our clinical setting, a low HTC value adds independent value when diagnosing cobalamin deficiency. Studies of the clinical utility of HTC in patient management are ongoing.

## **HSANZ – Stem Cell Transplantation III**

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### **Infusion of allogeneic mesenchymal stem cells delays GVHD onset in both a full mismatch and MHC matched, multiple minor mismatch murine transplant model**

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A novel means of immunosuppression in graft-versus-host disease is the use of mesenchymal stem cells (MSC). MSC have been shown to be both non-immunogenic and actively suppress T cell function and we hypothesise that due to these effects, GVHD can be ameliorated by treatment with MSC. In our study we examined the *in vivo* effects of intraperitoneally injected donor-derived MSC in a murine model of HSCT. In a major histocompatibility mismatched model, BALB/c (H-2<sup>d</sup>) mice were myeloablatively conditioned (cyclophosphamide + irradiation) and transplanted with UBI-GFP/BI6 (H-2<sup>b</sup>) bone marrow and spleen cells ± increasing doses of MSC infused one day post-transplant, then monitored for onset and severity of GVHD. We showed that mice who received higher doses of MSC ( $3 \times 10^5$  or  $4 \times 10^5$ ) had significantly greater survival than control mice that did not ( $p = 0.009$  and  $p = 0.0166$  respectively) and death from GVHD was delayed by up to 33 days as compared to controls. Interestingly, at day +6, mice treated with the higher doses of MSC showed a decrease in percentage of activated host dendritic cells (DC) in the bone marrow. Furthermore, in a MHC matched, multiple minor mismatch model (UBI-GFP/BI6 (H-2<sup>b</sup>) → BALB.B (H-2<sup>b</sup>)) conditioned as above ± MSC, we showed a similar result with death by GVHD delayed by up to 44 days and an increase in percentage of activated donor DC in both the bone marrow and the spleen. Further elucidation of MSC and their role in attenuation of GVHD could potentially allow for wider application of HSCT.

## Unrelated donor HSCT for AML in Australia – increase in activity and improvement in survival

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### Introduction

There is evidence that survival probabilities post haemopoietic stem cell transplant (HSCT) have increased in recent years due to improvements in a range of clinical practices. This study examines the outcome of unrelated donor (UD) HSCT for the indication of acute myeloid leukaemia (AML) performed for Australian adults, to evaluate whether HSCT outcome in this setting has improved over time.

### Patients and Methods

HSCT recipients were selected from the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR). Study subjects received first allogeneic HSCT for the indication of AML in Australia between 1992 and 2004, were aged between 16 and 67 and received marrow or peripheral blood stem cells from unrelated donors matched at A-, B- and DR- HLA loci. Data was collected on patient and disease characteristics, pre-transplant CMV and GVHD prophylaxes, post-transplant relapse and overall survival.

### Results

A total of 185 UD-HSCT were included in the study. Five-year overall survival probability was significantly higher for HSCT performed in recent years (1998 to 2004, 45%) compared to earlier years (1992 to 1997, 20%,  $P=0.02$ ). In a multivariate Cox regression survival analysis, the independent risk factors for reduced overall survival among this study group were earlier year of transplant (1992 to 1997,  $P=0.01$ ), disease status past 1<sup>st</sup> remission ( $P=0.002$ ), recipient age 40 and above ( $P=0.001$ ) and donor aged 39 and above ( $P=0.03$ ). Stem cell source and type of conditioning did not significantly affect survival.

### Discussion

There is a clear improvement in outcome in recent years for this group after adjusting for other patient and clinical factors possibly due to better post-transplant care. The results also indicate that younger donors confer a survival advantage. The ABMTRR is a valuable national data resource providing relatively large sample numbers for studies, enabling the detection of sensitive trends such as change over time.

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### RIC allogeneic HSCT with sirolimus-based GVHD prophylaxis in high risk recipients

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### Introduction

A challenge with reduced intensity conditioning (RIC) allografting is to adequately manage GVHD without negating a graft versus leukaemia/lymphoma effect.

Initially we adopted Dana Farber GVHD prophylaxis with dose modification for azole antifungal prophylaxis. The RIC entailed fludarabine 30mg/m<sup>2</sup> x5 and melphalan 140mg/m<sup>2</sup>. Tacrolimus was administered at 0.02mg/kg daily, sirolimus 1mg daily and methotrexate 5 mg/m<sup>2</sup> (days 1,3,6 and 11).

