SUNDAY17/10/2004

0930 to 1100 John Batman Theatre

HSANZ Plenary Session 1 - Update on Lymphomas

Chair: Andrew Grigg

1

Application of New Techniques to the Diagnosis & Management of Lymphoma

Randy Gascoyne

The diagnosis and classification of non-Hodgkin's lymphomas (NHL) has undergone significant change in the past few years. Previously based solely upon morphology, modern classification systems now utilize data from various sources, including immunophenotype, cytogenetic, molecular genetic studies and clinical information to accurately sub-classify NHLs. Some cases can be accurately diagnosed using routine H&E morphology, while others may require immunostains and/or interphase fluorescent in-situ hybridization (FISH) studies. It is now well recognized that within any given NHL disease entity there is a diverse spectrum of clinical behavior that reflects the underlying molecular genetic alterations inherent within tumor cells. Thus, beyond a specific diagnosis, it is the determination of additional molecular genetic alterations that may provide important insight into the clinical behavior and help to stratify patient risk. To what extent any or all of these techniques are used to establish diagnoses and determine prognosis varies with the individual case. Current pathology practice not only requires that an accurate diagnosis is rendered, but value-added information is provided to help determine prognosis and plan targeted therapies. The integration of these data to arrive at a specific diagnosis and provide additional prognostic information is a large part of the changing science and art of 'lymphoma diagnosis'. The completion of the human genome project and novel developments in nanotechnology set the stage for large scale genome-wide analyses required to move towards a molecular classification of tumors. Most lymphoma subtypes have now been studied, setting the stage for these techniques to take their place in the current test menu. Additionally, these data can now be complemented with high-resolution cytogenetic data from array comparative genomic hybridization (CGH) studies and will soon develop more texture as proteomic techniques mature and data are generated for NHLs. How these approaches are used to make a diagnosis and in particular, how they might translate into the clinic will be the focus of the discussion.

2

Application of New Techniques to Diagnosis/Managment for Lymphoma

Joseph M Connors

sponsored by Baxter

Major new insights into the biology of lymphomas are being derived from the use of micro-array gene expression technology to elucidate their underlying biology and to identify novel pathways for therapeutic intervention. Already these studies have substantially improved our understanding of four of the most common B-cell lymphomas: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FOLL), mantle cell lymphoma (MCL) and primary mediastinal large B-cell lymphoma (PMBCL).

Lymphomas display enormous variation in their natural history and response to treatment, usually associated with differing histologic diagnoses or easily measured clinical factors such as LDH level or patient performance status. However, even with apparently equal tumor burdens at presentation some patients are cured and others succumb to lymphoma. These differences must reflect intrinsic gene expression variations but have been difficult to characterize with single gene expression studies (essentially, immunohistochemical staining or, occasionally, quantitative PCR). Micro-array based gene expression studies, with their ability to characterize whole families of gene expression pathways, provide novel insights into the basic biology of each lymphoma that cannot be gathered in any other way.

The key insights provided thus far by gene expression studies have differed from one lymphoma to another. In DLBCL, a fundamental distinction has been found separating cases into germinal center B-cell type (GCB) and activated B-cell (ABC) lymphomas with the latter preserving many of the gene expression patterns of normal germinal center B-cells and displaying at least twice as good a prognosis when compared to the latter conferring a prognosis much worse than that seen in the GCB type. Studies in FOLL have provided the major new insight that pathways that appear to reflect common immune responses in the reactive cells associated with the lymphoma dramatically affect outcome. In MCL studies have confirmed the homogenous nature of this BCL1 driven lymphoma but also show that increasing recruitment of proliferation genes leads to a five-fold difference in prognosis. Finally, gene expression analysis of PMBCL has revealed its close kinship with Hodgkin lymphoma and, interestingly, a pivotal role for NF?B.

1130 to 1245 John Batman Theatre
HSANZ Plenary Session 2 - Microarrays in Haematology Practice: esoteric or essential?
Chair: David Ma

3

Discovery of Novel Molecular Classification Schemes and Genes Predictive of Outcome in Leukaemia

Cheryl L. Willman

A major challenge for the treatment of acute leukemia is to improve and refine diagnosis and risk classification schemes in order to precisely tailor therapeutic approaches to the biology of the tumor and the genotype of the host, and, to develop more effective therapies for resistant forms of disease. To meet this challenge, many have hypothesized that genomic technologies that measure global patterns of gene expression in leukemia patients will yield orderly and systematic profiles that may ultimately be useful for: 1) the identification of intrinsic biologic groups of acute leukemia not currently appreciated by current diagnostic techniques; 2) the develop of "molecular classification schemes" based on gene expression profiles; 3) the identification of novel genes that can refine risk classification; and 4) the identification of new therapeutic targets. Our research team at the University of New Mexico Cancer Research and Treatment Center and our collaborators at the UNM High Performance Computing Center and Sandia National Laboratory have completed comprehensive analyses of gene expression profiles using Affymetrix oligonucleotide arrays (U-95A.v2 containing 12,625 genes/ESTs) in several retrospective cohorts of pediatric and adult leukemia patients, including: 1) 127 infants with leukemia (79 ALL, 48 AML, 57/127 with MLL rearrangements) registered to Pediatric Oncology Group (POG)/Children's Oncology Group (COG) clinical trials; 2) 254 pediatric ALL patients registered to POG trials stratified for the presence of recurrent cytogenetic abnormalities and remission vs. fail within each cytogenetic group; and 3) 325 cases of adult AML registered to various trials conducted by the Southwest Oncology Group (SWOG). Gene expression data were correlated with a large number of biologic and clinical co-variables (including AML/ALL type, cytogenetics, clinical outcome with long term follow-up) using novel algorithms, new data visualization tools, and parallel high performance computing approaches at the UNM High Performance Computing Center and Sandia National Laboratory in Albuquerque. This presentation will briefly summarize this work in the context of a discussion of the different methodologies for computational data analysis, an extremely critical component of these investigations. Although each of the statistically defined cohorts that we have studied represents different types of leukemia, there were some consistent themes:

1. Although strong correlations exist in certain forms of leukemia, the intrinsic biologic clusters found in acute leukemias in infants, children, and older adults are often uniquely identified through gene expression profiling and are not precisely identified or defined by traditional morphologic features, phenotype, or cytogenetics, suggesting that incorporation of the results of gene expression profiling indeed refine diagnosis and risk classification.
2. The biologic groups of leukemia (clusters) identified through gene expression profiling are highly dependent on the specific cases under investigation and the statistical design of the patient cohort; this information is critical when comparing results obtained by different laboratories.
3. Using powerful, supervised machine learning algorithms in well-designed statistical cohorts, one can identify gene expression profiles and frequently novel genes that are associated with or predictive of class (cluster), outcome, or other clinical and biologic parameters. We and others have identified novel genes highly predictive of outcome in infant and pediatric ALL and adult AML

4

Gene Expression Profiling – Esoteric Tool or Essential

Keith Stewart

Recently the biology of myeloma has been interrogated in studies which reveal that patients have unique gene expression clusters which correlate with disease severity. Further analysis has begun to produce powerful prognostic models based on as few as 3 genes. Gene expression profiling (GEP) has also proved of value in defining therapeutic targets. For instance GEP can identify hallmark IgH translocations and other common structural genetic changes which impart prognostic significance. GEP has also been employed in defining new therapeutic targets. We have employed high throughput global and kinome specific sequencing and cDNA arrays to this end. Finally, molecular profiling has been demonstrated to be of value in pharmacogenomic studies predicting response to therapy and revealing novel therapeutic targets. Thus there is no doubt that gene expression profiling has revealed much about myeloma in a relatively short period of time. In particular, it appears that novel prognostic risk factors, tailored therapies and new molecular targets will all eventually derive from expression profiling data. Nevertheless, the links of microarray data to clinical outcomes are only now being made and as yet only in a handful of laboratories worldwide. Thus for now expression profiling must remain a research tool. Those caveats aside, in the years ahead, the manner by which multiple myeloma will be diagnosed, treated and monitored seems likely to be built on the back of current genomic studies.

5

Gene Expression Signatures in Childhood Leukaemia - Current Studies and Future Perspectives

Ursula Kees

Telethon Institute for Child Health Research, Division of Children's Leukemia and Cancer Research, WA

Cancer therapy has optimal chance of success if tailored to the exact tumour type of the patient, and high specificity of therapeutics can be achieved when they are designed for particular alterations in cancer cells. Novel high-throughput genomic technologies, such as microarrays, provide new avenues for the molecular diagnosis of malignancies. Acute lymphoblastic leukaemia (ALL) is the most common cancer in children and long-term survival has reached 75-90%. Despite this, therapy resistant forms are a leading cause of cancer-related deaths in children. Our studies focus on the gene expression profile of paediatric ALL in order to improve our understanding of the biology of the disease and the response to current therapy. We have analysed 121 patient specimens using Affymetrix HG-U133A arrays. Data were normalised and gene expression profiles were compared using the Random Forest algorithm. Genes identified as differentially expressed between groups of specimens were further analysed by quantitative RT-PCR. The patient specimens obtained at the time of diagnosis were examined to identify gene expression profiles correlated with clinical outcome. Since contemporary therapeutic protocols involve many drugs, we have designed studies to examine whether there is a link between sensitivity to individual chemotherapeutic agents and gene expression profile. This complementary approach makes use of our panel of 17 cell lines established from paediatric ALL specimens. Our studies showed that microarray technology has the potential to lead to more accurate prognostic assessment for patients, and is expected to ultimately lead to test that allow the clinician to select therapies optimally suited to each patient.

1415 to 1530 John Batman Theatre
HSANZ Plenary Session 3 - Cytokines and Transplantation
Chair: Jeff Szer

6
How do Cytokines Mobilise Stem Cells?
Jean-Pierre Levesque

Adhesive Interactions and Cell Trafficking Laboratory, VIC

Despite the extensive use of mobilised blood as a source of transplantable haematopoietic stem cells (HSCs), the mechanisms that lead to the mobilisation of HSCs into the peripheral blood in response to cytokines such as G-CSF are still poorly understood, delaying the design of more efficient mobilising agents. We have shown that the bone marrow haematopoietic microenvironment is profoundly altered during mobilisation with loss of expression of the cell adhesion molecule VCAM-1/CD106 and decreased concentration of soluble chemokine SDF-1/CXCL12, two molecules expressed by bone marrow stromal cells and essential to the retention of HSCs within the bone marrow. We further demonstrated that administration of mobilising agents results in a dramatic increase of granulocyte numbers in the bone marrow and release of neutrophil proteases that cleave and inactivate VCAM-1 and CXCL12 in the bone marrow. Consequently, we proposed a model by which HSCs are mobilised due to proteolytic cleavage of VCAM-1 and CXCL12 as a consequence of neutrophil proteases accumulating in the bone marrow in response to mobilising agents. In favour of this model, several laboratories have shown that either the loss of VCAM-1 expression or antagonising CXCL12 is sufficient to mobilise HSCs. However, we have recently shown in mice lacking genes encoding neutrophil proteases that, whilst the loss of VCAM-1 expression is protease-dependent, neutrophil proteases are not absolutely required for mobilisation of HSCs nor for the reduced expression of CXCL12. This implies that parallel protease-independent mechanisms, such as the reduction of CXCL12 transcription, contribute to mobilisation.

7
Optimisation of Stem Cell Mobilisation to Separate GVHD and GVL
Geoff Hill

QIMR , QLD

Stem cell mobilization modulates the ability of donor T cells to induce graft-versus-host disease although the mechanism remains unclear. We have studied the effects of G-CSF together with the more potent newer progenipoietins and pegylated versions of G-CSF in order to dissect the mechanisms involved. We have found that G-CSF induced two main populations of regulatory cells within the transplant donor. One is a myeloid precursor that presents host antigen after SCT in a tolergenic fashion. The subsequent expansion of IL-10 producing regulatory T cells in turn promotes tolerance. In addition the function of regulatory T cells in the transplant donor is preferentially augmented by G-CSF. Both these effects are G-CSF dose dependent with both the pegylated-G-CSF and progenipoietins having significantly enhanced regulatory properties. Surprisingly, these cytokines do not inhibit graft-versus-leukaemia effects as would be expected. A phase I/II clinical trial utilising pegylated-G-CSF to mobilise stem cells for allogeneic transplantation has now commenced in Australia.

8

Cytokine Blockade Strategies for GVHD Prophylaxis and Treatment

James Ferrara

Acute Graft versus host disease is a major cause of morbidity and mortality after allogeneic bone marrow transplantation (BMT). Primary treatment of GVHD with corticosteroids achieves a complete response (CR) rate of 20-40%. Extensive pre-clinical data have identified inflammatory cytokines, including tumor necrosis factor-alpha TNFα as one of the important mediators of GVHD. This session will describe the role of cytokines in the pathophysiology of this disease, and new approaches to circumvent the problem. A clinical study of TNFα blockade to treat GVHD will also be described. Patients were treated with solumedrol (2mg/kg) and etanercept as initial therapy for acute GVHD. Etanercept was begun at 25 mg subcutaneously two times a week for 8 weeks. It was started within 72 hrs of the first dose of solumedrol. Solumedrol was maintained at 2 mg/kg for at least 1 week. Patients were monitored for response and toxicity for 4 weeks after the final dose of etanercept. Twenty patients were enrolled and the response rate was 70%, double that of historical controls.

In additional studies, we have developed a plasma proteomic analysis for the diagnosis of acute GVHD. No single diagnostic blood test for GVHD is available at the current time. The vast dynamic range of protein abundance in human plasma presents formidable challenges to any single bioanalytic technique. In order to achieve greater resolution and sensitivity in the analysis of plasma proteins we have developed an orthogonal, three-dimensional, intact protein-based separation system. This strategy employs an immunoaffinity chromatography column for removal of the most abundant proteins (albumin, transferrin, IgA, IgG, haptoglobin and α_1-antitrypsin) in order to increase resolution of low abundance proteins. Paired plasma samples were collected from five BMT patients two weeks prior to the diagnosis of GVHD and on the day of diagnosis. Pooled samples were concentrated and labeled with Cy5 (pre-diagnosis) or Cy3 (at diagnosis). Labeled samples were then were mixed and separated in an orthogonal, three-dimensional system according to pI, hydrophobicity, and molecular mass. Differences in protein levels prior to the diagnosis of GVHD and at the time of diagnosis were quantitatively analyzed by differential gel electrophoresis. Approximately eight hundred fractions each then resolved in a one dimensional SDS PAGE and visualized using a Typhoon scanner. Using capillary HPLC ESI Q-TOF MSMS, we have identified approximately eighty unique proteins whose expression levels in plasma changed at the onset of GVHD, including forty that were upregulated and forty that were downregulated. Several of these identified proteins include growth factors and interleukins in nanomolar concentrations. This strategy thus provides a novel combination of comprehensive profiling and quantitative analysis which will help define alteration in circulating proteins associated with this major complication of bone marrow transplantation.

1600 to 1730 Bellarine Room 1
HSANZ Plenary Session 4A - Haemopoietic Stem Cell Biology
Chair: Ngaire Elwood

9

SCL - A Master Regulator of Adult Haemopoiesis

David J Curtis, Mark A Hall, Nicholas J Slater, Stephen M Jane, C Glenn Begley

Rotary Bone Marrow Research Laboratory, Royal Melbourne Hospital, Melbourne, Australia

Expression of DNA-binding helix-loop-helix transcription factors (bHLH) are critical for lineage commitment and differentiation of many cell types (muscle, blood, neural). In the haemopoietic system, the aptly named stem cell leukaemia gene (SCL) is one such bHLH transcription factor. Without it, mice die at the onset of haemopoiesis due to complete lack of erythropoiesis and defective endothelial development. Subsequent studies utilising SCL-null embryonic stem cells have confirmed that SCL is critical for the differentiation of the presumptive hemangioblast. In the adult, SCL is expressed in multi-potent progenitors including stem cells as well as the erythroid, megakaryocyte and mast cell lineages. To circumvent the embryonic lethality of SCL knockout mice, we have used a conditional knockout approach. Unlike development of haemopoiesis, deletion of SCL in adult haemopoiesis was not lethal. SCL-null mice survived for greater than 12 months with a relatively mild anaemia and thrombocytopenia, suggesting that other factors could partially compensate for loss of SCL. More stringent assays of haemopoiesis revealed a role for SCL at both the stem cell and progenitor level. Most striking was the complete loss of the major erythroid and megakaryocyte progenitors and a 10 to 50-fold increase in mast cell progenitors. Furthermore a DNA-binding mutant of SCL rescued megakaryocyte growth but not erythroid cell growth in SCL-null haemopoiesis. We propose that expression and different functional domains of SCL regulate the cell fate of a common erythroid/megakaryocyte/mast cell progenitor.

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Applying Haemopoietic Stem Cell Biology for Therapeutic Gain

David Haylock, Susan K Nilsson, Paul J Simmons

Peter MacCallum Cancer Institute, VIC

The biological potential of haemopoietic stem cells (HSC) is well recognised. These relatively rare cells have the ability to sustain production of mature blood elements for life. Transplantation of unmanipulated haemopoietic stem and progenitor cells (HPC) is now commonly performed and has predictable outcomes and risks. In contrast, novel therapeutic procedures with HSC/HPC including transplantation with ex vivo expanded/propagated cells have not been widely adopted. Nevertheless, at least 23 clinical trials with ex vivo expanded cells have been reported. A total of 323 patients have been transplanted with ex vivo expanded cells with no reports of toxicity or serious adverse events. However, there is a wide discrepancy in the documented effects on haemopoietic recovery (HR) attributed to infused ex vivo expanded cells and no studies provide information on the mechanisms responsible for improved outcomes with ex vivo cell transplants. Cord blood (CB) transplantation is a unique clinical setting where application of HSC biology could lead to significant therapeutic gain. Paradoxically, cord blood HSC exhibit extensive proliferative potential in vitro, yet patients transplanted with CB have delayed HR; which is considered a direct consequence of the low dose of stem cells transplanted. Accordingly, transplantation with ex vivo expanded CB could improve HR dramatically. Although many of the key regulators responsible for inducing division and proliferation of HSC have been described those combinations of factors that influence stem cell self-renewal remain poorly understood. We believe that a comprehensive analysis of the haemopoietic stem cell niche is important for identifying novel molecules involved in this process. Moreover, presentation of these molecules in a manner similar to that in vivo will be necessary to achieve HSC expansion.