Due to high cost and renal dysfunction/thrombotic thrombocytopenic purpura, tacrolimus was substituted with intravenous cyclosporin 0.5mg/kg twice daily in a second cohort (n=6).

### Results

*Tacrolimus, Sirolimus and Methotrexate:*

Fifteen patients (11M:4F) received this regimen. Median age was 56 (38-67) with 4 over 60. 9/15 were MUD transplants, the remainder sibling. Acute GVHD grade I was seen in 2/15, grade II in 4/15 and grade III in 3/15 (one when immunosuppression was withdrawn). 8 of the 9 patients who developed GVHD were MUD recipients. 3 had problems that lead to withdrawal of Tacrolimus.

At a median follow up of 532 days (300-951) 3/15 patients had died of relapse and another of sepsis (day 258). The remainder maintain complete remission (CR).

*Cyclosporin, sirolimus and methotrexate:*

Six patients (5M:1F) received this regimen. Median age was 53 (44-63) with one over 60. 5/6 were sibling transplants, the other MUD. Acute GVHD grade II was seen in 1/6 and grade III in 1/6 (the MUD recipient).

At a median follow up of 101 days (56-421) 1/6 had died of sepsis (day 351), one had persistent disease and 4/6 maintain CR.

### Conclusion

We conclude from this preliminary data that a Flu/Mel RIC with sirolimus-based GVHD prophylaxis yielded acceptable results with

manageable GVHD in this high risk group. At this stage cyclosporin appears to be more easily managed than tacrolimus.

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### **Allogeneic transplant outcomes in imatinib-refractory chronic myeloid leukaemia (CML) are similar to transplant outcomes in non-imatinib refractory CML and appear to be predicted by the EBMT risk score.**

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#### Aims

To review the outcome of allogeneic stem cell transplantation (SCT) in imatinib refractory chronic myeloid leukaemia (CML).

#### Methods

Outcomes of all allogeneic transplants performed after January 2001 for CML at our institution were retrospectively reviewed. Imatinib-refractory CML was defined as either lack of any cytogenetic response (CGR) after at least 6mths of imatinib, loss of CGR or progression to a more advanced disease stage (accelerated or blast phase) during imatinib therapy. Using the EBMT risk score (Lancet 1998; 352: 1087), transplant outcomes for imatinib refractory CML were compared with all other CML transplants performed during the same time period. Survival analysis was performed using the Kaplan-Meier product-limit and comparison of survival data via the log-rank test.

#### Results

Of 32 allogeneic transplants performed for CML, 12 (8M; 4F) had been performed in patients with imatinib refractory CML, including 3 patients with no CGR to imatinib, 3 patients with loss of CGR and 6 patients with CML progressing to either accelerated phase (3 patients) or blast crisis (3 patients). Median age at SCT was 39yrs (range 21-63yrs). Conditioning regimens used included Cy/TBI (10 cases), Bu/Cy (1 case) and Flu/Mel (1 case); CsA + MTX was used as standard GVHD prophylaxis. EBMT risk scores were 2 (2 cases), 3 (3 cases), 4 (2 cases), 5 (2 cases) and 6 (3 cases). At median follow-up post-SCT of 44mths (range 17-50mths), median PFS, EFS and OS are not reached, 33mths and 33mths respectively. Causes of death post-SCT included progressive CML in 4 cases and transplant-related complications in 2. For patients with EBMT risk scores of 1 or 2 *versus* 3 or 4 *versus* 5 or 6, OS at 2 yrs post-SCT is 100%, 60% and 60% respectively ( $p=0.32$ ). In comparison to 20 transplants performed in non-imatinib refractory CML during the same time period, no significant differences in PFS, EFS or OS were observed in any defined subgroup based on EBMT risk score. For the whole cohort of 32 transplants, EBMT risk score was significantly associated with OS ( $p=0.03$ ; see Figure).

#### Conclusion

Our experience suggests that survival post-SCT for imatinib-refractory CML is similar to non-imatinib refractory CML. The EBMT risk score appears to remain useful in predicting survival post-SCT in imatinib-refractory CML.