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Retroviral Gene Transfer into Haemopoietic Stem Cells - State of the Art

John Rasko

Centenary Institute of Cancer Medicine and Cell Biology, NSW

Gene therapy promises compelling advantages for the treatment of diverse diseases with a genetic component, including many that affect the blood. These advantages include the production of protein(s) by endogenous cells, minimisation of exposure to exogenous pathogens, long-term correction of inherited disorders and novel therapeutics for diseases that currently lack treatment options. However, despite hundreds of clinical trials using various delivery systems, many of these promises had not translated into reality until 1999 when the first report of success in a handful of young children with severe combined immunodeficiency was published. The excitement generated through the initial success of this proof-of-principal trial has been greatly tempered by reports that two of the children have developed leukaemia. This setback is related to insertion of the retroviral gene therapy vector near to a known oncogene. In this talk we will explore the tools available to achieve gene transfer and the technical hurdles that currently hinder their widespread clinical application. The outcomes of a Phase I Study of AAV-Mediated, Liver-Directed Gene Transfer for Hemophilia B will also be summarised.

1600 to 1730 John Batman Theatre
HSANZ Plenary Session 4B - Update on Myeloproliferative and Myelodysplastic Disorders
Chair: Lynda Campbell

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Molecular Biology and Therapy of Chronic Myeloproliferative Disorders
Junia V Melo
sponsored by Novartis

Chronic myeloid leukaemia (CML) is caused by a t(9;22)(q34;q11) chromosomal translocation that gives origin to a fusion gene, encoding a constitutively activated tyrosine kinase, the Bcr-Abl oncoprotein. Bcr-Abl became the paradigm for molecularly targeted therapy when it was shown to be efficiently inhibited by the tyrosine kinase inhibitor imatinib mesylate. This drug induces haematological remission in over 90% and a major cytogenetic response in approximately 70% of patients with chronic phase CML. However, molecular remissions are so far rather rare, and relapse under imatinib therapy occurs at a high frequency in patients with advanced disease. These unwanted phenomena dictate the need for the discovery of alternative forms of therapy for CML, either to enhance the effect of imatinib and/or to replace it when irreversible drug resistance takes place. Several other signal transduction inhibitors (STIs) have recently become available for pre-clinical investigations, and some have been shown to synergise with imatinib, both in imatinib-naïve and -resistant cells. Among these STIs are inhibitors of key downstream effectors of Bcr-Abl, such as the PI-3, MEK, Ras and Src kinases. Furthermore, the possibility of combining STIs and other molecularly targeted forms of therapy, such as RNA interference and immunomodulatory strategies will probably improve considerably the chances of achieving complete elimination of the leukaemic clone in CML. It is also important to note that BCR-ABL-negative myeloproliferative disorders caused by similar fusion genes, such as those containing activated forms of PDGFRB, PDGFRA, KIT and FGFR1 receptors, are equally amenable to the effect of specific inhibitors of these kinases. The successful example of molecularly targeted therapy in CML has opened the field for similar approaches in other forms of leukaemia and solid tumours.

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Update on Myelodysplasia
Pierre Fenaux

MONDAY18/10/04

0830 to 1030 John Batman Theatre
HSANZ Plenary Session 5 - New Developments in Lymphoma Management
Chair: David Joske

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Hodgkin Lymphoma: Limited, Advanced and Recurrent. What's New?
Joseph M Connors
sponosred by Baxter

Hodgkin lymphoma is cured in more than 80% of patients. Indeed, our challenge today is not to cure the disease but rather to do so with the least long-term toxicity. Limited stage Hodgkin lymphoma, stage I or II disease without B symptoms or without bulky disease (>10 cm) is found in about 30% of patients at diagnosis. The current international standard of care for such patients is two cycles of ABVD chemotherapy followed by involved field radiation. The time has come, however, to move beyond that standard. The pivotal Canadian trial of chemotherapy alone versus combined modality treatment shows that overall survival exceeding 90% can be achieved without radiation and will be presented in detail. Advanced Hodgkin lymphoma is present in about 70% of patients at diagnosis and is best treated with an extended course of chemotherapy, usually ABVD. Newer regimens such as escalated BEACOPP and Stanford V may, in special circumstances, be a better choice for certain patients, however, for the more than 80% of patients with moderate to good prognosis advanced Hodgkin lymphoma neither of these regimens has proven to be desirable, primarily because of excessive toxicity and lack of improvement in overall survival.

Hodgkin lymphoma recurs in less than 5% of cases of limited and 25% of cases of advanced stage disease. Fortunately, and in distinction to most other human neoplasms, even recurrent disease can be cured. High dose chemotherapy followed by autologous stem cell transplantation is the treatment of choice for almost all patients and cures more than 50% of patients with relapse after an initial complete response and even 25% to 30% of patients with disease that progressed despite initial ABVD. As we move into the era when all patients with Hodgkin lymphoma should be treated with chemotherapy alone another step has been taken in maintaining very high cure rates while reducing even further long-term toxicity.

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Management of Early Stage Hodgkin's Lymphoma
Andrew Wirth

Peter MacCallum Cancer Institute , VIC

Early stage Hodgkin's lymphoma (HL) is now curable in the majority of cases with the use of chemotherapy and/or radiotherapy (RT). The major goals of modern therapy are to maintain a high cure rate while minimising the toxicity of therapy. Patients with lower and higher risks of relapse can be identified, and the intensity of therapy can be adapted accordingly. For favourable disease, 3-4 months of ABVD and involved field RT cure over 90% of patients, and recent preliminary trial data suggest that as little as two cycles of ABVD and low dose (20 Gy) involved field RT may be adequate therapy. For patients with risk factors, such as bulky disease or B-symptoms, 4-6 cycles of ABVD are appropriate, with ongoing studies evaluating the incorporation of more intensive chemotherapy (BEACOPP) based on excellent results in patients with advanced disease. Several trials suggest that the use of full dose chemotherapy alone may be less effective than short course chemotherapy and involved field RT. However, for some patients, such as children or young women with mediastinal/axillary disease, the late risks of RT are an important consideration. For such patients, the selective omission of RT is an option. It is possible that early metabolic response to initial chemotherapy may assist in decision making regarding consolidative RT. Lymphocyte predominant HL represents a distinct sub group that may be treated with involved field RT alone. Meticulous imaging and RT technique are important to optimise outcomes when RT is used.

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Critical Issues Surrounding Radioimmunotherapy of B Cell Lymphoma

J.Harvey Turner

The University of Western Australia, Fremantle, WA

Non-myeloablative radioimmunotherapy (RIT) of relapsed or refractory non-Hodgkins Lymphoma (NHL) with single dose 90Y- ibritumomab tiuxetan Zevalin½ or 131I-tositumomab Bexxar½ murine anti-CD 20 Mabs is FDA-approved and shows efficacy in low grade follicular or transformed B cell NHL (ORR 70-83% CR 37% with durable response of > 1 year in 37%). 131I-rituximab anti CD 20 chimeric Mab (MabThera½) has similar durable ORR with CR > 50%. Zevalin½ is prescribed as an empiric activity whereas Bexxar½ and 131I-rituximab are administered as a predetermined 0.75Gy radiation absorbed dose to bone marrow after prospective individual patient dosimetry. Grade IV haematological toxicity (NCI) after Zevalin½ 13%, 33%, Bexxar½ 2%, 14%, and 131I-rituximab 2%, 15%, for platelets and neutrophils after RIT is self-limited. Myeloablative RIT Bexxar½ or 131I-rituximab in combination with chemotherapy and stem cell rescue has reported ORR 86%, CR 76% in aggressive follicular NHL and Mantle Cell lymphoma without significant toxicity. Non-myeloablative repeated administrations of Zevalin½ Bexxar½ or 131I-rituximab for initial responders who relapse after > 12 months show renewed response with acceptable toxicity. Combination of RIT with chemotherapy achieved ORR 95% CR 50% in mantle Cell lymphoma. After frontline chemotherapy of follicular NHL supplementary RIT improved the CR to 95% with manageable overlapping toxicity. Upcoming RIT agents include 186Re / 188Re chimeric rituximab, humanized 131I/188Re/177Lu/ 90Y-epratuzumab anti CD 22 Mab targeting NHL and 131I-chimeric anti-tenascin Mab targeting matrix protein of NHL tumour stroma vasculature. Hodgkins lymphoma RIT with 131I-basiliximab anti-CD 25 Mab and 90Y-daclizimab is also in clinical trial.

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N Radioimmunotherapy with Iodine-131 anti-CD20 chimeric monoclonal antibody (Rituximab) for Relapsed or Refractory Indolent Non-Hodgkin's Lymphoma: Results of an Australian Phase II Trial for the Australian 131I-Mabthera Radioimmuotherapy Investigators

M F Leahy, J F Seymour, R J Hicks, J H Turner

Peter Maccallum Cancer Insitute

Radioimmunotherapy is an effective therapy for patients with relapsed/refractory indolent non-Hodgkin's lymphoma (NHL) with two commercial products licensed in Europe and the USA. An Australian phase II multicentre study was conducted to explore the efficacy and safety of ^{131}I radioimmunotherapy using the chimeric anti-CD20 antibody Mabthera using an "in house" conjugation method. Eligibility included relapsed/refractory indolent NHL, including mantle-cell NHL, no evidence of histologic transformation, confirmed CD20 expression, > 6 months from prior Mabthera exposure, ANC >1.5 and platelets >100 and no prior stem-cell transplantation. From 2000–2004, 107 patients with a median of 3 prior therapies (range 1–8) have been enrolled; median age 63 years (35–84), 60% male, 68% stage III/IV. Histology was predominantly follicular (74%) but included small lymphocytic/marginal zone (16%) and mantle-cell (8%). Patients were pretreated with Lugol's iodine, and had a tracer dose after "cold" Mabthera 375 mg/m^2, 3 whole-body dosimetry scans, and 7–14 days later a therapeutic dose of ^{131}I-Mabthera to deliver 0.75Gy red marrow radiation dose, again following "cold" Mabthera. Patients were monitored weekly until wk12, and restaged at 3, 6, and 12 months. The cohort treated at Peter MacCallum also had prospective molecular and PET evaluations. Haematologic toxicity was modest. Median nadir values (time to nadir) were; Hb 118 (wk8), ANC 1.5 (wk7), and platelets 74 (wk5). Grade 4 haematologic toxicity was uncommon; Hb 0%, neutrophils 21%, platelets 4%. Only 2 patients required IV antibiotics and 1 G-CSF. 13 patients have developed hypothyroidism, and 2 MDS (both RCMD). 95 patients are evaluable for response; 51% CR/CRu, 23% PR (ORR 74%). Lower response rates were observed with mantle-cell NHL (38%), high LDH (64%), but not influenced by stage or bulk (≥10 cm). The median PFS was 13Mo, and median response duration 8Mo for PR and 21Mo for CR, with 40% of CR patients in continued remission beyond 2yrs. The 4-year actuarial survival of all treated is 63 ± 7%. These results establish the safety of this approach and demonstrate both short- and long-term efficacy comparable to those reported using commercial products.

1100 to 1200 John Batman Theatre
HSANZ Plenary Session 6 - Advances in Stem Cell Transplantation
Chair: Ken Bradstock

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New Concepts in Haemopoietic Stem Cell Transplantation
James Ferrara

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Adoptive Immunotherapy for Prophylaxis of CMV Post Allogeneic Transplantation
David Gottlieb

Westmead Hospital, NSW

CMV is a common human pathogen that persists after an initial episode in healthy individuals as a latent infection producing few clinical symptoms. However, during periods of immune suppression after allogeneic stem cell transplantation (SCT) CMV may reactivate causing significant morbidity and mortality. The importance of the cellular immune response in controlling CMV proliferation has been well documented in both healthy and immune suppressed individuals. In immunocompromised patients, the regeneration of functional CMV-specific immunity is essential to limit problems associated with CMV reactivation. Reconstitution of CMV-specific immunity using cloned donor-derived cells has been achieved post-allogeneic transplant, but is not logistically feasible as a routine clinical procedure. Clinical acceptance will require simplification of CMV-specific cell generation along with development of GMP grade methods satisfying regulatory authorities. Potential antigens include lysates of whole CMV-infected cells, peptides derived from CMV proteins and antigen presenting cells transduced with vectors containing genes encoding immunogenic CMV proteins. Trials will need to identify those antigen(s) best suited to producing CMV-specific cytototoxic T cells, the number of such cells required and the ideal time of infusion. It is likely that a balance of CMV-specific CD4+ and CD8+ lymphocytes will be required. Since the antigens presented during acute infection probably differ from those expressed chronically and surveyed by the immune system during periods of quiescence, cellular immunotherapy for prophylaxis of reactivation and for treatment of active infection may involve direction of the immune response towards different antigenic targets.

1200 to 1300 John Batman Theatre
Carl De Gruchy Oration
Chair: Mark Hertzberg

'Six Degrees of Separation - with a Flake of my Life'
Graham Young

'Six Degrees of Separation - with a Flake of my Life' The de Gruchy Oration in 2004 will be a little different from previous years, and will tell a personal tale based on the book and movie 'Six Degrees of Separation'.

1400 to 1530 John Batman Theatre
HSANZ Free Communications 1 - Clonal StemCell Disorders / Molecular Haematology
Chair: Peter Bardy

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Determination of the IC50 for Imatinib Mesylate in de-novo CML Patients on the TIDEL Study is Predictive of Molecular Response.

Deb White [1,2], Verity Saunders [1,2], A.Bruce Lyons [1,2], Timothy Hughes [1,2,3]

[1] Institute of Medical and Veterinary Science
[2] Hanson Institute
[3] Australasian Leukaemia Lymphoma Study Group

The ability to predict those CML patients who will do poorly on imatinib may facilitate a more aggressive approach to therapy in these patients. We have used western blotting to assess the phosphotyrosine content of the adaptor protein Crkl (p-Crkl) -a major substrate for BCR-ABL in the TIDEL Study. PB was collected from 54 patients prior to the initiation of therapy and IC50 for imatinib was determined. The median IC50 for imatinib was 0.6 uM (R 0.375 - 1.8uM). IC50's were then grouped into low and high relative to the median, and compared to molecular response achieved at 3 and 12 months. Using log rank survival analysis there was a significant difference in the probability of achieving a 2 log depletion of BCR-ABL by 3 months between the two groups (Low IC50 33%; High IC50 5%; p=0.01). Further to this there was a significant difference in the probability of achieving a 3 log reduction by 12 months (High 48%; Low 18%; p=0.022). We then analysed patient data to assess whether the IC50 was a surrogate measure of Sokal or Hasford , or was an independent predictor of response. When IC50 was compared to the prognostic indicators there was no correlation between either score and the IC50 (R2 = 0.009 for Sokal and 0.0281 for Hasford) Similarly when IC50 was compared to the percentage of blasts in the PB prior to imatinib therapy the R2 value was 0.038. In this analysis a small, but distinct group of patients who had a low IC50 and a high blast %, were identified. Interestingly, despite having a low IC50, none of these patients achieved a 3 log reduction of BCR-ABL by 12 months. Confining the analysis to patients with less than 2% blasts there was a greater difference in probability of achieving a 3 log reduction by 12 months in the low v high IC50 groups (High 73%; Low 15%: p =0.003). This preliminary evidence would suggest that the presence of blasts in the PB prior to imatinib therapy may be an independent unfavourable risk factor. We conclude that intrinsic sensitivity to imatinib as measured by IC50 is predictive of molecular response in imatinib treated patients.

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Philadelphia Negative Clones Identified in Patients with Chronic Myeloid Leukaemia Treated with Imatinib (Glivec) are Associated with Significant Risk of Transformation into Secondary Acute Myeloid Leukaemia

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Aim: To review the outcome of imatinib (Glivec) treated CML patients who developed Philadelphia chromosome negative (Ph-) clones identified on routine cytogenetic analysis of bone marrow samples processed in our laboratory.

Method: Cytogenetic results of all bone marrow examinations performed in CML patients on Glivec therapy were retrospectively reviewed. In order to confirm that any identified Ph- clones did not harbour a silent t(9;22), in all samples with an apparent Ph- clone on standard cytogenetics, interphase FISH was performed utilizing the BCR/ABL probe as well as a probe specific for the identified non-Ph abnormality.

Result: Of a total of 69 CML patients on Glivec therapy, 3 (4%) were identified with Ph- clones. Interphase FISH analysis confirmed the absence of t(9;22) in all 3 cases. All 3 patients were diagnosed in chronic phase 1, had initially been treated with interferon (IFN) before commencing Glivec, and had achieved a complete cytogenetic response on Glivec prior to development of the Ph- clones. Median time to development of Ph- clones was 31mths (range 18 to 82mths) post diagnosis of CML, and 12mths (range 6 to 28mths) post commencing Glivec. In 2 cases (a 63yr male and a 77yr male), development of Ph- clones [45 XY,-7 and 46,XY,del(5)(q13q31) respectively] was associated with rapid transformation into Ph- acute myeloid leukaemia (AML). In case 3 (47, XXX), no change in peripheral blood counts has been noted at 2mths follow-up.