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### **CI-SCaT – Collaboration, consensus and common sense**

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# Seconded project officer

To deliver optimal treatment to patients, clinicians require a comprehensive understanding of the latest research, key evidence and internationally accepted standards. The Cancer Institute NSW has developed CI-SCaT, a single web-based Australian repository of standardised evidence-based treatment protocols. Launched in August 2005, this uniquely holistic resource provides clinicians with comprehensive information for the safe administration of treatments and also provides consumers with a lay version of each treatment and its side-effects.

Originally the brainchild of Professor Robyn Ward, St Vincent's Hospital Sydney, a dedicated team at the Cancer Institute NSW has continued to develop this resource with strong Australia-wide clinician buy-in complementing a successful clinical governance model. Rather than each individual facility writing, reviewing and updating treatment protocols, the CI-SCaT team, in conjunction with clinicians, identify existing protocols for inclusion. With the assistance of drug evaluators who identify key evidence, comparative efficacy and toxicity and required dose modifications, the team develop draft versions for dissemination to an expert reference group. Each protocol undergoes rigorous scrutiny at the reference group workshop until a consensus is achieved. Approved protocols are then uploaded to the website and reviewed annually. Protocols that are not afforded consensus are further investigated and, where appropriate, are resubmitted at the next reference group workshop with supporting evidence.

Initially focused on medical oncology and haematology protocols, the site is expanding to include radiation oncology and bone marrow transplant treatment (including from conditioning up to Day +100) pathways, supportive care and palliative care. Continued use of, and HSNZ Oral Abstracts, HAA, 15-18 October, 2006

contribution to, this resource by clinicians will strengthen, expand the available information and guide the future direction of CI-SCaT.

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### **Outcome of autograft in patients with Hodgkin lymphoma is not reliably predicted by either duration of first response, response to salvage or persistence of metabolically active disease**

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#### Introduction

The outcome of autografting for relapsed Hodgkin lymphoma (HL) is consistently reported to be adversely affected by resistance to salvage chemotherapy. Other previously reported adverse indicators are age >50 and a higher number of prior therapies, duration of first CR <1 year, B symptoms at relapse and extranodal disease. Functional imaging has also recently been associated with an inferior outcome.

#### Methods

We retrospectively analysed the clinical characteristics at relapse and functional imaging results of 50 sequential patients, median age 32 (range 18-62), 25 male, undergoing autologous SCT for treatment of the initial relapse of HL at RMH or PMCC. Response to salvage was determined as absence/presence of disease using the worst result of imaging post salvage (No PET+CT(CR)= CR; PET(CR)+CT(CR)= CR; PET<sup>+</sup>+CT(CR)= PR etc). Differences in EFS and OS for each of these variables were calculated by Kaplan-Meier analysis.

#### Results

Fifty patients (55% male) were assessed, median age 32 (range 18-62). Salvage regimens included DHAP or similar (n=28), ICE or similar (n=14), and a range of other regimens (n=8)

Pre-transplant conditioning utilised included BEAM (n=30), Cyclo/ BCNU/VP-16 (n=10), fractionated TBI/Cyclo/VP-16 (n=6), other (n=4). The 5 year actuarial OS and EFS were 71% and 46% respectively. Post salvage functional imaging (gallium (n=17) or PET (n=19)) was performed in 70% (n=36) of cases (of which 33% were negative). A trend towards adverse 5 year OS was observed in patients with CR vs PR vs no response to salvage therapy (89% vs 69% vs 38%) and when relapse was associated with B symptoms (82% vs 67%). EFS did not differ according to duration of first CR longer or shorter than 6 months (59% vs 46%) or persistence of gallium/PET positivity (55% vs 39% p=0.23) post salvage.

#### Conclusions

Our findings reinforce the importance of chemosensitivity on outcome of autografting for HD. Other factors failed to reliably predict for adverse outcome from autografting, and suggests that a significant proportion of patients with apparent poor risk HD may still benefit from autografting.

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#### **Novel therapies for myeloma**

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For decades, chemotherapy for multiple myeloma has consisted of standard combinations of alkylating agents, anthracyclines and steroids with or without hematopoietic stem cell rescue. While these therapies can provide rapid responses and result in modest gains for patients, the disease eventually relapses in all patients and becomes resistant to treatment. More recently, novel immunomodulatory agents, like bortezomib, thalidomide and its derivative lenalidomide, have shown promise for treatment of patients with multiple myeloma.

Singhal et al first reported that thalidomide induced partial or complete responses in nearly one-third of patients with resistant myeloma.<sup>1</sup> This was followed by the observation that thalidomide and dexamethasone was active, and possibly synergistic, in approximately 45% of resistant patients even if previous treatment with thalidomide and dexamethasone was unsuccessful.<sup>2</sup> This synergy has also been noted in vitro as well.<sup>3</sup> Subsequently many combinations have produced response rates ranging from 45-65% and a phase III trial in untreated pts demonstrated superior response and time to progression of thalidomide-dexamethasone (TD) compared with dexamethasone (D) alone in patients with previously untreated MM.<sup>4,5,6</sup> However, this trial also demonstrated that there was no survival benefit of TD compared with D for patients who did not subsequently proceed to autologous stem cell supported therapies.<sup>6</sup> This is in contrast to 2 phase III trials of melphalan-prednisone (MP) vs MP-thalidomide (MPT) in which patients treated with MPT had superior response, TTP, and OS compared with those who received MP alone; the trial by Facon also demonstrated superiority of MPT compared with melphalan 100mg/M<sup>2</sup> with autologous stem cell support x 2.<sup>7,8</sup> Thus, these 2 trials provide the first direct evidence of superior survival compared with MP. It is important to note that for patients who are candidates for myeloablative therapy with stem cell support, prolonged administration of alkylating agents should be delayed until after stem cell collection.

Bortezomib is a proteasome inhibitor that not only blocks NF- $\kappa$ B and subsequent IL-6 production, but also affects cellular adhesion, signaling pathways and apoptosis of myeloma cells. A phase II trial of bortezomib in MM pts resulted in a 27% > PR rate, median survival for all pts was 16 months and median TTP was 7 months; nonresponding pts received dexamethasone and another 18% of pts responded.<sup>9</sup> This was followed by a phase III trial that was conducted in pts who failed to respond or relapsed after front line therapy for MM. Patients who were treated with bortezomib had higher a higher response rate (38% Vs 18%), longer TTP (6.2 Vs. 3.5 mos), and greater overall survival at 1 year (80% Vs 66%) than counterparts who received dexamethasone despite crossover of dexamethasone pts at progression.<sup>10</sup> Based on these findings in refractory and relapsing pts, Jagannath et al reported a 90% response rate in previously untreated patients who received bortezomib or botezomib with dexamethasone (nonresponding pts)<sup>11</sup>. Several trials have now been reported with excellent results of various combinations including agents such as doxorubicin, thalidomide, melphalan, cyclophosphamide, and dexamethasone.

Due to the encouraging activity of thalidomide in multiple myeloma, immunomodulatory derivatives (ImiDs) have been developed in an attempt to avoid troubling side effects of thalidomide such as teratogenicity, neuropathy and thrombosis. Lenalidomide is a derivative of thalidomide with more potent biologic activity than the parent compound. This drug inhibits tumor necrosis factor- $\alpha$  secretion, down regulates interleukin-6 and nuclear factor kappa-B, activates caspase 8, may promote natural killer cell-mediated myeloma cell death and directly induces apoptosis of myeloma cells.<sup>12</sup> Such in vitro activity was confirmed by the 20-25% partial response rate (PR) in 2 phase I trials among patients with relapsing or refractory myeloma.<sup>13,14</sup> A follow-up phase II trial comparing different schedules of lenalidomide demonstrated that a daily dose of 30 mg was well tolerated and produced at least a partial response in 25% of patients with relapsed and refractory MM.<sup>15</sup> An additional 33% of patients that had not responded to lenalidomide as a single agent achieved partial remission with the addition of intermittent pulses of dexamethasone.

Recently the results of 2 double-blind, randomized phase III trials of lenalidomide-dexamethasone versus dexamethasone-placebo were reported.<sup>16,17</sup> In both trials, response, time to progression and overall survival were significantly superior in the group receiving lenalidomide-dexamethasone ( $p < .001$ ). In the North American trial the rate of thromboembolic events was significantly higher among patients treated with lenalidomide-dexamethasone and were highest among those patients who received concomitant erythropoietin. The incidence of grade 3-4 neuropathy was < 2%. Recently a phase II trial of LD in previously untreated patients with MM has resulted in an excellent response rate of >90% and a low rate of thromboembolic events in this group of patients who were treated with concomitant prophylaxis with aspirin. Currently many trials of new combinations with lenalidomide including combinations with melphalan-prednisone, bortezomib-dexamethasone, and doxil-vincristine-dexamethasone.