Conclusion: Our experience suggests that development of Ph- clones in CML patients treated with Glivec is associated with significant risk of transformation into secondary AML. As such, we recommend that patients who develop such clones be closely monitored and if appropriate, referred early for consideration of allogeneic stem cell transplantation.

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Fusion of the Platelet-Derived Growth Factor beta to a Novel Gene KIAA1509 on Chromosome 14 in an Atypical Myeloproliferative Disorder Associated with Eosinophilia

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Aim: Platelet-derived growth factor beta(PDGFRB), a tyrosine kinase receptor, has recently been shown to be constitutively activated by fusion to different gene partners in atypical, bcr-abl negative, myeloproliferative disorders. As a consequence, the activated PDGFRB gene is a potential target for the tyrosine kinase inhibitor imatinib mesylate. The objective of this study was to further characterise a t(5;9;14) translocation found in a patient with a myeloproliferative disorder.

Case study: A 22-year-old female was diagnosed with an atypical myeloproliferative disorder with eosinophilia and a lichenoid eruption involving her skin and oral mucosa. G banded cytogenetics on her bone marrow revealed t(5;9;14)(q33;q22;q32) in 38 of 40 metaphases examined. Fluorescence in situ hybridisation using a CSF1-R probe suggested a breakpoint involving the PDGFRB gene locus on chromosome 5q33 with an unknown partner gene on chromosome 14.

Result: 5' RACE studies using a PDGFRB primer generated a product of approximately 220bp that on sequencing revealed a novel gene KIAA1509 fused to the 3' end of PDGFRB in exon 11. KIAA1509 has previously been mapped to chromosome 14 and encodes a protein of 1935 amino acids that shows homology to HOOK proteins and contains coiled coiled domains. The resulting in frame fusion gene preserves the tyrosine kinase domains of PDGFRB but lacks the transmembrane domain, and would be predicted to result in constitutive activation of the tyrosine kinase receptor. In view of these findings, the patient was commenced on imatinib, achieving a complete cytogenetic response after 3 months therapy.

Conclusion: In this study a novel KIAA1509 - PDGFRB fusion gene has been characterized. These findings highlight the importance of investigating the molecular basis of the myeloproliferative disorders to identify potential novel targets for imatinib and similar molecules.

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Is all Chronic Idiopathic Thrombocytosis Essential Thrombocythemia? - ENU Mutagenesis Screen Identifies a Novel Model of Idiopathic Thrombocytosis

William Stevenson, Donald Metcalf, Doug Hilton, Andrew Roberts, Warren Alexander

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Approximately 30% of women with a clinical diagnosis of ET have polyclonal granulocytes when clonality is assessed by X chromosome inactivation, suggesting that they do not have a clonal stem cell disorder. In order to identify novel genes that when mutated cause thrombocytosis we randomly induced point mutations into the mouse genome with ENU, and screened pedigrees for heritable thrombocytosis. Mice with the Plt2 mutation were identified as a model of non-clonal idiopathic thrombocytosis. Platelet counts for Plt2 homozygote mice were 1997 ± 642x109/L (mean ± 2SD; n=51), 140% of those observed for wild-type mice (1487 ± 372; n=56) and Plt2 heterozygote mice (1402 ± 554; n=76). Analysis of Plt2 animals showed increased numbers of megakaryocyte precursors (as measured by meg-CFC/5x105 cells to maximal cytokine stimulation) in the bone marrow (Plt2 40 ± 20 cf wild-type (WT) animals 28 ± 8; n=7) and spleen (Plt2 16±6 cf WT 8 ± 3; n=7, p=0.01). Mature megakaryocytes were increased in frequency in bone marrow (BM) sections (Plt2 86.8 ± 28.4 cf WT 56.8 ± 18.5) and demonstrated a similar maturation profile to control animals when their nuclear ploidy is measured by flow cytometry. Reciprocal BM transplantation experiments demonstrate that the Plt2 mutation is acting extrinsically on the haemopoietic system and despite having an increased platelet count, Plt2 mutant mice have inappropriately elevated serum thrombopoietin levels (Plt2 5420 ± 2842; n=15) compared to controls (2947 ± 2310; n=13; $p<0.0001$). Mapping experiments have excluded both thrombopoietin and its receptor, c-mpl, as the mutated gene. Further experiments will aim to map this mutation, which may affect a novel regulator of thrombopoietin, and assess its role in ET.

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Molecular Characterisation of Centerin, a Novel Germinal Centre Cell Serpin

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Centerin is a novel serine protease inhibitor (serpin) whose expression is restricted to germinal centre B cells and lymphoid malignancies with germinal centre B cell maturation. Serpins play critical roles in regulating proteolytic enzymes involved in a variety of vital processes including blood coagulation, fibrinolysis, complement activation, inflammation, cell migration and apoptosis. Centerin contains motifs typical of an active serpin with an arginine residue at its active site indicating preferential inhibition of trypsin-like serine proteases to mediate its biological functions. The centerin gene maps to the A clade serpin cluster on chromosome 14q32.1. We have isolated a centerin cDNA from the Burkitt's lymphoma cell line Raji and produced a His-tagged recombinant in E coli. The protein has been purified to homogeneity. Biochemical and biophysical studies including transverse urea gradient gels and circular dichroism show that centerin undergoes the stressed-relaxed conformational change typical of inhibitory serpins. In vitro assays confirm inhibitory activity against trypsin-like proteases. An additional unusual feature of centerin is its high pI of 9.5 with clustering of positive surface charge which suggests that it will bind negatively charged entities such as glycosaminoglycans or nucleic acid. A polyclonal antibody to centerin is being produced for use in immunihistochemistry and immunoassays. The tightly restricted expression of centerin suggests an important role in immune function and B cell physiology. Expression of centerin in malignant B cell lymphomas may also be useful as a diagnostic or prognostic marker in lymphoid malignancies.

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Methylation Profiling of Peripheral Blood Mononuclear Cells Reveals Extensive Somatic Methylation of Cancer Associated Genes in Normal Tissue

Michael Raynor [1], Renee Malowney [2], Sally-Anne Stephenson [1], Alexander Dobrovic [2]

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Aim: Altered DNA methylation patterns have much promise as a marker for cancer. However, this relies on being able to readily distinguish cancer cells from normal cells. We sought to determine whether low levels of methylation for a panel of genes that are known to become methylated in cancer were present in normal peripheral blood mononuclear cells.

Method: We used sensitive methylation specific PCR (MSP) assays to examine peripheral blood mononuclear cells from normal individuals for promoter region methylation of the ABO, APC, BRCA1, CCND2, CDH1, RARB2 and TWIST1 genes.

Result: All genes were found to be MSP positive in at least some individuals. The frequency of individuals with detectable methylation varied markedly, ranging from 58% for the promoter region of CDH1 to 3% for the promoter region of BRCA1. Most (75%) individuals showed a positive MSP for at least one of the promoter regions and 28% had 4 or more promoter regions methylated. Although the number of methylated promoters detected per individual tended to increase with age, some younger individuals showed methylation at most of the loci examined and some quite elderly individuals showed no detectable methylation.

Conclusion: Most cancer related methylation can occur in normal somatic tissue. Propensity for methylation may vary between individuals. It is important to determine the levels of background methylation when using methylation as a cancer marker particularly for minimal residual disease.

1400 to 1530 Bellarine Room 1
HSANZ Free Communications 2 - Leukaemia Diagnosis
Chair: Ruth Spearing

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Minimally Differentiated Acute Myeloid Leukaemia (FAB AML-M0) Is Not Associated with an Adverse Outcome in Children: A Report from the Children's Cancer Group Studies CCG-2891 and CCG-2961

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Aim: To assess the impact of FAB AML-M0 morphology in children, we reviewed two sequential Children's Cancer Group paediatric AML trials.

Method: CCG-2891 accrued 1173 patients, including 189 with Down syndrome (DS). CCG-2961, which excluded DS children, accrued 888 patients. Bone marrow (BM) morphology and cytogenetics were centrally reviewed. Presenting characteristics and outcomes for AML-M0 patients were compared to those with AML-M1-M7. The Chi-squared, Fisher's exact, and Mann-Whitney tests were used to evaluate significance of differences in proportions. Survival estimates were calculated using the Kaplan-Meier method.

Result: Among the non-DS patients, 80/1756 (4.6%) had FAB AML-M0. In addition, 10/189 (5.3%) DS children on CCG-2891 had DS-associated AML-M0. The pre-treatment characteristics differing significantly between the non-DS M0 and non-M0 patients were lower WCC (p=0.003), lower incidence of chloroma (p=0.022), and higher BM blast percentage (p=0.001) in the M0 patients. Cytogenetic analyses indicated a higher incidence of non-constitutional trisomy 21 and hyperdiploidy, and a lower incidence of t(8;21) in the non-DS M0 subgroup. Combined CCG-2891 and -2961 outcome analyses demonstrated no significant difference in remission induction rate or OS between the non-DS M0 and non-M0 patients. DFS was, however, inferior in the M0 group (29%-vs-55%; p=0.021). Within the DS-associated patients, only the diagnostic BM blast percentage (p=0.017) differed significantly between the M0 and non-M0 patients. There was no significant difference in any outcome measure between the DS AML-M0 and non-M0 patients.

Conclusion: Children with AML-M0 treated with intensive chemotherapy do not have a significantly different outcome to that of non-AML-M0 children.

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Characterisation of MYC-Containing Double Minutes by FISH Mapping Shows 'DNA hot-spots' for Amplification

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Aim: Double minutes (dmin) represent a form of gene amplification in malignancy, detectable on routine cytogenetics . FISH may identify the origin of dmin and could also provide further delineation of the mechanisms behind dmin formation.

Method: Two patients with primary acute myeloid leukaemia (AML) and one with secondary AML following a lymphoma had complex cytogenetics with dmin on bone marrow analysis. The dmin were shown to consist of ***CMYC*** (Vysis) on simple FISH. Further FISH mapping utilised a contig of 63 BAC and 1 PAC clone (containing the ***MYC*** gene) covering a region of approximately 10.5Mb. The proximal and distal breakpoints were mapped in the three patients.

Result: ***MYC*** amplification of key clones was present in the dmin. Two of the cases were also deleted on one chromosome 8 [del(8)(q24q24)] for the amplified region, while the other case was not deleted. When compared with six other cases investigated by the same protocol (Storlazzi et al., Hum Mol Gen, May 26, 2004), the breakpoints (both proximal or distal regions) fell within the "hot-spots" for amplification. In one patient the distal breakpoint was more telomeric to previously reported "hot spot" clones, indicating a large deletion.

Conclusion: Dmin arising from ***MYC*** amplification are not necessarily the same in all patients and may be accompanied by other rearrangements involving chromosome 8(q24).

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The Canonical Wnt/beta-catenin Pathway is Active in Pre-B Acute Lymphoblastic Leukaemia (ALL) Cells and Augments their Growth and Survival

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Wnt signalling is known to play an important regulatory role in haematopoietic progenitors/stem cells during both foetal and adult development. Recently this pathway has been implicated in several haematological malignancies, however, its precise role in pathogenesis of pre-B ALL remains unknown. In this study we examined the expression of genes required for Wnt signalling in 12 ALL cases, normal bone marrow mononuclear cells, bone marrow stroma and the effect of Wnt-3a stimulation on leukaemic cell growth and survival. RT-PCR analysis revealed expression of Wnt family members 2b, 5a, 10b and 16b in most ALL cases. BM stroma expressed Wnt-5a alone while BM mononuclear cells expressed Wnts 3a, 5a, 10b and 16b. Wnt receptors Fz-7 and 8 were widely expressed on ALL cases while Fz-3 and 4 showed restricted expression. Together this suggests that Wnt signalling pathway may be active in pre-B ALL cells. To characterise functional consequences of Wnt pathway activation, we investigated the effects of Wnt-3a on proliferation, survival and cell cycle status of blasts from three pre-B ALL cell lines and one newly derived stromal dependent cell line (STYG) under 3-day serum-free culture conditions. Exposure to Wnt-3a resulted in a mean 5.3-, 5.2-, 1.7-, and 2.7- fold stimulation of 3H-thymidine incorporation in Nalm6, Reh, LK63 and STYG cells, respectively. Cell cycle analysis based on propidium iodide staining confirmed an increased proportion of cells progressing from G0/1 through to S/G2/M phases. Wnt3a augmented survival in all leukemic cell lines by 18.6% (range 11.7% - 24.6%, $p<0.001$) in comparison to untreated cells under serum-free conditions as measured by Annexin V/PI staining. Immunofluorescence microscopy revealed elevated nuclear beta-catenin levels in Wnt-3a stimulated cells confirming activation through the canonical pathway. Our results suggest that Wnt proteins may exert a proliferative/survival effect on leukaemic cells through an autocrine/paracrine regulatory loop and antagonising it may prove to be a valuable therapeutic strategy.

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SDF-1 Antagonists Inhibit Homing of Acute Lymphoblastic Leukemia Cells into the Bone Marrow of NOD/SCID Mice

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SDF-1 is a key regulator of normal and leukemic B cell progenitors facilitating their proliferation, survival and migration in vitro and in vivo. Several pharmacological agents can modulate the responsiveness of normal and malignant pre B cells to SDF-1. We have have previously shown that the polyphemusin analogue TC14012 and the bicyclam AMD3100 are effective in modulating various cellular responses of acute lymphoblastic leukemia cells to SDF-1 in vitro, including chemotaxis, migration and proliferation. In this study we examined the ability of these SDF-1 antagonists to inhibit the homing of the pre B ALL cell line NALM6 and a primary ALL sample to the bone marrow of NOD/SCID mice. CFSE labelled cells were administered intravenously to mice treated with AMD3100 or TC14012. Mouse femurs were harvested 4 hours after tail vein injection, and CFSE labelled cells in the marrow quantified and corrected for the total number of cells present. We found both AMD3100 and TC14012 to significantly inhibit the homing of NALM6 to the bone marrow by 65% and 67% respectively and of the primary ALL sample by 42% and 57% respectively. These results demonstrate effective inhibition of SDF-1 mediated trafficking of pre B ALL cells invivo by AMD3100 and TC14012 and suggests that these agents may be a useful for containing and minimising the spread of pre B ALL cells throughout the body.

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Quantification of Mitochondrial Mutations in Remission for Acute Leukaemia

Scott Grist, Katrina Patsouris, Alec Morley

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Comparisons of DNA sequence of the mitochondrial D-loop at diagnosis and remission has shown that diagnostic mutations are present in 32% of patients with acute myeloid leukaemia (AML), and 58% of patients with acute lymphoblastic leukaemia (ALL). These mutations represent potential molecular markers for the diagnostic leukaemic clone which can be used to monitor that clone throughout the course of disease. We have shown by simple electrophoretic means that the diagnostic marker is present in some remission samples of both AML and ALL, however the lack of sensitivity of this method limits it's utility. We are currently investigating a number of new methods to sensitively detect and quantify these molecular markers, including single nucleotide primer extension (SNuPE), primer mismatch, oligonucleotide ligation assay (OLA) and MALDI-TOF mass spectrometry. In mixing experiments we have been able to detect the diagnostic mutation at levels of 1x10-3 by SNuPE and MALDI-TOF, and levels of 1x10-5 by two-primer mismatch. In biological samples, we have used SNuPE to successfully quantify residual disease in the remission marrow of a number of AML patients; this method has shown good correlation with existing methods but promises superior sensitivity. We believe each of these methods, individually have the capacity for improvement towards detection of even lower levels of disease, but are already potentially useful, at least for AML, which tends to show higher levels of disease at remission. For ALL, we predict a future approach combining two or more of these methods to achieve very sensitive detection will be able to monitor disease in remission.

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WT1 Transcripts as a Measure of Residual Disease in AML

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Aim: The aim of this project was to develop and validate robust 5' nuclease assay for the quantification of WT1 transcript numbers for use as a measure of MRD in AML.

Method: PCR generated fragments of WT1 and ABL were cloned in to TOPO vector (Invitrogen). Following screening linearised plasmid was prepared and the concentration determined spectrophotometrically. Dilutions were prepared and linearity of the assay was determined. PCR conditions were optimised and the reaction for both WT1 and ABL amplification and detection were multiplexed. Results were expressed as WT1 copies per 104 copies ABL WT1 transcription levels were determined on Bone Marrow samples from 29 newly diagnosed AML patients. Similarly bone marrow levels where determined on 5 normal bone marrow samples. Using this data cut off levels for sensitivity were determined. Longitudinal studies monitoring treatment were performed on a number of patients and compared with existing data from longitudinal studies using fusion gene transcript assays.

Result: Cloned standards for WT1 and ABL (control gene) where developed as a basis for a standard curve assay. Assays for both WT1 and ABL were found to be linear over 6 log decades with R2 values reproducibly greater than 0.99 and efficiencies greater than 0.9. Median values for WT1 expression from diagnostic leukaemia samples were found to be 2290 copies of WT1/104 copies of ABL (11-38471) whilst median samples from normal samples were found to be 32 copies of WT1/104 copies of ABL (18-51). A significant difference was found between these two groups ($P < 0.05$). Early longitudinal studies indicate a high level of concordance between fusion gene levels and WT1 levels

Conclusion: These results indicate that WT1 may be a suitable candidate for use in monitoring MRD and predicting relapse.

1400 to 1530 Bellarine Room 2
HSANZ Free Communications 3 - Lymphoma I
Chair: Robin Filshie

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Treatment of Primary Central Nervous System Lymphoma (PCNSL) with High-dose Methotrexate (HD-MTX) Monotherapy: A Six Year Experience.