Of particular interest is a recent phase I trial of bortezomib and lenalidomide for treatment of pts with refractory and relapsed MM.<sup>18</sup> The preliminary response rate of 58% (>PR) is encouraging. Similar results have been reported by Orlowski with liposomal doxorubicin and bortezomib (response 73%).<sup>19</sup> These excellent response rates for salvage regimens without concomitant steroids and in the absence of autologous stem cell support are unprecedented, and if confirmed, these regimens will be particularly useful for pts with diabetes and for those with increased susceptibility to steroid toxicity.

Many novel agents appear to be synergistic with bortezomib in vitro. Preclinical studies suggest Hsp90 is required for unfolding of ubiquitinated proteins and proteasome degradation. A trial with the Hsp90 inhibitor, KOS-953, demonstrated activity in MM and preliminary results of a more recent trial in combination with bortezomib suggests that KOS-953 overcomes bortezomib resistance.<sup>20</sup> Other promising novel agents include FGR and MAP kinase inhibitors and work with these and other novel agents continue to provide promise for myeloma in the future.

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### Minimal residual disease detection in AML; implications for trial design

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Treatment outcome for patients with acute myeloid leukaemia (AML) can be broadly predicted on the basis of a number of pre-treatment factors including age, as well as P-glycoprotein expression, FLT3 and NPM1 mutation status and karyotype of the leukaemic clone. Current risk-stratified treatment approaches to the management of AML typically take into account age/performance status, cytogenetics and response to induction therapy<sup>1</sup>. In many protocols, patients with "favourable risk" AML, i.e. with t(8;21)/*AML1-ETO*, inv(16)/t(16;16)/*CBFB-MYH11* or acute promyelocytic leukaemia (APL) with t(15;17)/*PML-RARA* are no longer routinely subject to transplantation in first complete remission (CR), since any benefit in terms of reduction in relapse risk is offset by transplant-related mortality<sup>2,3</sup>. While transplantation in younger patients with AML with adverse karyotypic features remains standard practice, the optimal management for patients with "standard risk" disease remains uncertain. While cytogenetics has provided a valuable framework for determining treatment approach to AML for over a decade, it fails to precisely pinpoint which patients will be cured following first line therapy and those destined to relapse. Therefore, there is considerable interest in minimal residual disease (MRD) monitoring to determine whether it can provide a more reliable tool for accurately distinguishing these subsets of patients, thereby affording the opportunity to give additional therapy to patients who would otherwise be destined to relapse, whilst conversely, potentially sparing unnecessary treatment-related toxicity in patients who will be cured with first-line therapy.

A number of studies have shown that MRD monitoring using flow cytometric or PCR-based approaches provide an independent prognostic factor, identifying patients at high risk of relapse within cytogenetically defined risk groups (reviewed<sup>4,5</sup>). Use of flow cytometry to detect MRD relies upon the identification of leukaemia associated phenotypes (LAPs) defined on the basis of cross-lineage antigen expression, asynchronous antigen expression or antigen over- or under-expression, with assay sensitivity being determined by the relative frequency of normal bone marrow progenitors with a phenotype akin to the LAP<sup>6-10</sup>. Typically flow cytometric detection of MRD affords sensitivities of 1 in 10<sup>3-4</sup> and carries the distinct advantages that the methodology is relatively fast and importantly is applicable to virtually all cases of AML<sup>10</sup>. However, a key drawback relates to the stability of the LAP, since relapse can occur from minor subclones that may be barely detectable at time of diagnosis (reviewed<sup>5</sup>). Therefore, flow cytometric detection of MRD may be best applied relatively early during treatment (post-induction and immediately post-consolidation) to make more reasoned decisions as to the need for additional consolidation therapy in any given patient, rather than as a means for serial longitudinal disease monitoring. While MRD detection using flow cytometry has been used to determine treatment approach in childhood acute lymphoblastic leukaemia for quite some time, this technology is only now beginning to be integrated into national trials to enhance risk-stratification amongst patients with AML.