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We aimed to investigate whether HD-MTX is an effective and non-toxic treatment for PCNSL. From Jan 1998 to Jan 2004, 21 immunocompetent RMH PCNSL patients were treated with intravenous HD-MTX without whole brain radiotherapy (WBRT) or intrathecal chemotherapy. The median age was 65 years (range 28-78 years) and ECOG performance status was ±2 in 48% of patients. HD-MTX consisted of 8gm/m2 MTX fortnightly for 3 cycles followed by 3.5gm/m2 monthly for 3 cycles. Overall response rate was 58%, (complete response 29%, partial response 29%). Median OS was 26 months and median PFS was 7 months. Median OS and PFS for patients with a complete response were 55.7 months. Seven patients had primary refractory disease while 4 patients who achieved an initial partial response progressed during HD-MTX. Three patients received second line chemotherapy and achieved OS of 6.6+, 26.7 and 63.9+ respectively. Eight patients received salvage WBRT, only one survived longer than 12 months. Patient age >60 years, ECOG performance status ±2, elevated LDH, involvement of deep structures and multi-focal lesions at diagnosis appeared to be associated with decreased response rates, PFS and OS using log-rank tests. Toxicity included: Grade III/IV Haematological toxicity (2%), Grade III Transaminitis (16%) and Grade II renal toxicity (9%). HD-MTX without WBRT is well tolerated in this representative population. HD-MTX can achieve durable long term responses in a subset of patients but the complete response rate in this patient cohort was disappointing. The emergence of resistance during therapy and early relapse suggests new treatment strategies are required.

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Results of Allogeneic Stem Cell Transplantation in Hodgkin's Lymphoma: the Queensland Experience

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Aim: To review the outcome of allogeneic stem cell transplantation (SCT) performed for Hodgkin's lymphoma (HL) at our institution.

Method: Cases were identified from the institutional database and outcomes retrospectively reviewed. Overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method.

Result: A total of 11 allogeneic transplants for HL were performed between 1994 and 2004. Patients were generally heavily pre-treated, with 82% receiving ≥2 prior chemotherapy regimens, 91% previous radiotherapy, and 73% a prior autologous SCT. Disease status at SCT included 3 cases with primary refractory HL and 8 cases with relapsed disease (4 with chemosensitive relapse, 1 with chemoresistant relapse and 3 untested). Donor source was fully matched sibling donors in 6 cases, partially mismatched sibling donors in 3 and fully matched unrelated donors in 2. Myeloablative conditioning was used in 6 cases (Cy-TBI 3 and Bu-Cy 3), and non-myeloablative preparative regimens in 5 (Flu-TBI 3, Flu-Cy 1 and Flu-Mel 1). At a median follow-up of 5mths (range <1-41mths), only 4 patients remain alive, including 2 patients transplanted within 5mths of the current review. D100 transplant related mortality (TRM) was 36%, and was significantly associated with use of myeloablative vs. non-myeloablative conditioning regimens (p=0.02). Disease progression occurred in 5 of 7 patients (71%) surviving >3mths post transplantation, with 3 patients subsequently dying from progressive lymphoma. 12mth OS, PFS and EFF was 38%, 27% and 17% respectively. Of note, advanced stage (grade2-3) acute GVHD had previously occurred in all patients who subsequently suffered relapsed HL.

Conclusion: Our results suggest that allogeneic SCT has only a limited role in the treatment of relapsed and / or refractory HL, with high TRM and high rates of HL relapse seen despite the occurrence of advanced stage GVHD.

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Fludarabine, Cyclophosphamide & Rituximab (FC-R) Achieves a High Rate of Molecular Remissions and Prolongs Remission Duration Compared with FC Alone in Patients with Indolent Lymphoid Malignancies

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Aim: We examined the efficacy of FC-R, including rates of 'molecular complete remission' (mCR), in the treatment of patients with indolent lymphoid malignancies.

Method: 59 patients (median age 61 [range 30-89], previously untreated 31%, #prior therapies 2 [0-9], median time from diagnosis 38 months [0-196]) received a median of 4 (range 1-6) cycles of intravenous FC-R (F 25mg/m2 x 3, C 250mg/m2 x 3, R 375mg/m2 x 1). Histology: CLL 41%, follicular lymphoma (FCL) 34%, mantle cell lymphoma (MCL) 10%, other 15%. Outcomes were compared to a historical cohort treated with FC (n=64).

Result: Toxicity was similar to FC except for infusional reactions related to R (26% first cycles). Overall response (OR) and complete response (CR) rates were 84% and 46% respectively, with lower CR in pretreated patients (33% vs 72% for previously untreated, p=0.01). All patients with previously untreated CLL (n=8), FCL (n=7) or MCL (n=1) responded, with CR in 6/8, 6/7 and 1/1 patients respectively. Most evaluable patients who achieved CR had no evidence of minimal residual disease as assessed by either flow cytometry (CLL 6/7) or disease-appropriate molecular studies (CLL 5/6; FCL 6/6; MCL 1/1). For pretreated patients, OR / CR rates were: CLL, 94% / 13%; FCL, 83% / 67%; MCL, 60% / 40%. At median follow-up of 13.2 months (range 0.5-40), remission duration was significantly longer compared with patients treated with FC (2yr remission 56±18% vs 41±7%, p=0.03).

Conclusion: FCR is more effective than FC, without additive toxicity, and achieves a high rate of mCR.

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A Risk-Adapted Outpatient Approach to Relapsed or Refractory Lymphoma

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[4] Fremantle Hospital, Fremantle
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Aim: Interim analysis of the safety and efficacy of a risk-adapted outpatient-based approach to lymphoma salvage therapy.

Method: Patients were stratified - Group 1 (G1) (good risk - first relapse following durable CR1); Group 2 (G2) (poor risk primary refractory, >1 relapse, or non-durable CR1); or Group 3 (G3) (relapse post-ASCT). Two regimens were evaluated: VGF (vinorelbine 25mg/sqm days 1 and 8, gemcitabine 1000mg/sqm days 1 and 8, pegfilgrastim 6mg SC day 9) (G1/3) and F-GIV (VGF plus ifosfamide 3000mg/sqm day 1) (G2). Following 2 cycles all patients were re-staged. Responsive patients (>50% reduction in disease and functional imaging negative) received 2 further cycles of the same therapy, the remainder 'escalated' therapy to F-GIV (G1/3) or IVAC (G2) (inpatient ifosfamide, VP-16 and Ara-C).

Result: 45 of a planned 90 patients, median age 56 years, are evaluable (G1 = 16, G2 = 23, G3 = 6). Diagnoses were Hodgkin's lymphoma n = 9 and NHL, n = 36 (DLC = 24, follicular = 6, others = 6). To date G1 and G2 have received 127 cycles of VGF or F-GIV, with grades 3/4 neutropenia or thrombocytopenia in 31% and 17% (VGF) and 74% and 78% (F-GIV) of patients, respectively. Febrile neutropenia, admission, treatment delay or dose-reductions occurred with 13%, 28%, 12%, 6% of cycles, respectively. Based on ITT the ORR is 59% (CR 33%).

Conclusion: VGF and F-GIV can be safely administered on an outpatient basis and show significant activity against advanced lymphoma.

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Preliminary Results of the NSW Study of Risk Factors for Non-Hodgkin Lymphoma (NHL)

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Aim: NHL incidence has increased over several decades, but its causes remain largely unknown. We studied infectious, immunologic and occupational factors and exposure to solar ultraviolet radiation (UVR) as potential causes of NHL.

Method: In a population based case-control study in adults aged 20-74 years in NSW and the ACT, cases were identified through the Cancer Registry (n=704), and controls (n=694) randomly selected from State electoral rolls. Risk factors were assessed by questionnaire and interview.

Result: Self-reported history of atopic diseases, including hay fever, asthma, eczema and food allergies were associated with reduced NHL risk; this reduction was significant for hay fever (OR (odds ratio) 0.65, 95% CI 0.52-0.82) and food allergies (OR 0.29, 95% CI 0.20-0.42). Compared to a fourth or later born child, the odds ratios (OR) were 0.52 (95% CI 0.32-0.84) for an only child, 0.55 for a first-born child, and 0.70 and 0.81 for second and third-born children. No infectious disease was significantly associated with NHL. Risk of NHL fell with increasing UVR exposure. Relative to 1.0 for the lowest quarter of total sun exposure hours, the ORs for successively higher quarters were 0.72 (95% CI 0.53-0.98), 0.66 (0.48-0.91) and 0.65 (0.46-0.91). The risk of NHL was increased by about 30% with exposure to all solvents combined, and to aliphatic and aromatic solvents other than benzene.

Conclusion: We have identified several factors significantly associated with NHL. Their validity as risk factors will be evaluated in pooled analyses with results obtained by collaborators in other countries.

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Patterns of Failure in Australasian Leukaemia and Lymphoma Group (ALLG) Intermediate Grade Non-Hodgkin's Lymphoma (intNHL) Studies

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Consolidative radiotherapy (RT) in aggressive NHL assumes relapse in original sites likeliest . We examined patterns of failure in patients (pts) following systemic chemotherapy (CT), to determine factors predicting for site of recurrence.

Method: Of 519 pts in 2 ALLG intNHLstudies, 234 (45%) achieved complete remission (CR) with anthracycline CT. 169 pts with retrospectively collected RT data had median age 55 years (range 19-76); ECOG PS =<2 in 94%; bulky disease (>=10 cm) in 42 (25%); elevated LDH in 47%; 14 (8%) stage I, 49 (29%) stage II, 37 (29%) stage III and 69 (41%) stage IV. Regimens were CHOP (45), MACOP-B (48), standard CEOP (38) and high dose CEOP (38). Ten pts received consolidative RT.

Result: At median follow-up 7.5 years (range 2.3-11.6), 69 pts (41%) had relapsed and 9 (5.3%) died. Relapse sites were: 24 pts local (14.2%), 21 remote (12.4%), 24 both (14.2%). Log-rank tests revealed stage to be associated with time-to-first-failure (p=0.039) but not with age, arm, histology (B or T cell), initial response (CR vs. CRu), bulk, RT or IPI. At 4 years the estimated percentages of failure-free patients were 71%, 78%, 45% and 67% in Stages I to IV respectively. Early stage predicted local vs distant relapse (at minimum follow-up) (p=0.027) (Fisher's exact test). Percentages of failures at original sites only were 0, 2, 13.5 and 14.5% in stages I to IV respectively.

Conclusion: In this study, 70% of relapses included original sites 35% solely so. Advanced stage was the strongest predictor of early and local relapse. No other criteria were associated with probability of relapse at original sites. RT potentially could prevent 1/3 of relapses and contribute to prevention of another 1/3, as systemic therapy becomes more effective.

1400 to 1530 Bellarine Room 3
HSANZ Free Communications 4 - Myeloma I
Chair: Noemi Horvath
session sponsored by Janssen-Cilag

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Multiple Myeloma (MM) Cells Undergo TRAIL-induced Apoptosis Through TRAIL-R1 and are Protected by the Bone Marrow Microenvironment

Karly C Koutrouvelis [1,2], Andrew Spencer [1,2]

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[2] Department of Medicine, Monash University, Central and Eastern Clinical School, Alfred Hospital

Aim: To elucidate and compare the mechanisms of TRAIL-induced apoptosis through the individual TRAIL-effector receptors, TRAIL-R1 and TRAIL-R2 in human multiple myeloma cell lines (HMCL).

Methods and Result: Four authentic HMCL, RPMI-8226, LP-I, U266 and NCI-H929 which express both TRAIL-R1 and TRAIL-R2 were studied. TRAIL-sensitive (RPMI-8226) and resistant (U266) HMCL were treated with LZ-TRAIL, and agonist antibodies to TRAIL-R1 (TRM-1) and TRAIL-R2 (TRM-2 and KTRM-2) and apoptosis levels were compared. MTS analysis of the treated HMCL indicated that TRM-1 was as effective as LZ-TRAIL whilst TRM-2 and KMTR-2 were not. This was further supported by immunoblot analysis demonstrating that LZ-TRAIL and TRM-1 cleaved PARP to similar levels where agonist antibodies to TRAIL-R2 did not. TRAIL-R1 and TRAIL-R2 levels on all four HMCL were upregulated as a result of 24hr incubation with a sub-lethal dose (5uM) of VP-16. Despite an increase in expression of both receptors, only NCI-H929 exhibited a significant (p-value: 0.01) increase in sensitivity to TRM-2. Treatment of primary myeloma cells with LZ-TRAIL and agonist antibodies for 24hrs exhibited modest efficiency. RPMI-8226 co-cultured with normal bone marrow MNC but not HS-5 condition media exhibited reduced levels of sensitivity to both LZ-TRAIL (50%) and TRM-1 (30%) when compared to treatment of RPMI-8226 alone. This suggests the existence of cell-adhesion mediated resistance to death receptor ligand mediated apoptosis.

Conclusion: TRAIL-induced apoptosis in HMCL is driven primarily through TRAIL-R1. In vivo resistance to TRAIL-induced apoptosis may be modulated by cell adhesion mediated mechanisms.

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Cytotoxicity Testing in vitro Using Primary Myeloma Cells: An Approach to Therapy Selection in Relapsed/Refractory Multiple Myeloma

Christopher Ward [1,2], Qiang Chen [1], Marie-Christine Morel-Kopp [1]

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Multiple myeloma is characterised by bone marrow infiltration with clonal plasma cells. Myeloma responds to intensive chemotherapy and stem cell transplantation but relapse is almost universal. Response rates to emerging novel therapies for resistant myeloma are difficult to predict in an individual. We optimized techniques for the isolation and culture of myeloma cells from bone marrow aspirates, and in vitro cytotoxicity testing. Myeloma cells were purified using anti-CD138 coated magnetic beads and cultured in the presence or absence of purified IL-6. In vitro therapies tested included steroids (dexamethasone and methylprednisolone (MP)), standard cytotoxic agents (doxorubicin, cyclophosphamide) and novel agents (arsenic trioxide (ATO) plus ascorbic acid (AA)). Myeloma cell apoptosis was quantitated by flow cytometric uptake of 7-AAD and Annexin V-FITC binding. Primary myeloma cells from 10 patients have been tested in vitro with variable patterns of response. Doxorubicin (0.1 μ g/ml) was the most efficient drug with >95% cell death after 24 hours for all patient samples. Intermediate response rates were observed with MP (2 mg/ml)(4 samples tested: 20 to 74% cell death) or ATO (20 μ g/ml). Enhanced myeloma cell apoptosis was noted when AA (0.5 mM) was added to ATO (6 samples tested: 29 to 72% cell death). No significant in vitro cytotoxicity was seen with dexamethasone (0.2 or 0.4 μ g/ml) or cyclophosphamide (1 mg/ml). Modifications of the system, including myeloma cell co-culture with heterologous stromal cells and cell proliferation assays using fluorescent CFSE will be described. We hypothesise that this assay system can be used to test for the development of drug resistance in a single patient over time, and may aid in the selection of an optimal second-line therapy.

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Immunohistological Detection Of Plasma Cell Microaggregates With CD138 Is A Predictor Of Earlier Relapse Post High-Dose Therapy For Plasma Cell Myeloma

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[2] Peter MacCallum Cancer Institute

Aim: Standard criteria for defining complete response (CR) after treatment for plasma cell myeloma include the absence of serum and urine paraprotein by immunofixation and fewer than 5% plasma cells in the bone marrow aspirate and trephine. Despite this, most patients eventually have disease progression, implicating the presence of residual disease. We hypothesised that immunohistological detection of plasma cells using CD138 would improve the ability to detect minimal disease in the bone marrow after high-dose therapy and that this would predict earlier relapse.

Method: 45 patients with plasma cell myeloma who received high-dose therapy and had fewer than 5% plasma cells on aspirate analysis by 3 months after chemotherapy were included in the study. CD138 (Clone MI15; DAKO) staining of paraffin embedded decalcified bone marrow allowed identification of plasma cell microaggregates, defined as non-perivascular plasma cell collections comprising at least 10 contiguous plasma cells. The volume of disease was determined by comparison with a pictorial reference standard (Tuzuner and Bennett, 1994). Progression-free survival (PFS) was determined by linear regression analysis using the Graph Pad Prism vOS9 statistical software program. Progression was defined as a 25% increase in paraprotein with an absolute increase of at least 5g/L or an increase in bone marrow plasma cells to more than 10%.

Result: In the first 3 months after high-dose therapy 27% of patients with less than 5% plasma cells on aspirate analysis had evidence of plasma cell microaggregates by immunohistology. The median PFS in patients with microaggregates was 18 months compared with 44 months in those without microaggregates (p=0.0251). 38% patients with less than 5% plasma cells achieved a CR. Median PFS of those in CR was 44 months compared to 27 months in those not achieving CR (p=0.2545).

Conclusion: In patients with less than 5% plasma cells in the bone marrow aspirate after high-dose therapy for plasma cell myeloma, immunohistological detection of plasma cell microaggregates by 3 months is predictive of earlier disease progression.

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Clonally Expanded T Cells with a Late Memory/Effector Phenotype in Patients with Myeloma are Associated with an Improved Survival and Express High Levels of Interferon Regulatory Molecules in Microarrays.