Approximately 40% of AML cases carry a fusion gene marker, enabling MRD to be detected by reverse transcription polymerase chain reaction (RT-PCR) <sup>4</sup>. The clinical value of MRD monitoring is most firmly established in APL. Using conventional “end-point” RT-PCR assays for the *PML-RARA* fusion, which typically achieve a sensitivity of 1 in 10<sup>4</sup>, patients destined to relapse can be identified on the basis of molecularly persistent disease or molecular relapse. This enables additional therapy to be targeted solely to the highest risk group of patients, who could not have been reliably identified on the basis of pre-treatment characteristics (reviewed <sup>11</sup>). Preliminary evidence from the Italian GIMEMA group has suggested that pre-emptive therapy at the point of molecular relapse of APL can improve outcome, as opposed to re-treatment once full-blown relapse has ensued <sup>12</sup>. Moreover, MRD monitoring has been shown to predict outcome following autologous transplant procedures undertaken in second CR and is of value as a means of guiding the need for additional therapy in the post-transplant setting <sup>11</sup>. These concepts have been integrated into the ongoing UK Medical Research Council AML15 trial, whereby molecular monitoring using “real-time” quantitative PCR (RQ-PCR) is being evaluated prospectively as a means of directing treatment approach in APL. Patients identified as being at “high risk” of relapse on the basis of persistent PCR positivity or molecular relapse receive additional molecularly targeted therapy in the form of arsenic trioxide, followed by stem cell transplantation. Key aims of the study are to define the optimum frequency and material (PB vs BM) for MRD assessment and to determine whether regular MRD monitoring coupled with pre-emptive therapy can reduce rates of frank relapse, thereby improving overall cure rate.

While early molecular monitoring studies in AML were based on “end-point” qualitative RT-PCR assays; these have now been largely superseded by quantitative PCR technologies, which provide a considerable number of advantages <sup>13</sup>. In particular, they allow quantification of fusion gene transcripts in comparison to endogenous control gene transcripts, thereby enabling accurate assessment of kinetics of response to therapy and prediction of relapse on the basis of a rising fusion transcript level. Parallel quantification of control gene transcripts also permits determination of the sensitivity of each MRD assay, thereby eliminating “false negative” results due to poor sample quality. Moreover, RQ-PCR assays enable more rapid sample throughput than nested RT-PCR assays and are readily standardised, facilitating analysis within multi-centre clinical trials and comparison of data between laboratories. Standardised methodologies and optimised assays for the common leukaemia-associated fusion genes have been established through a large European collaborative study <sup>14,15</sup>.

While MRD monitoring using PCR-based assays is now routinely used to determine treatment approach in APL, use of molecular monitoring for clinical decision making in other subsets of AML is less well established. In the core binding factor (CBF) leukaemias, end-point assays have revealed detectable *AML1-ETO* transcripts in patients in long-term remission, while prolonged PCR positivity was a common feature of *CBFB-MYH11*-associated AML, precluding reliable distinction of patients destined to relapse from those cured of their disease <sup>4</sup>. Subsequently, RQ-PCR analyses have revealed that the prolonged PCR positivity observed in these subsets of AML (as compared to APL) is correlated with a relatively higher level of fusion gene expression in the leukaemic blasts leading to greater assay sensitivity <sup>14</sup>. Preliminary evidence suggests that quantitative PCR assays can more reliably predict relapse in CBF leukaemias <sup>16,17</sup> and opens the way to investigation of the use of pre-emptive therapy in this subset of AML in the future. A further key issue is the identification of novel molecular targets for MRD detection in AML cases that lack a fusion gene marker. One candidate is the detection of *NPM1* mutations which are present in at least half of normal karyotype AML and which appear to provide sensitive and stable targets for MRD detection <sup>18,19</sup>. Considerable interest has also focused upon the *WT1* gene which is over-expressed in the majority of fusion-gene negative AML <sup>20-23</sup>. The relative value of *WT1* monitoring to the development of risk-stratified treatment approaches is being addressed in the context of the European LeukemiaNet initiative ([www.leukemia-net.org/](http://www.leukemia-net.org/)) and is critically dependent upon defining *WT1* expression levels in normal steady state and regenerating blood and marrow. Overall, these studies set the scene for the next generation of AML trials in which MRD monitoring is used to rapidly identify and target additional therapy to patients who are not cured by front-line therapy. Moreover, MRD detection is likely to be used increasingly in the context of stem cell transplantation, not only potentially influencing choice of transplant type, but also used to determine the need for additional therapy in the post-transplant setting.