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Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, NSW

This study aimed to characterize the expanded CD8+CD57+ T cell clones in patients with myeloma which we have previously demonstrated to be associated with a significantly prolonged overall survival. Recently, it has been shown that the expression of the costimulatory receptors CD28 and CD27 on CD8+ T cells can be used to differentiate three distinct memory/effector phenotypes: CD28+CD27+, CD28-CD27+ and CD28-CD27- which correspond to 'early', 'intermediate', and 'late' subsets respectively on a putative linear differentiation pathway (Nat Med 8:379). We performed 5-color flow analysis on 39 expanded T cell receptor Vb (TCRVb) clones in 32 patients and found that the majority (78%) of patients have clonally expanded CD8+CD57+TCRVb+ T cells of the late subset; while the remaining 22% were of the intermediate subset. The late T cell phenotype was associated with a significantly improved prognosis ($p<0.001$). Longitudinally, the loss of late T cell clones was associated with disease progression. Although late T cell clones are reportedly associated with CMV but not hepatitis C, HIV or EBV infection, there was no correlation between the presence of T cell clones and positive CMV serology in our patients (Chi sq=2.7; p=NS; n=32). Furthermore, only one out of 12 HLA-A2+ myeloma patients with CD28-CD27- TCRVb expansions had a CMVpp65 (NLVPMVATV) HLA-A2-tetramer positive expanded clone. A 19,000 gene microarray analysis of mRNA expression in CD27+ and CD27- purified clones demonstrated high expression of Interferon regulatory factor 5, Interferon-related developmental regulator 1, Interferon-like protein precursor and granzyme B in late T cells clones. Thus the clonally expanded memory/effector T cells in patients with myeloma have a late T cell phenotype consistent with chronic antigen presentation and T cell recognition, are rarely CMV-specific but are associated with improved survival and the expression of molecules with potential anti-tumour activity.

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Comparison of Cytotoxic T Lymphocyte Induction by Blood Dendritic Cells and Monocyte-derived Dendritic Cells and Preclinical Studies of Blood Dendritic Cell Immunotherapy for Multiple Myeloma

Cameron Turtle [1,2], Kristen Radford [1], Andrew Kassianos [1], Kerry Taylor [2], Derek Hart [1,2],

[1] Mater Medical Research Institute
[2] Mater Adult Hospital

Aim: Blood dendritic cells (BDC) may be the optimal cellular adjuvant for multiple myeloma (MM) immunotherapy. We compared CD11c+ BDC and CMRF-56+ BDC preparations with monocyte-derived dendritic cells (MoDC) as a prelude to BDC immunotherapy clinical trials in MM patients.

Method: Expression of cytokines, TLR3, activation and costimulatory molecules was assessed on CD11c+ BDC and MoDC (n=4) with and without microbial, viral and/or cellular activation. Activated and non-activated CD11c+ and CMRF-56+ BDC- and MoDC-induced recall and tumour-associated antigen-specific CTL responses were assessed by IFN-gamma ELISPOT, tetramer and chromium release assays. The ability of the clinically applicable immunomagnetically selected CMRF-56+ BDC preparation to induce MHC class I- and II-restricted immune responses was examined in MM patients.

Result: Changes in BDC phenotype/subsets were noted with different activators. Activation marker and costimulatory molecule expression was higher on CD11c+ BDC than on MoDC. Poly I:C appeared to be the optimal activator for BDC and the cytokine cocktail was optimal for MoDC. Antigen-specific CTL induction was more efficient after priming with activated, rather than non-activated CD11c+ BDC or MoDC. The optimal antigen presenting cell preparation was donor-dependent, CD11c+ BDC being optimal in some donors, and MoDC in others. CMRF-56+ BDC preparations and MoDC were equivalent in CTL induction and CMRF-56+ BDC preparations were capable of inducing MHC class I- and II-restricted responses in MM patients.

Conclusion: BDC are as efficient as MoDC at inducing in vitro CTL responses and clinically applicable functional preparations can be isolated from MM patients for immunotherapy.

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Clarithromycin (Blaxin½), Low-Dose Thalidomide and Dexamethasone (BLT-D) is Highly Effective in Patients with Treatment Refractory Multiple Myeloma

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Aim: Clarithromycin has regulatory effects on tumour cytokine production and angiogenesis, and demonstrates synergistic anti-myelomatous activity in combination with thalidomide and dexamethasone. We report our results of the clarithromycin, thalidomide and dexamethasone regimen (BLT-D) in patients with advanced myeloma.

Method: BLT-D (thalidomide 50-200mg/day, dexamethasone 20-40mg/week & clarithromycin 500mg b.d.) was administered to 14 patients with advanced plasma cell dyscrasias (13 multiple myeloma, 1 plasma cell leukaemia; IgG 67%/IgA 25%/LCD 8%). Patient characteristics : median age 61.5 (range 51-74), male 79%, stage III disease 71%, performance status 2 (1-3), time from diagnosis 38 months (16-122), #prior therapies 5 (2-10). 10 (71%) and 11 (79%) patients had failed previous stem cell transplantation and thalidomide respectively.

Result: 11 patients were evaluable : 6 objective response (OR 55%; CR 9%/ PR 46%), 1 minor response (MR 9%), 1 stable disease (SD 9%) and 3 progressive disease (PD 27%) were observed. Significantly, all patients with PD despite ongoing single agent thalidomide (n=5) responded to BLT-D, with one patient achieving CR. In total, 7 of 10 patients with PD experienced a response or disease stabilization. Main toxicities were peripheral neuropathy, proximal myopathy and hyperglycaemia; one patient developed a DVT. At median follow-up of 62 weeks (range 4-100), median remission duration and overall survival were 30 weeks and 52 weeks respectively.

Conclusion: BLT-D is highly active in patients with advanced myeloma. The inclusion of clarithromycin achieves higher response rate in thalidomide-refractory patients than is expected for thalidomide/dexamethasone alone, without additional toxicity.

1400 to 1530 Bellarine Room 4
HSANZ Free Communications 5 - Stem Cell Transplantation I
Chair: Jeff Szer
session sponsored by Gilead

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Evaluation of G-CSF-Induced CD34+/133+ Endothelial Progenitor Cell-Mobilisation in Patients with Refractory Ischemic Heart Disease

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[2] Victor Chang Cardiac Research Institute, St Vincent's Hospital Sydney

Myocardial ischaemia remains a major cause of morbidity and mortality in Western society. To test the hypothesis that CD133+/CD34+ endothelial progenitor cells (EPCs) can revascularise ischaemic myocardium and improve cardiac function, we have initiated a safety trial of G-CSF-mediated stem cell mobilisation, followed by a double-blinded controlled study of intracoronary-administered, G-CSF-mobilised unfractionated PBSC versus immuno-isolated CD133+/CD34+ cells, in 20 patients with chronic stable ischaemia on maximal medical therapy, not amenable to further conventional revascularisation. Here we report the effects of G-CSF alone in the first 5 patients (one 36 y.o and four >60 y.o) studied. Platelets, WBCs, and CD133+/CD34+ cells (0.15% of total WBCs) in blood, as well as the number of CFU-GEMM and CFU-GM increased and peaked after 5 days of G-CSF (10 ?g/kg/d). 100% CD133+ cells were positive for CD34, c-kit and HLA-DR, and 62% were positive for CD38. These CD133+ cells also expressed endothelial cell markers, CD31 (100%) and vWF (70%), but only 21-23% were positive for CD54, CD62E or CD144. There was an age-related trend towards lower numbers of G-CSF mobilized CD34+ cells in the patients, as compared to a group of younger (<60 y.o) normal male donors (N=66). At 12 hours after the 5th dose of G-CSF, one patient developed chest pain and a small rise in troponin I, but with no change in ECG or in CK-MB levels, and recovered without sequelae. Thus, G-CSF can be used with apparent safety to effectively mobilise CD34+/133+ EPCs in patients with refractory ischaemic heart disease.

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Allogeneic Bone Marrow Transplantation: Improved Transplant-Related Mortality. The Royal Melbourne Experience

Alwyn D'Souza [1], Rosemary Hoyt [1], David Curtis [2], Jeff Szer [1], Andrew Roberts [1]

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Transplant-related mortality (TRM) remains a major deterrent to referral for potentially curative allogeneic bone marrow (including blood stem cell) transplantation (BMT). Recent improvements in supportive care, molecular matching and the use of blood stem cells may have impacted on rates of TRM. We conducted an analysis of TRM in all patients receiving allogeneic transplantation at RMH between January 1992 and March 2003 and analysed the risk of TRM in bi- or triennial periods. Method: Three hundred and ninety five consecutive BMT patients were included in this analysis. Data were collected prospectively and all patients were followed for a minimum of 1 year with data closure as at March 31 2004. TRM was defined as any death other than that due to relapse or progression of the underlying haematological condition. Result: 196 patients were alive and 199 dead, 95 from TRM and 104 from the underlying disease. Overall risks of TRM after 100 days, 1 year, 2 years or 5 years were 14.5%, 22% 24% and 25% respectively. There was a significant reduction in TRM in recent years (p=0.001). In the most recent time period (2001-2003), TRM was 5 ± 2% after 100 days, 6 ± 3% after 180 days and 10 ± 3% after 1 year. Cox regression demonstrated year of BMT (p=0.008), age group (p=0.009) and donor type (p=0.023) to be significant factors for TRM. We conclude that TRM after allogeneic BMT is multifactorial in origin and has significantly reduced at RMH over the last decade.

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Unrelated Cord Blood Transplantation for Hematological Malignancies in adults: Results from the Westmead BMT Service

Ken Bradstock, Mark Hertzberg, Ian Kerridge, Vicki Antonenas, David Gottlieb

BMT Service Westmead Hospital, Westmead, NSW

Unrelated cord blood (UCB) is a potential source of hemopoietic stem cells for adult patients requiring an allogeneic transplant but lacking a compatible related or unrelated donor. We explored the use of UCB for adults in these patients. Criteria for acceptability for an UCB were a nucleated cell dose of > 2.0 x 107 per kg patient weight, and matching for>=4 HLA antigens. Seven patients aged 22 to 49 years (median 29) underwent UCB transplant between October 2000 and November 2004. Diagnoses consisted of T-LL CR2, B-NHL, refractory AML, secondary AML CR1, high risk ALL CR1, AML CR2 (2). Conditioning therapy consisted of TBI 12 Gy, Cyclophosphamide 120 mg/kg, ATG 90 mg/kg, with Methylprednisolone and Cyclosporin as GVHD prophylaxis. Cell doses given were 1.5-2.6 x107/kg. Two patients died (intracranial bleeding day 14, RSV pneumonia day 20) without evidence of engraftment. All others had neutrophil (ANC 0.5 median day 24; 13-34) and platelet (Platelets >20 day 42; 41-115) engraftment. Acute GVHD grades I-III occurred in 4/5 evaluable. One patient died of bacterial sepsis on day 53, another of multi-organ failure on day 42. Three patients are alive and in remission 7, 13, and 56 months post-transplant. UCB transplantation is a feasible option for adults lacking adult allogeneic donors, although associated with high treatment-related mortality.

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Allogeneic Stem Cell Transplantation For Advanced Stage Multiple Myeloma: Comparison of a Targeted Preparative Regimen Incorporating 153Sm-EDTMP (Quadramet) with Cyclophosphamide-TBI

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Aim: To determine the relative efficacy of a myeloablative preparative regimen substituting a radiolabeled bone-seeking lanthanide complex (153Sm-EDTMP; Quadramet) for TBI in allogeneic stem cell transplantation (SCT) in multiple myeloma (MM).

Method: Outcomes of 9 patients who received allogeneic SCT with Quadramet - cyclophosphamide conditioning as part of previous phase I / II trials were retrospectively reviewed and compared with outcomes of 33 MM patients who received cyclophosphamide - TBI (Cy-TBI) based conditioning during an over-lapping time period. Overall survival (OS) and progression-free survival (PFS) were calculated using the Kaplan-Meier method. Multivariate analyses were performed using standard Cox proportional hazard regression modeling.

Result: With the exception of immunoglobulin subtype, the 2 transplant groups were well matched with respect to underlying disease characteristics. Quadramet based conditioning was associated with acceptable toxicity, stable engraftment and similar rates of acute and chronic GVHD compared to conditioning with Cy-TBI. On univariate analysis, SCT with Quadramet was associated with significantly inferior complete response rate (25% vs. 74%; p=0.032) and progression free survival (median PFS 12mths vs. 66mths; p=0.004) with respect to Cy-TBI. On multivariate analysis, only IgA type MM (HR 13.9; 95% confidence interval 2.9 to 65.4; p=0.001) and stable disease status at SCT (HR 0.03; 95% confidence interval 0.005 to 0.21; p<0.001) remained predictive for PFS. Median survival was not significantly different between the 2 transplant groups (24mths for Quadramet vs. 30 months for Cy-TBI respectively; p=0.92).

Conclusion: Given the reduced response rate without a demonstrable OS benefit, our experience does not support the use of Quadramet to replace the TBI component of myeloablative preparative regimens in MM.

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Myeloablative Allogeneic Stem Cell Transplantation for Advanced Stage Multiple Myeloma: The Queensland Experience

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Division of Oncology, Royal Brisbane Hospital

Aim: To review the outcome of myeloablative allogeneic stem cell transplantation (SCT) performed for multiple myeloma (MM) at our institution.

Method: Records of all patients who received myeloablative allogeneic SCT for MM were retrospectively reviewed. Overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method.

Result: A total of 47 transplants had been performed. Results for 10 transplants were subsequently excluded from this analysis due to syngeneic donor (1case) and use of an experimental conditioning regimen incorporating a radiolabeled bone-seeking lanthanide complex (Quadramet) in place of TBI (9 cases, to be reported separately). A majority of patients had stage III disease at presentation (54%), and had received >1 prior treatment regimen prior to transplantation (57%). A significant number of patients also had MM refractory to previous therapy (46%) or with PD at SCT (32%). Conditioning regimens used included Cy-TBI (33 cases), Mel-TBI (3 cases) and Bu-Cy (1 case). Donor source included HLA-identical siblings (32 cases), fully matched non-sibling related donors (2 cases) and fully matched volunteer unrelated donors (3 cases). D100 TRM was 32%. A majority of patients surviving >D100 achieved a CR (72%), with only 1 patient failing to obtain a disease response post-SCT. Grade 2-4 acute GVHD occurred in 18 patients (49%), and extensive stage chronic GVHD in 7 (28%) of patients surviving >D100. Median OS, PFS and EFS was 28mths, 66mths and 13mths respectively, with 5yr OS, PFS and EFS of 40%, 53% and 26%.

Conclusion: Our results suggest that allogeneic SCT, even when performed in advanced stage, heavily pre-treated MM, may still result in long term OS and PFS in a significant number of patients. Further studies of allogeneic SCT in MM are warranted.

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A Randomised Renal Safety and Pharmacokinetic Analysis of Combination Zoledronic Acid and Thalidomide Therapy in Multiple Myeloma

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[5] Novartis Australia Pty Ltd., Sydney

Aim: To evaluate the renal safety of zoledronic acid (ZA) with or without concomitant thalidomide (T) in patients with myeloma.

Method: As part of an ongoing multicentre Phase III trial of post-ASCT maintenance patients receive ZA (4mg IV over 15 minutes every 28 days) with or without T (50 - 200mg daily). A subset of patients (n= 24) have completed ZA Cmax and AUC (0-48) determinations for the first 2 ZA doses. Differences in creatinine (Cr) between arms over time were determined by fitting an interaction between dose and arm, with dose and arm treated as linear predictors.

Result: To-date 136 patients have commenced maintenance therapy (T=66) and received a median of 10 ZA doses. The arms were well matched for gender, age, pre-ASCT B2microglobulin and baseline Cr. Higher Cr levels were associated with male gender ($p < .001$) and B2microglobulin >4mg/L ($p < .001$). Furthermore, the relationship between Cr and ZA dose demonstrated no significant difference between the two arms. The PK of ZA in the 2 groups was similar, with peak levels falling rapidly such that very low levels were detectable after 24 and 48 hours. Peak drug concentrations and overall drug exposure were similar between the two arms (p = ns, student t-test), and there was no suggestion of accumulation or changed PK profile after the second ZA dose.

Conclusion: There is no evidence for a PK interaction between ZA and T. Furthermore, ZA was found to be safe in this population irrespective of concomitant T administration.

00830 to 1030 18/10/04
Bellarine Room 6
Diagnostic Laboratory Science Plenary Session 1 - Laboratory Science 'The Cutting Edge'
Chair: Paul Monagle

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Flow Cytometry in Haematologic Malignancies - What's New?
Surender Juneja, Andrew Wei

Royal Melbourne Hospital

In recent years, the number of novel applications for flow cytometry (FCM) and immunophenotypic analysis in haematologic diseases has increased. The technology has particular utility in cell phenotype designation for disease classification, analysis of biological markers to increase our understanding of disease behaviour and specific enumeration of minor populations in a heterogeneous background (minimal disease detection). Some recent examples of progress in identification of disease phenotypes include, dendritic subtypes of acute leukaemia, myelodysplastic syndromes, paroxysmal nocturnal haemoglobinuria/aplastic anaemia and mastocytosis. Detection of biological markers for prognostic and therapeutic application include apoptotic proteins such as Bcl-2 and Bax, proliferative indices such as Ki-67 and S-phase quantitation, surrogate markers of germline immunoglobulin rearrangements such as ZAP-70 and CD38 in CLL and monoclonal antibody targets such as CD20, CD117, CD33 and CD52. Flow cytometry is a powerful tool for detecting minimal disease and is established for the prognostic classification of patients with acute leukaemia and multiple myeloma after intensive therapy. Technological advances like 5 or 6 colour analysis and five dimensional automated phenotyping analysis are valuable in enhancing the diagnostic capabilities of FCM. The capability to detect 4 or more colours has resulted in change of flow panels used in routine diagnostic service. Emerging technologies like immunomicroarray, gene expression profiling & immunohistology are increasingly likely to complement and/or replace immunophenotyping by FCM.

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Molecular Genotyping and Detection of Blood Group Polymorphisms
Robert Flower

PaLMS and Northern Blood Research Centre, University of Sydney, Sydney, Australia

Agglutinating antibodies have been the single most important tool in the history of immunohaematology. Specific monoclonal antibodies and polyclonal antisera have been used to detect and define red blood cell (rbc) antigens. The genetic bases of polymorphisms producing the major clinically-significant rbc antigens are known. Point mutations produce null phenotypes as well as single defined antigens. Chromosomal crossover and gene conversion sometimes produce complex phenotypic change. Application of genetic techniques provides a basis for definition and inexpensive typing of rbc polymorphisms. In addition, there are new possibilities, such as typing of unborn babies by amplification of foetal DNA from the maternal circulation. Various techniques can be used for genotyping, each of which has advantages and limitations. Nucleic acid amplification may fail to detect new mutations and, for reliability, requires high standards in process design and laboratory procedures. Results can be difficult to interpret when the same phenotype can be produced by several different genetic changes. It is to be hoped that molecular genetic terminology does not become so complex that it is too unwieldy for routine use. This may be the case if the same terminology is used for genetic variation that defines phenotypic change and for silent genetic variation.

Conclusion: Systematic investigation based on molecular genetics has the potential to improve practice, the most obvious examples being availability of procedures for typing of DAT-positive patients and multi-transfused patients. However, these techniques should be introduced with caution as they come with their own new range of pitfalls and complications.

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Paediatric Reference Ranges and Point of Care Testing - How do we get it right?

Paul Monagle

Royal Children's Hospital, Melbourne, Victoria, Australia

The concept of developmental haemostasis was developed in the late 1980's by Andrew et al. The Royal Children's Hospital has completed the largest study of normal ranges for coagulation proteins in a paediatric population using Stago analyzer and reagent systems. While confirming the concepts defined by Andrew et al, as expected the actual ranges differ with analyzer and reagent systems. The RCH normal APTT ranges for different ages are:

Age	Day 1	Day 3	<1 year	1-5 years	6-10 years	11-16 years	Adults
APTT(sec) Mean (95% popl'n)	38.7 (30-54.5)	36.3 (28-69)	38 (20-53)	37 (30-45)	37 (29-46)	37 (31-44)	33 (28-38)
Number of subjects	21	25	30	41	53	46	42

The implications of this in children are immense, in terms of misdiagnosis, wasted resources for the investigation of normal children, and appropriate monitoring of clinical conditions and anticoagulant therapy. All laboratories involved in the coagulation testing of children must consider the use of appropriately developed age related reference ranges.

The monitoring of anticoagulation therapy in children has many inherent differences to monitoring anticoagulation in adults, and raises specific challenges. Point of Care monitoring has obvious advantages in sample collection, and convenience for families and children, especially reducing lost school time. However, issues of quality control, ensuring accurate monitoring techniques, and parental and child education must be addressed. Using a nurse coordinated anticoagulation clinic model we have developed a system for home monitoring of anticoagulation in children that is safe and effective.

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The ALLG National Tissue Bank; Practical Issues

Russell Saal Paula Marlton

Qld Health Pathology Service

1400 to 1530 Bellarine Room 6
Diagnostic Laboratory Science Plenary Session 2 - Laboratory Science 'The Cutting Edge'

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Laboratory Tests for Heparin-induced thrombocytopenia (HIT)
Beng Chong

Department of Medicine, St George Clinical School and Centre for Vascular Research, University of New South Wales, Sydney

Laboratory tests for heparin-induced thrombocytopenia (HIT) can be divided into two groups, namely (1) functional tests and (2) immunoassays. The functional tests measure HIT antibody-induced platelet activation changes as end-points. The commonly used tests are platelet aggregation test, 14-C-serotonin release assay and the heparin induced platelet aggregation assay (HIPA). Other tests include platelet lumiaggregometry, the flow cytometry and platelet microparticle release assays. Immunoassays are anti-PF4-heparin and anti-PF4-polyvinylsulphonate antibodies Enzyme-linked Immunosorbance Assay (ELISA) and particle gel immunoassay (DiaMed). Functional tests are technically more difficult to carry out. They measure HIT antibodies that are more relevant clinically. Sensitivity of the tests can be maximized with the use of platelets from individuals known to react strongly with the HIT antibodies and using washed platelets instead of platelet-rich plasma. Test specificity can be increased by the adoption of the two-point system in which a test result is considered positive only if a positive reaction is obtained with a low or therapeutic concentration of heparin e.g. 0.1 to 0.5 U/ml and a negative reaction with a high heparin concentration (100U/ml). Immunoassays are more sensitive and technically easier to perform - they are recommended for hospital laboratories even though they may detect clinically irrelevant antibodies in some patients. Referral laboratories should be able to

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Thrombophilia testing
Paul Ockelford

Departments of Haematology, Auckland City Hospital and Diagnostic Medlab, Auckland, New Zealand

The term thrombophilia means a predisposition to thrombosis. Individuals diagnosed with venous thromboembolism by definition have thrombophilia. This diathesis may manifest itself spontaneously or following a provoking risk factor. Such events may be associated with a linking inherited or acquired risk marker and some are familial. The presence of a blood test marker does not independently define a person as having 'thrombophilia' as test abnormalities are identified in normal cohorts in case-control studies. They are however associated with an up-regulation of thrombotic risk. First degree relatives of an individual with thrombosis have an increased risk of a thrombotic event which is increased a further 3-10 fold if there is a linking laboratory marker. This might increase the baseline risk of 1:10,000 (for an age-matched 40 year old control) to 1% or even higher. On the other hand, for an individual who has already had a thrombosis, FVL heterozygosity does not increase the risk of a recurrence. This implies that the risk arising from the index event far outweighs any additional risk due to a blood test abnormality, perhaps with the exception of elevated FVIII levels or the lupus anticoagulant. Pregnancy related thrombosis risk also appears to be higher in individuals with an inherited test abnormality especially if the event was spontaneous. Testing is therefore justified in selected patients including those with thrombosis occurring at a young age and in unusual sites. For their relatives the presence of a linking marker may assist with risk-profile counselling or lower the threshold for considering thromboprophylaxis.

1400 to 1530 Bellarine Room 6

Diagnostic Laboratory Science Plenary Session 3 – Morphology & QAP

Chair:Elizabeth Duncan

Juneja Surender, Janine Campbell, \Katherine Marsden

TUESDAY 19/10/2004

0800 to 1015 Bellarine Room 6
HSANZ Plenary Session 7 - Myeloma: New Concepts and Treatments
Chair: H. Miles Prince

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Advances in Multiple Myeloma Treatment
Keith Stewart

Recent advances in myeloma herald an era of molecular classification and risk adapted therapy. Patients lacking IgH chromosome translocations are often hyperdiploid, over-express cyclinD2 and harbor a relatively favourable prognosis. In contrast IgH translocations affecting 50% of patients involve cyclinD1 and D3, the maf transcription factor family and the fibroblast growth factor receptor 3 (FGFR3). Both maf and FGFR3 translocations harbor a very poor prognosis once therapy is required. Deletions of chromosome13 and p53 also impart a poor prognosis. Thus molecular based prognostic models are emerging which will allow tailored therapies. As an example of the latter the dysregulation of FGFR3 by the translocation t(4;14)(p16;q32) occurs in 15% of multiple myeloma (MM) patients and confers a growth and survival advantage to malignant plasma cells. Patients with a t(4;14) have a significantly shorter event-free and overall survival following both conventional and high dose therapy when compared to other patients This reflects early relapse rather than primary chemotherapy resistance with initial response rates to corticosteroids and thalidomide being high and response rates to alkylating agents disappointing. Even tandem autologous transplant does not seem to benefit these patients.

As FGFR3 is therefore a poor prognostic indicator and a molecular target we assessed the therapeutic potential of two FGFR-specific tyrosine kinase inhibitors SU5402 and CHIR258 in MM. Subsequent data indicate that both drugs potently inhibit FGFR3 and have activity against t(4;14) human MM cells both in xenograft models and primary patient cultures. These findings support the development of clinical trials of early intervention with FGFR3 inhibitors in t(4;14) myeloma.

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New Insights into Myeloma Pathogenesis

P. Joy Ho

Institute of Haematology & Centenary Institute, Royal Prince Alfred Hospital and University of Sydney.

Multiple myeloma is characterised by a multitude of chromosomal and genetic abnormalities, which have provided important insights into disease pathogenesis. Numerical abnormalities of chromosomes are important predictors of disease behaviour, as non-hyperdiploid myeloma have a significantly worse prognosis than hyperdiploid tumours. Monosomy 13 or del 13q occur in 30-55% and also confer a poor prognosis; whether this effect is independent of hypodiploidy is still to be clarified. Candidate genes include Rb on chr. 13 and the activation of IGF1R and other cell cycle genes. Chromosomal translocations into the IgH genes occur in 55-70%. There are 5 recurrent partners - 11q13 (15%), 6p21 (3%), 4p16 (15%), 16q23 (5%) and 20q11 (2%), from which candidate genes may be dysregulated by IgH enhancers. Translocations t(4;14), t(14;16) and t(14;20) are associated with a poor prognosis, while t(11;14) confers improved survival. Several genes are potentially dysregulated by t(4;14) - FGFR3, IgH-MMSET and TACC3. FGFR3 is a tyrosine kinase receptor, overexpression of which increases IL-6 dependent cellular proliferation. However, the unfavourable prognostic impact of t(4;14) is independent of FGFR3 expression, leading us to consider possible effects of FGFR3 expression on the response to thalidomide, a known inhibitor of bFGF. The study of t(4;14) and FGFR3 may have important therapeutic implications, such as the potential efficacy of tyrosine kinase inhibition. Translocations t(14;16) and t(14;20) dysregulate transcription factors of the maf family, through which Cyclin D2 can be upregulated. The D Cyclins promote the transition from G1 to S phases of the cell cycle. Recent studies have shown that c-maf overexpression is not confined to t(14;16)+ myeloma, but can occur in up to 50% of myeloma. In contrast, t(11;14) and (6;14) dysregulate Cyclin D1 and D3 respectively. Although increased Cyclin D1 expression should promote cell cycling, t(11;14) confers an improved prognosis, especially for patients on high dose therapy. The dysregulation of Cyclin proteins has been proposed as the unifying oncogenic change in myeloma: Cyclin D1/D3 can be upregulated by t(11;14) and t(6;14) (20%), some myeloma (predominantly hyperdiploid) overexpress Cyclin D1 without IgH translocations (40%), while others upregulate Cyclin D2 or D3 by IgH translocations through c-maf or undefined mechanisms (40%). Five disease groups have been proposed, with implications on biological behaviour, prognosis and therapeutic options. Other molecular abnormalities include those affecting c-myc, ras, the Rb pathway, p53 and PTEN, as well as epigenetic anomalies. More recently, gene expression profiling has defined clinically relevant disease categories, and appears to be useful in predicting responsiveness to novel therapies such as Bortezomib.

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Australian studies examining new agents in Myeloma

H. Miles Prince, Linda Mileshkin, Dirk Honeman

Haematology Service, Peter MacCallum Cancer Centre, Melbourne, VIC

Standard therapy for newly diagnosed and relapsed myeloma is evolving. High response rates (RR) have been demonstrated for patients with newly diagnosed and relapsed MM in Phase II trials with thalidomide (T), revemid (R) and bortezomib (B) either alone or in combination with dexamethasone (D). We have demonstrated that T alone in relapsed disease can induce RR in approximately 30% of patients with factors predicting durable PFS and OS being age <60y, low B2M, low LDH and normal creatinine. Chrom 13del appears to predict for a poor response. Recent analysis of biomarkers demonstrates that in addition to these clinical prognostic factors, immunohistochemistry (IHC) of marrow CD57 and serum VEGF, HGF and IL-6 are predictors of outcome while IHC for CD34, VWF (angiogenesis) and mast cell tryptase are not predictive. Serum MUC-1 is also an adverse predictive factor. MUC-1 is tumour associated antigen on plasma cells (PC) and is a potential target for immunotherapy. Our centre has recently completed a trial examining the tracking of dendritic cells pulsed with MUC-1 in MM. A Lewis antigen and Wue-1 are also potential immunotherapy targets. Combinations of T with D or prednisolone (P) increase RR and a trial of the DT-PACE combination is underway; preliminary data indicate that RR are high and analysis of the impact on stem cell collection yield is ongoing. In vitro data indicates that PC growth may be influenced by the cyclo-oxygenase pathway and a recently completed multi-centre study of 65 relapsed patients have demonstrated an improved RR when celecoxib is administered with T (42% v 29%) but does not influence PFS or OS. A randomized placebo-controlled study (RPCT) of D v T+D for initial treatment of MM is underway. A RPCT of D +/- R for relapsed MM has recently been completed; the major toxicity associated with R is myelosuppression - efficacy results should be available soon. A randomised trial of M+P +/- bortezomib is planned to open later in 2005. Increasing reports on osteonecrosis of the jaw associated with bisphosphonate therapy are a concern and the influence of these new agents on its development need ongoing investigation. Currently there are no trials in Australia examining the role of farnesyl transferase inhibitors or histone deacetylase inhibitors. A Phase II trial examining a VEGF inhibitor (GW786034) has recently opened for accrual in Melbourne.

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Autologous Stem Cell Transplantation (ASCT) for Multiple Myeloma (MM)

Andrew Spencer

Clinical Haematology & Bone Marrow, Transplantation, Alfred Hospital, Prahran Vic

Between 1997 and 2003 1396 Australasian MM patients underwent ASCT - 580 during 2002 and 2003 and 816 during the preceding 6 years. Additionally, 5 ASCT trials were completed, 3 under the auspices of the ALLG. ALLG MM4 was a single arm multicentre trial evaluating PCAB induction followed by a BuMel conditioned ASCT. Fifty patients were accrued and final analysis is awaited. The ALLG amifostine trial was a randomised trial of 90 patients undergoing a MEL200 conditioned ASCT with or without amifostine. Amifostine pre-treatment significantly reduced the incidence of severe (grades 3/4) OM (33% vs 12%, p = .02). Subsequently, an ALLG pilot study demonstrated 0% severe OM in 10 patients receiving MEL220 with amifostine. Two other multicentre ASCT trials have recently been reported. The first evaluated oral induction (Cyclophosphamide, idarubicin, dexamethasone) with early ASCT in 36 de novo MM patients. Response rates were comparable to those seen with VAD and 86% of patients successfully proceeded to ASCT. The second explored a tandem ASCT approach with the first ASCT acting as an in vivo purging strategy prior to a second PBSC collection. A five-fold reduction in tumour contamination between the first and second collections was demonstrated. Presently, the ALLG MM6 study is exploring the role of thalidomide maintenance post-ASCT. Over 200 patients have been accrued. The triple maintenance combination of Zometa-prednisolone-thalidomide is well tolerated and interim safety analyses have demonstrated that the addition of thalidomide is associated with superior renal function preservation post-ASCT when compared with Zometa-prednisolone maintenance.

1045 to 1200 Bellarine Room 2
HSANZ Free Communications 6 - Myeloma - II
Chair: Philip Campbell

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Improving Outcomes in AL Amyloidosis - Clinical and Free Light Chain Responses to Low Dose Thalidomide

Philip Campbell [1], Roger McLennan [2]

[1] Clinical Haematology, Geelong Hospital, Barwon Health
[2] Medical Oncology, Geelong Hospital, Barwon Health

Therapeutic options for patients with Primary Systemic Amyloidosis (AL) are frequently limited by age and extent of end organ involvement. The serum free light chain assay (FLCA) is a highly sensitive nephelometric immunoassay which has recently been shown to correlate with amyloid protein load, treatment response and overall survival . Thalidomide is an active agent in myeloma but it's limited use to date in AL has been associated with substantial toxicity, incomplete clinical responses and no information on FLCA response. We describe 2 patients with AL responding to low dose thalidomide including 1 complete biochemical response (FLCA). Case 1. 76 year old male presenting in 11/2000 with hepatomegaly, thickening of right vastus medialis, abnormal liver function and nephrotic syndrome. Investigations confirmed a diagnosis of AL with an IgGk paraprotein on EPG, amyloid infiltration on muscle biopsy and no evidence of myeloma. Initial therapy consisting of melphalan and prednisolone (5 cycles) was associated with disease progression. In 6/2001 he commenced thalidomide 100mg daily with alternate day prednisolone (50mg) resulting in clinical improvement, reduced paraprotein (PR) and suppressed free kappa light chains in the absence of significant toxicity. Treatment was subsequently discontinued after 2 ' years due to disease progression. Case 2. 62 year old male presented to the Cardiology Department in 12/2002 with biventricular failure secondary to a restrictive cardiomyopathy and evidence of amyloid infiltration on endomyocardial biopsy. Further investigations demonstrated an IgGl paraprotein, elevated l free light chains and no evidence of myeloma. Low dose thalidomide was commenced (100mg daily) in combination with pulsed dexamethasone which was replaced with alternate day prednisolone (25mg) due to fluid retention. Significant clinical improvement has occurred over 18 months coinciding with a complete response (absent paraprotein, normal FLCA). A dual chamber pacemaker was implanted early in the course of his disease due to cardiac conduction defects. Low dose thalidomide and corticosteroids have the potential to be an effective regimen in AL as reflected by FLCA and clinical response. Early FLCA assessment may be of prognostic significance and useful in avoiding the toxicity of ongoing thalidomide in non-responders. Implantation of a pacemaker is recommended in patients with significant cardiac involvement.