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### Using surrogate endpoints to aid evaluation of new drugs - the CML experience

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Achievement of excellent clinical responses to rationally designed therapies requires not only good targeted therapies, but excellent response monitoring, and an in-depth understanding of the mechanisms facilitating response. The hallmark haematological disease for rational treatment strategies is CML, and the drug imatinib. Imatinib has resulted in excellent and sustained clinical responses, in most patients with CML. There remains however response diversity, with some patients performing less well. Central to improving outcomes in these patients is the ability to predict their response pre, or in the early treatment phase. Classical prognostic criteria such as Sokal Score, detection of 9q deletions and the presence of additional chromosomal abnormalities have demonstrated some predictive value in the imatinib era. These indicators however provide little information on the interaction of imatinib with the target leukaemic cell, and thus provide little scope for therapeutic manipulation.

As CML results from a reciprocal translocation juxtaposing the BCR and ABL genes, the depth of patient response can be accurately quantitated by RQ-PCR. This sensitive assay provides a sharper criterion than cytogenetic analysis to set endpoints for the assessment of imatinib efficacy. Utilising Crkl, a downstream phosphorylation partner of BCR-ABL, we have developed assays to predict response to imatinib, based on these endpoints. The in-vitro IC50 assay performed pre therapy, has demonstrated surprising diversity in intrinsic sensitivities between patients to imatinib induced kinase inhibition. Significantly, the IC50, provides a predictor of longer term molecular response. Further, using p-Crkl, we have demonstrated the degree of kinase inhibition achieved in-vivo over the first 28 days of imatinib therapy is a potent predictor of longer term molecular outcome. These assays developed for imatinib, now include evaluations for the HSNZ Oral Abstracts, HAA, 15-18 October, 2006

second generation Abl kinase inhibitors (AKI) nilotinib and dasatinib. Response prediction is invaluable for tailoring individual AKI therapies. Of equal importance however, such developments potentiate a greater understanding of the underlying biology of CML cells, and the intrinsic factors mediating response. Current research in our laboratory, based on our previous findings, is focussing on the importance of membrane transport pumps in the achievement of good response outcomes, and the differential relevance of these pumps to the 3 current AKI's.

By retrospectively assessing molecular outcomes in the context of intrinsic factors pre-therapy, and assessing changes which may occur on therapy, rationally tailored treatment strategies in CML can be further developed to improve long term therapeutic outcomes.

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### **Treatment outcomes in follicular lymphoma in 2006 - on the improve**

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Follicular lymphoma (FL) represents 20-25% of all non-Hodgkin's lymphomas. The majority of patients with FL present with advanced disease and cannot be cured with conventional therapeutic approaches. Conventional chemotherapy produces high response rates but patients inevitably relapse and ultimately become resistant to treatment or transform to a higher-grade lymphoma. The median survival of patients with FL is 6-10 years. Recently, new treatment modalities have been developed that offer the hope of improving the long-term outcome. The anti-CD20 monoclonal antibody rituximab has proven single-agent activity in FL. In two phase II studies, the combination of rituximab with chemotherapy (CHOP or Fludarabine) produced overall response rates of over 90% with elimination of bcl-2 positive cells in the majority of responders. Recently, four prospective randomized phase III studies comparing concurrent rituximab plus chemotherapy (R-Chemo) versus chemotherapy alone (Chemo) in previously untreated patients have been published or presented at international meetings. In all four studies, R-Chemo produced significantly higher response rates and longer time to treatment failure. Furthermore, in three of these studies, there was a significantly longer overall survival. The combination of rituximab with chemotherapy is the new standard for the treatment of patients with advanced stage FL. Once a response is achieved, maintenance therapy with rituximab was compared to observation in three randomized controlled trials. In each study, a prolongation of time to disease progression was demonstrated. In the EORTC 20981 study, patients with relapsed/refractory FL were randomized to CHOP or R-CHOP-21. Patients achieving CR or PR were then randomized to maintenance rituximab (3 monthly for 2 years) or observation. Both progression-free and overall survival were significantly prolonged by maintenance rituximab. Other therapeutic modalities which have demonstrated promising results in FL include high-dose therapy with autologous stem cell transplantation. Radioimmunotherapy with radioisotopes conjugated to monoclonal antibodies are showing encouraging results in phase II studies and prospective randomized studies are planned. With these treatment modalities, we now have increasing evidence that the survival of patients with FL is on the improve.