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Flow Cytometric Immunophenotypic Analysis of Multiple Myeloma

Dinesh Bhurani [1], Peter Chapple [2], Andrew Wei [3], Surender Juneja [1]

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[2] Diagnostic Haematology, Royal Melbourne Hospital
[3] Molecular Genetics of Cancer, Walter and Eliza Hall Institute of Medical Research, Parkville Vic

Aim: Advances in technology and new monoclonal antibodies allow a sensitive method for detection of plasma cells (PCs) by flow cytometry. Analysis of bone marrow by multiparameter flow cytometry allows the immunophenotypic distinction between normal and malignant plasma cells, which display an aberrant antigenic profile in a majority of cases. There is scant literature on this subject especially with regard to the optimum gating strategies & the appropriate combination of antibodies to be used. Therefore, we have analysed the phenotype of PCs by multiparameter flow cytometry. The aim was to identify the optimum gating strategy, the frequency of expression of various antigens by plasma cells and the occurrence of aberrant phenotypes.

Method: Bone marrow samples were obtained from 134 consecutive patients with plasma cell myeloma (WHO criteria) diagnosed at the Royal Melbourne Hospital, between 2000 and 2003. Leucocytes were stained with a panel of monoclonal antibodies (Coulter) including CD45, CD38, CD138 CD56, CD19, CD86, CD117, CD20 and kappa and lambda light chain. Analysis was performed on an EPICS-XL Flow Cytometer. The plasma cells were identified by bright positivity for CD138 or CD38. CD38 bright-positive cells were then analysed for the co-expression of other antigens. Reactivity with a particular antibody was considered positive when it was detected on more than 20% PCs relative to isotype control.

Result: Of the bright CD38 positive cells, an aberrant phenotype was identified in 75% cases. 56% were CD56+/ CD19 - and aberrant plasma cell co-expression of CD86, CD117 or surface light chain restriction was detected in 56%, 38% and 37% of myeloma cases, respectively. The exclusion of normal B cells by gating on low side-scatter /CD138 positive plasma cells was found to improve the specificity of detecting aberrant B cell antigens such as CD20.

Conclusion: Multiparameter flow cytometry provides useful information in the evaluation of plasma cell myeloma. In this study, the optimal gating strategy for plasma cells was determined. An aberrant plasma cell immunophenotype was detected in 75% of patients. The utility of this technique in the setting of minimal residual disease detection post high-dose therapy is currently being studied.

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Flavopiridol Demonstrates Pre-clinical Efficacy Against Human Myeloma Cell Lines and Primary Tumours

Randy Suryadinata, Sung-Lin Yeh, Karly Koutrouvelis, Andrew Spencer

Myeloma Research Group, Alfred Hospital, Melbourne

Aim: Characterise the activity of the broad-spectrum kinase inhibitor Flavopiridol (F) against myeloma cell lines (HMCL) and primary tumours (PMM).

Method: The viability of 5 HMCL was determined by MTS assay following treatment for 24 and 72hrs with escalating concentrations (0 - 50μM) of F. Subsequently, 3 HMCL (U266, LP1 and NCI H929) were evaluated in more detail using 100nM F. Cell cycle status and apoptosis induction was measured with ApoBrdU and AnnexinV/PI flow-cytometry. Cell cycle and apoptosis-related protein expression was determined by immunoblot analysis of whole cell lysates. Viability of HMCL was determined following sequential treatment with F then cytotoxics and vice-versa. The ability to sensitise HMCL to TRAIL-receptor ligands was tested by pre-treatment with F. Finally, the effect of F on the viability of 4 PMM was measured.

Result: MTS assay showed <20% viability of all HMCL at 72hrs with >100nM F. ApoBrdU and Annexin V/PI demonstrated both an accumulation of cells in GO/G1 with decreased S and G2/M phase cells and 30-40% apoptosis induction maximal at 48-72hrs. Immunoblots revealed a variable reduction in Bcl-XL and Cyclin A expression and a marked reduction in p21 expression. Sequential treatment with F-cyctotoxics or cyctotoxics-F either demonstrated no additive effect or was antagonistic (etoposide/vincristine), however, F enhanced apoptosis induced by TRAIL-R2 specific ligands. F induced a median of 20% (range, 5% to 25%) apoptosis of 4 PMM in short-term (24-48hrs) cell culture.

Conclusion: Flavopiridol shows promising efficacy against myeloma. The potential role of p21 modulation in myeloma cell apoptosis-induction warrants further investigation.

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Genetic Polymorphism in Microsomal Epoxide Hydrolase and Risk of Multiple Myeloma

Lisa F Lincz [1], Fiona E Scorgie [1], Ian Kerridge [2], Andrew Spencer [3], Arno Enno [1,4]

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[2] Clinical Haematology Department, Westmead Hospital, Wentworthville, NSW
[3] Myeloma Research Group, Department of Clinical Haematology and Bone Marrow Transplantation and Department of Medicine, Monash University, The Alfred Hospital, VIC,
[4] Hunter Area Pathology Services

Aim: We have recently identified a gene polymorphism which reduces the activity of glutathione S-transferase theta (GST T1), an enzyme involved in benzene detoxification, as a risk factor for multiple myeloma (MM). Previous epidemiological studies attempting to link benzene exposure to increased frequency of MM have been inconclusive, however no one has investigated genetic factors which may increase susceptibility to benzene toxicity. Thus we have expanded this case-control study to include other xenobiotic genes involved in benzene metabolism.

Method: PCR-RFLP was used to detect gene polymorphisms for glutathione S-transferases theta (GST T1), microsomal epoxide hydrolase (mEH) Tyr113His, myeloperoxidase (MPO) -463 G/A, and cytochrome P-450 (CYP2E1 Rsa1/Pst1) in 102 patients with MM and 210 healthy Australian Caucasians. Genotype frequencies were compared using Fisher's exact tests (two-tailed).

Result: There was a significantly increased incidence of the reduced activity mEH 113HH genotype in MM cases compared with controls (29% vs. 17%, p= 0.0005). When combined with previous results, inheritance of both high risk genotypes of GSTT1 null + mEH 113 YH/HH was found to contribute to a 3-fold increased risk of MM (95%CI: 1.25 - 7.92, p=0.01). In contrast, there was no difference in frequencies between cases and controls for MPO -463 G/A and CYP 2E1 Rsa1/Pst1 polymorphisms.

Conclusion: This study supports the notion that environmental exposure to benzene may contribute to the development of MM, and further suggests that risk of disease may be dependent on inherited polymorphisms in genes responsible for particular metabolic pathways for this substance, namely GST T1 and mEH.

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Elevated Serum Levels of SDF-1 alpha is Associated with Increased Osteoclast Activity and Osteolytic Bone Disease in Multiple Myeloma Patients

Andrew Zannettino, Amanda Farrugia, Angela Kortesidis, L. Bik To, Stan Gronthos

Myeloma and Mesenchymal Research Group, Matthew Roberts Foundation Laboratory, Division of Haematology, Institute of Medical and Veterinary Science

Multiple myeloma (MM) is an incurable plasma cell (PC) malignancy able to mediate massive destruction of the axial and craniofacial skeleton. The aim of this study was to investigate the role of the potent chemokine, stromal derived factor-1 alpha (SDF-1alpha) in the recruitment of OC precursors to the BM. Our studies show that MM PC produce significant levels of SDF-1alpha protein and exhibit elevated plasma levels of SDF-1alpha when compared with normal, age-matched subjects. The level of SDF-1alpha positively correlated with the presence of multiple radiological bone lesions in individuals with MM, suggesting a potential role for SDF-1alpha in OC precursor recruitment and activation. To examine this further, PB-derived CD14+ OC precursors were cultured in an in vitro OC-potentiating culture system in the presence of recombinant human SDF-1alpha. Whilst failing to stimulate an increase in TRAP+, multinucleated OC formation, our studies show that SDF-1alpha mediated a dramatic increase in both the number and the size of the resorption lacunae formed. The increased OC motility and activation in response to SDF-1alpha was associated with an increase in the expression of a number of bone resorption-related genes including, RANKL, TRAP, matrix metalloprotease-9 (MMP-9), Carbonic Anhydrase II (CA-II), and Cathepsin K. The synthesis of high levels of SDF-1alpha by MM PC may serve to recruit OC precursors to local sites within the BM and enhance their motility and bone resorbing activity. Our findings therefore suggest that inhibiting the activity of MM PC-derived SDF-1alpha may provide an effective means of treatment for MM-induced osteolysis.

1045 to 1200 Bellarine Room 3
HSANZ Free Communications 7 - Stem Cell Transplantation - II
Chair: Anthony Schwarer

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Rhabdomyolysis as a Manifestation of Chronic Graft Versus Host Disease - A Case Report

Julie Crawford, Paul Cannell

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Introduction: We describe a case of acute myonecrosis associated with myoglobinuric renal failure in the setting of chronic graft versus host disease post allogeneic bone marrow transplantation.

Case History: A 47 year-old man was diagnosed with lymphoid blast crisis chronic myeloid leukemia (CML) in March 2002. He underwent allogeneic stem cell transplantation in May 2002. Moderate gastrointestinal graft versus host disease which responded to increased immune suppression complicated his early post transplant course. The patient presented with progressive myalgias & proximal muscle weakness 14 months post transplant. This was accompanied by myoglobinuric renal failure; peak creatinine kinase 2850U/L & creatinine 195umol/L & an acute flare of liver GVHD with raised alkaline phosphatase & alanine aminotransferase; measuring 570U/L & 2470U/L respectively. Muscle biopsy revealed monophasic myonecrosis without evidence of infection, consistent with rhabdomyolysis. Liver biopsy showed some panlobular necrosis; interpreted as being consistent with GVHD. The diagnosis reached was chronic GVHD (CGVHD) resulting in myonecrosis. The biochemical abnormalities symptoms responded promptly to increased immunosuppression. Weaning of immunosuppression was accompanied by a transient deterioration in liver function. Three months after the re-institution of high dose immunosuppression the patient was diagnosed with a pleural mucormycosis & he remains on long term amphoterocin therapy. His myonecrosis has not recurred.

Conclusion: Myonecrosis is a very uncommon manifestation of CGVHD. In this patient it proved responsive to intensification of immunosuppression & has not recurred despite persistent CGVHD of other organs (gastrointestinal & hepatic). The diagnosis should be considered in patients presenting with myalgia post transplantation.

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Measurement of Mortality in Long-Term Haemopoietic Stem Cell Transplant Survivors

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[2] Westmead Hospital
[3] St Vincent's Hospital Sydney
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Aim: To measure mortality rates in long-term haemopoietic stem cell transplant survivors and compare these with the general population.

Method: Records of patients who had survived more than two years post autologous or allogeneic stem cell transplant were drawn from the ABMTRR database. Annual mortality was calculated for this group and compared to the mortality of the Australian population using indirect age-standardised mortality rates.

Result: Among 1,502 allogeneic recipients disease free at 2 years, there were 36 deaths (18 from non-malignancy related causes) in the third year compared to an expected 3. Among 778 allogeneic recipients disease free at 5 years, there were 3 deaths in the sixth year deaths (2 from non-malignancy related causes) compared to an expected 1. In contrast, among 2,741 autologous recipients disease free at 2 years, there were 52 deaths (8 from non-malignancy related causes) in the third year compared to an expected 9. Among 1,188 autologous recipients disease free at 5 years, there were 14 deaths in the sixth year (6 non-malignancy) compared to an expected 3.

Conclusion: The non-malignancy mortality of both allogeneic and autologous HSCT recipients approaches population norms for those who survive more than 5 years post transplant. Indirect age-standardised mortality calculations offer a relatively straightforward mortality analysis strategy. The ABMTRR is an important national data resource providing timely information on transplant activity and outcome across Australia and New Zealand.

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Optimal Timing of Peripheral Blood Stem Cell (PBSC) Mobilisation in Patients with Hematological Malignancies Treated with the Hyper-CVAD Chemotherapy Regimen

P Mollee[1] S Gibbs [2], C Keane [1], A Mills [1], K Grimmett [1], R Van Kuilenburg [1], R Saal [1], D Gill [1], H M Prince [2], J F Seymour [2], P Marlton [1]

[1] Princess Alexandra Hospital
[2] Peter MacCallum Cancer Centre

Aim: We have previously demonstrated that Hyper-CVAD adversely affects PBSC mobilisation (ASH 2003). We now examine the optimal timing of PBSC collection with this regimen.

Method: A retrospective analysis was performed at two centres of consecutive patients treated with Hyper-CVAD in whom an attempt was made to collect PBSC, usually on recovery from cycle A or B with daily G-CSF 10mcg/kg. Patients were analysed according to timing of mobilisation.

Result: 74 patients were identified: median age 44yrs(range,15-69yrs); 69% male; diagnoses (ALL n=14, NHL n=60); marrow involvement 53%. Overall 66% and 42% of patients obtained a minimum (CD34+ >2x106/kg) and optimal (CD34+ >5x106/kg) PBSC collection, respectively. Only 3 of 17 patients (18%) collected beyond cycle 3B successfully mobilised. Two patients mobilised prior to 3B but receiving alternate mobilising chemotherapy were not analysed. Of 55 remaining patients, 13, 21, 14 and 7 mobilised following cycle 1B, 2A, 2B and 3A with apheresis occurring (median+SD) on day 12+0.6, 13.5+1.4, 13.5+2.4 and 15+2.6, respectively. Cycle 1B compared to 2A was not significantly better in obtaining a minimum graft (100% vs. 81%, p=0.14), but was superior in terms of total CD34+ yield (21.4 vs. 3.4x106/kg, p<0.001) and in achieving both a minimum (92% vs. 19%, p<0.001) and optimal (77% vs. 0%, p<0.001) PBSC collection with a single apheresis. There were no significant differences in PBSC yields following cycles 2A, 2B and 3A.

Conclusion: If marrow involvement is cleared, mobilisation following cycle 1B of Hyper-CVAD allows the most predictable timing of PBSC collection and provides a significantly better PBSC graft.

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Incidence, Natural History and Management of Cytomegalovirus (CMV) DNAemia and Disease in Patients with Haematological Malignancies in the Non-allogeneic Transplantation Setting

Ashley Ng, Leon Worth, John Seymour, Monica Slavin, Karin Thursky

Peter MacCallum Cancer Institute

There is little information regarding CMV reactivation in the non-allogeneic transplantation setting. We report the incidence of CMV-DNAemia and CMV-associated disease in a cohort of patients treated for haematological malignancies at the PMCI from 6/99-6/04. Among the 36 patients identified, potential predictive factors were analysed, including underlying malignancy, previous therapy and therapy immediately preceding CMV-DNAemia/disease. Patients received a median of 2 prior therapies (range 1-9), age ranged from 35-77 years, with 19 males. Diseases were: acute leukaemia(6), CLL(6), NHL(21), HD(1), myeloma(2). Therapies immediately preceding CMV-DNAemia/disease were: 'conventional chemotherapy' (39%, including HyperCVAD 14%), fludarabine-based (19%), autologous BMT (22%), and antibody therapy (20%, including denileukin diftitox, 6%, alemtuzumab, 14%). Risk of CMV-DNAemia/disease was calculated from the total number of patients treated over the period of analysis: alemtuzumab, (6/12; 50%), denileukin (2/33; 6%), autologous BMT (9/223; 4%), fludarabine-based (8/177; 4.5%), HyperCVAD (5/77; 6.5%). 94% were symptomatic on investigation (fever 92%, CXR changes 25%, abnormal LFT's 3%, GI symptoms 8%, rash 6%). 7 had CMV-disease (3 pneumonitis, 2 oesophagitis, 2 enteritis/colitis). 11 had isolated fever. 14/19 treated with ganciclovir had possible/proven CMV-related illness; in 12, fever, CMV-DNAemia or symptoms resolved. 2 died from underlying disease or treatment complications. 3 asymptomatic patients with CMV-DNAemia on >1 occasion were observed until DNA negativity. 17/19 with CMV-DNAemia on only 1 occasion did not develop CMV-related illness. We recommend that patients receiving alemtuzumab, should have CMV surveillance to facilitate early therapy, though the role of ongoing surveillance/prophylaxis is unknown. Patients with persistent/rising CMV-DNA titres or evidence of CMV-disease, should be treated.

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Comparison of Cord Blood Donations Collected from a Tertiary Referral and a Medium Level Care Obstetric Centre with Respect to Ethnic Composition and Laboratory Acceptance for Processing

Robyn Rodwell [1,2], Robyn Nikolai [1], Diane Baldry [1,2], Jeremy Wellwood [1,2], Kerry Taylor [1,2]

[1] Queensland Cord Blood Bank At The Mater, South Brisbane QLD
[2] Haematology Division, Mater Health Services, South Brisbane QLD

Aim: To compare cord blood donations (CBDs) collected by the Queensland Cord Blood Bank (QCBB) staff at a tertiary referral (Mater Mothers Hospital) or medium level obstetric care centre (Logan Hospital) with respect to ethnic composition and laboratory acceptance criteria (LAC) for processing.

Method: At hospital enrolment mothers were provided with an information booklet and consent form and shown an educational video. Eligibility criteria included: age 18-45 years, >=36 weeks gestation and willingness to return at 180-days for follow-up testing. CBDs were collected with the placenta ex utero into a triple bag system (Stem cell set Macopharma) and transported to the QCBB in monitored temperature controlled units. LAC include transport temperature 4-25 degrees Celsius, volume >= 60ml, total nucleated cell (TNC) dose of 7.5 x 108, and absence of blood clots, critical labelling errors or known clinical risk factors for the transmission of genetic or infectious disease. Ethnicity, clinical and laboratory data were reviewed for the first year of operation of Logan (n=516 collections) and from a similar number of consecutive donations at the Mater from the date of Logan start-up. Data was analysed by Chi square analysis (significance level 0.05).

Result: Ethnicity data (only available for processed CBDs) showed minority groups were more common in CBDs from Logan 22% (90 of 410) compared to 6% (25 of 434) from Mater ($p<0.001$). Pacific Islanders were the predominant group at Logan and South East Asians at Mater (11% and 3% of all processed CBDs respectively). Logan compared to Mater had a significantly ($p<0.001$) higher proportion of CBDs that failed to meet LAC 27% (143 of 516) vs 6% (25 of 434). The only significantly ($P<0.001$) different rejection factor between Logan and Mater was for the volume collected 24% vs. 9%. No significant differences with respect to TNC dose (2.5% vs. 3%), blood clots (1.2% vs. 0.5%), transport temperature (0.7% vs. 0%) or clinical exclusion factors (5% vs. 3.5%) respectively were found.

Conclusion: The addition of a second collection centre has diversified the ethnic composition of CBDs in the QCBB with accrual of CBDs from Pacific Islanders as well as Australian Aborigines, groups unlikely to be represented in international banks. Knowledge of reasons for laboratory rejection of CBDs provides guidance for improving operational practices, the collection to banking ratios, and cost-effectiveness of our programme.

1045 to 1200 Bellarine Room 4
HSANZ Free Communications 8 - General Haematology

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Haemoglobinopathies in Cocos Islanders

Nina Chetty-Raju, Wendy Erber, John Prior, Erna Lim

WA Centre for Pathology and Medical Research

Aim: To determine the prevalence and types of haemoglobinopathies in residents of Cocos Island, a group of islands under Australian governance 3,000km NW of Perth, with a population of 630 comprised of Cocos Malays and Europeans.

Method: Over 6 years (1998-2004) PathCentre received blood samples from 44 Cocos Islanders for haemoglobinopathy investigations. FBC, film, HbH preparation, HPLC, Hb electrophoresis and DNA analysis were performed.

Result: Thirty-five of the 44 (79.5%) individuals had a haemoglobinopathy. The most common genetic defect was the alpha alpha/--FIL, a 30-34kb deletion involving the alpha1, alpha2 and zeta genes, present in 20 individuals. Heterozygotes had Hb 84-139g/L, MCV 62-77fL and 44% had positive HbH bodies. Ten individuals had -alpha 3.7 and 2 alpha alpha/--SEA. There were 7 HbH disease, six -alpha3.7/--FIL and one -alpha4.2/--FIL. There were 8 beta-thalassaemias, one beta-thalassaemia major and 8 traits. Genotyping showed 6 of 7 individuals to have the 45kb beta0-Filipino deletion, with high HbA2 levels (6.0-7.8%), HbF 1.0- 4.9%, haemoglobin 98-135g/L and MCV 63-68fL. No compound heterozygotes or variant haemoglobins were detected.

Conclusion: Haemoglobinopathies are prevalent in the Cocos Islands, with the most common genetic lesions being betaFIL and alpha alpha/--FIL. These are significant mutations leading to a high risk of beta-thalassaemia major and HbBart's hydrops fetalis. The incidence of the latter may be minimised by early fetal loss, due to absence of zeta chains with alpha alpha/--FIL. There is therefore a need for screening and preconceptual genetic counselling in this population with a limited gene pool.

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Prevalence of Haemoglobinopathies in Cambodia

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[3] Angkor Hospital for Children, Siem Reap, Cambodia

Background: Alpha-thalassaemia and HbE are common in Southeast Asia. The prevalence of these disorders in Siem Reap, Cambodia has not been determined since the genocide associated with the Khmer Rouge

Aim: To document the prevalence of anaemia, alpha and beta thalassaemia and other haemoglobinopathies in Siem Reap.

Method: 260 children between 5 months and 16 years of age and presenting sequentially to the out-patient department of the Angkor Hospital for Children were assessed with FBC, ferritin levels, HPLC, and DNA analysis for alpha and beta globin deletions and mutations.

Result: 110 anaemic subjects (42.3%) were identified with 61 cases of microcytic anaemia. Six iron deficient subjects and 137 children (52.7%) with a haemoglobinopathy were identified. The prevalence of Hb E (30%) is consistent with previous reports but Beta thalassaemia (0.8%) lower than previously reported. Alpha thalassaemia is prevalent (35%), with the majority having single gene deletions (82%). The frequency of double deletions is low (0.0096). 5% of subjects had alphaT thalassaemia; HbCS accounted for 92% of these cases. HbPS occurs at low prevalence (0.4%). Triplicated alpha gene frequency (0.008) is consistent with that described in other populations. Complex abnormalities are common with 22.6% of cases having alpha and beta globin gene abnormalities. Only 2 cases with clinically severe haemoglobinopathy (HbH disease and Eß thalassaemia) were identified.

Conclusion: Anaemia and haemoglobinopathies are common (52.7%) in Cambodian children and iron deficiency rare. The majority of globin gene abnormalities (-Alpha3.7 and Beta E) abnormalities are clinically insignificant. The low frequency of beta thalassaemia, alpha ° alleles or other clinically severe phenotypes suggests that intensive screening and genetic counselling is unlikely to be cost effective in Siem Reap population.

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A Comparative Study of Procoagulant Factor VIII and von Willebrand Factor Antigen in Myeloma, MGUS and CLL

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Venous thromboembolism (VTE) is a common complication of disseminated adenocarcinomas, but is less common in patients with haematological malignancy. Clinical trials of thalidomide and anthracycline chemotherapy (thal/CTX) in refractory multiple myeloma (MM) have shown an increased rate of VTE (incidence 25-30%). The prothrombotic mechanism(s) of thal/CTX remain to be defined, but elevated procoagulant factor VIII (FVIII:C) and von Willebrand Factor antigen (vWF:Ag) have been implicated. Elevated FVIII:C (>150%) has been associated both with VTE and recurrent VTE in the absence of malignancy. To provide a baseline for measuring the effect of thal/CTX on these parameters we assayed FVIII:C and vWF:Ag in currently untreated patients without thrombosis. We compared MM (n=15); monoclonal gammopathy of uncertain significance (MGUS; n=14) and chronic lymphocytic leukaemia (CLL; n=15). MM patients showed a significantly increased mean FVIII:C compared to the MGUS/CLL group (p=0.005). Correlations between FVIII:C levels and Durie-Salmon MM Staging established that for Indolent/Smouldering/Stage 1 MM, the mean FVIII:C was 156% compared to a mean of 204% for Stage 2/3 MM. vWF:Ag was within the reference range for all MM patients, showing no difference from the CLL/MGUS group. This unexpected finding could be explained by MM-related cytokines which have been reported to increase FVIII:C independently of vWF:Ag. This prevalence study demonstrates that FVIII:C, but not vWF:Ag, is elevated in MM compared to MGUS and CLL, and that the degree of elevation in MM is related to disease activity. Elevated FVIII:C may reflect the effect of myeloma-related cytokines, and may be implicated in prothrombosis. We propose that MM patients with elevated FVIII:C before thal/CTX may be at higher risk of VTE.

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Role of Cyclosporin A in Maintaining Response to ATGAM plus Cyclosporin A in Aplastic Anaemia, and Effectiveness of Retreatment for Relapse

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Aim: To assess the role of Cyclosporin A (CsA) in maintenance of response in aplastic anaemia to treatment with ATGAM/CsA, and to assess effectiveness of retreatment with ATGAM/CsA for relapse.

Method: A retrospective analysis was performed on 22 episodes of aplastic anaemia treatment using ATGAM/CsA in a single institution between January 1996 to January 2004. 11 were initial treatments and 11 were for first or second relapse. Data was abstracted regarding severity of aplastic anaemia at treatment, degree and duration of response, and freedom from relapse after CsA withdrawal. Minor treatment response was defined as improvement in only one of the three affected cell lines.

Result: Incidence of haematological improvement did not differ significantly between initial treatment and retreatment with ATGAM/CsA (91% vs 82%). Freedom from relapse in the overall group was not significantly different between patients on maintenance CsA or after CsA withdrawal (p=0.8, log rank). However,subgroup analysis indicated that in patients who achieved major responses (very good PR or CR), 6 of 8 relapsed 3 to 7 months after CsA withdrawal, and none of 4 relapsed on ongoing CsA. This contrasted with relapse in 5 of 7 patients with minor treatment response (p=0.01, Fischer exact test)whilst on CsA.

Conclusion: Retreatment with ATGAM/CsA for relapse appears as effective as initial treatment in aplastic anaemia. Ongoing CsA administration is associated with lower incidence of relapse in patients who have had a major treatment response, but not in patients with minor responses.

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Enzyme Replacement Therapy for Gaucher Disease In Australia

Jeff Szer [1], Jack Goldblatt [2], Janice Fletcher [3], Jim McGill [4], John A Rowell [6], Meredith Wilson [5]

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[3] Women's and Children's Hospital, North Adelaide
[4] Royal Children's Hospital, Brisbane
[5] Children's Hospital at Westmead, Sydney
[6] Queensland Health Pathology Service, Royal Brisbane Hospital, Brisbane

Aim: We studied the effectiveness of a specific national program of enzyme replacement therapy (ERT) for patients with severe forms of Gaucher Disease, a disorder of sphingolipid metabolism resulting from an inherited deficiency of the lysosomal enzyme beta-Glucocerebrosidase.

Method: The responses of haemoglobin (Hb) and platelet (plt) concentrations, liver and spleen volumes were assessed.

Result: Forty-eight (48) patients (40 with type 1 disease and 8 with 3B) aged 1-70 (median 24) yrs were treated with ERT for a minimum of 6 months. Duration of therapy at the time of analysis was 6-114 months. Thirty-six percent of patients started with a normal Hb increasing to 76% after 6 months. The mean improvement in Hb from baseline to the end of study period was 20g/L when the Hb was normal in 85% (41) patients. Thirty percent of patients had a normal plt count at start of therapy with a more gradual increase in the count at 6 monthly intervals of 50, 91, 108 and 142% of starting value. Seventy-five percent of patients had a normal plt count at the end of study. Spleen volumes reduced by a mean of 56% in 33 evaluable patients and the liver by 27% in 30 of 38 evaluable patients. Eight patients had an increase in liver volume of 28%.

Conclusion: ERT produced a spectrum of beneficial responses in patients with Gaucher disease, but all had some evidence of reversal of haematological complications and reduction in visceromegaly.

1400 to 1530 Bellarine Room 1
HSANZ Free Communications 9 - Haemopoietic Cell Biology
Chair: David Curtis

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CD34+-derived CD11c+++BDCA-1++CD123++ dendritic cells (DCs): Expansion of a Phenotypically Undescribed Myeloid DC1 Population to Clinically Useful Numbers

Kate Ward [1,2], Lisbeth Stewart [1], Anthony P Schwarer [1,2]

[1] Bone Marrow Transplant Programme, Alfred Hospital
[2] Department of Immunology, Monash Medical School, Monash University

Aim: DCs are commonly defined as HLA-DR+/Lin- cells that can be: CD11c+++CD123+/- termed DC1/myeloid DCs that induce a T-helper cell type 1 response (Th1): or CD11c- CD123+++ termed DC2/lymphoid DCs that induce a Th2 response. However significant heterogeneity within DC preparations is apparent and supports the existence of several distinct DC subpopulations. This study aimed to expand and characterise CD34+-DC for use in immunotherapy.

Method: G-CSF mobilised CD34+ cells were isolated, seeded at 1x10^5/mL and expanded for 14 days in RPMI + 10% autologous plasma supplemented with GM-CSF, IL-4, Flt-3L and SCF. Maturation was induced with TNF-alpha/ PGE2 for 2 days. DCs were analysed: morphologically; phenotypically with a panel of monoclonal antibodies to lineage, myeloid, lymphoid and DC markers; functionally in MLRs/T-cell assays and T-cell cytokine secretion by ELISA.

Result: Significant cellular expansion was observed: 60 +/- 5x10^6 DCs from 1x10^6 CD34+ cells (n=28). Morphologically DCs appeared vacuolated with dendrites. Phenotypically DCs were characterised as: HLA-DR++, CD11c+++, CD80++, CD83+, CD86++, CD123++, CD15++, CD33++, BDCA1++, CD4+ and Lin-. DCs displayed potent allo-stimulatory capacity and efficient presentation of KLH for the generation of naïve T-cell responses. KLH (naïve antigen)-pulsed DCs generated 11.2 fold greater proliferation of T-cells (T-cell:DC ratio 8:1) compared to autologous controls. DC-primed T-cells secreted IFN-gamma (Th1), however no IL-4 (Th2) was detected.

Conclusion: DCs generated here represent an undescribed population of myeloid CD34+-DCs functioning as DC1, however phenoytypically expressing markers characteristic of both DC1 and DC2. This novel DC population is capable of inducing naïve T-cell responses and can be expanded to clinically useful numbers. CD34+-DCs represent attractive candidates for use in immuno-therapeutic protocols.

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Efficient Electroporation of RNA into Cord Blood Derived Dendritic Cells for Adoptive Immunotherapy

Andy Hsu [1], Beverley Kerr [1], Derek Hart [2], Alison Rice [1]

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[2] Dendritic Cell Laboratory, Mater Medical Research Institute, Brisbane, QLD

We aim to develop an adoptive immunotherapy protocol to treat relapsed acute leukaemia post transplant. In order to generate anti-leukaemic cytotoxic T lymphocytes (CTL), dendritic cells (DC) derived from cord blood CD34+ stem cells will be transfected with leukaemic RNA as the antigen source. We have optimised an electroporation protocol using in-vitro transcribed (IVT)-green fluorescent protein (eGFP) mRNA as a model antigen. Variables investigated included electroporation voltage, capacitance, media, incubation, temperature, mRNA and cell concentrations and mRNA loading timecourse. Major findings that improved transfection efficiency and cell viability included lower electroporation voltage and capacitance, whilst the electroporation media and time of incubation after electroporation both contributed significantly (ANOVA) ($p = 0.02$ and $p = 0.023$ respectively). Increasing mRNA concentrations significantly increased eGFP fluorescence intensity ($p = 0.001$). Interestingly, incubating cells on ice post transfection resulted in lower viability (40-60%), compared to cells incubated at warm temperature (70-80%). Electroporation of DC with mRNA resulted in specific up-regulation of co-stimulatory molecules (CD40, CD80 and CD86) on DC. We have shown that total leukaemic RNA could be successively transfected into DC. CTL chromium release assays using flu matrix protein mRNA demonstrated that transfected mRNA were processed and presented as peptides to T cells. In summary, the optimal conditions defined in this study resulted in over 93% cell viability with up to 96% transfection efficiency, compared to non-optimised conditions with only 20% cell viability and 30% transfection efficiency. We expect that the systematic optimisation of antigen loading into DC will result in improved leukaemic CTL induction.

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Correlation of Telomere Length with Ex-vivo Proliferative Potential of Umbilical Cord Blood Stem / Progenitor Cells

Melissa Ferguson, Shan Li, Ngaire Elwood

Murdoch Children's Research Institute

Aim: The long-term aim of this study is to identify a more precise means of predicting, prior to transplant, which umbilical cord blood (UCB) units will be more likely to provide rapid and successful engraftment following transplant. This is necessary because following an UCB transplant many patients experience a delay in myeloid recovery compared to that observed following a bone marrow transplant. To this end, we have examined whether measurement of telomere length (TL) of UCB stem/progenitor cells may be useful for predicting the proliferative potential of a UCB unit.

Method: Telomere length may provide information about both the proliferative history and proliferative potential of a particular cell. A flow cytometry based method (flow-FISH) was established in our laboratory to measure the TL of UCB stem/progenitor cells prior to these cells being placed into culture in the presence of a defined cytokine combination. Cultures were maintained in an exponential growth phase until maximum proliferative potential was achieved. The starting TL was then correlated with the extent of ex-vivo cell growth.

Result: In general, UCB cells with longer telomeres generated greater numbers of cells in ex-vivo culture than those with shorter telomeres. Surprisingly, this relationship disappeared when TL exceeded a certain length.

Conclusion: Our data suggests an unexpected relationship between the TL of UCB cells and the ex-vivo proliferative potential where an optimum TL is observed for maximum proliferative potential. These findings may have direct clinical implications for predicting the in-vivo haematopoietic reconstituting ability of a UCB unit prior to transplantation.

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Side Population (SP) Cells are Increased in Ageing Bone Marrow

Matthew Greenwood [1,2], Peter Lansdorp [1,2]

[1] Terry Fox Laboratory, British Columbia Cancer Research Center
[2] The University of British Columbia

Aim: Evidence suggests that haematopoietic stem cells (HSC's) show functional impairment with age and may have limited replicative lifespans. Previous reports have suggested that HSC's, defined by phenotypic and in vitro assays, accumulate in the bone marrow (BM) of aging C57BL/6 mice. Using Hoechst 33342 (Hst) efflux capacity, a marker of ABCG2 transporter expression, and surface phenotype, we sought to further characterise the HSC compartment in old C57BL/6 (Ly5.1) mice.

Method: Whole BM was extracted from young (9-13 week) and old (95-108 week) C57BL/6 (Ly5.1) mice. BM cells were incubated with Hst and stained with anti-murine CD34, c-kit, and Sca-1. Six-colour flow analysis was performed on a FACS Vantage (BD) cytometer and data analysed using FlowJo software.

Result: In old versus young mice, whole BM CD34-ckit+Sca1+ cells (1.3% vs 0.3%) and SP cells were increased (2.1% vs 0.2%). The SP fraction contained a higher proportion of CD34-ckit+Sca1+ cells in both old and young mice. This proportion was increased in old mice. The proportion of CD34-ckit+Sca1+ cells increased with increasing Hst efflux, an effect also more profound in old mice.

Conclusion: With increasing age, Ly5.1 mice accumulate cells with phenotypic markers of HSC's. These cells also maintain high Hst efflux capacity. A higher proportion of cells within the SP fraction in old mice exhibit phenotypic markers consistent with HSC's. Cells with the highest Hst efflux capacity almost exclusively exhibit an HSC phenotype in old mice. Further work is needed to identify the role of the ABCG2 transporter in the aging HSC compartment.

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CD133+/CD34+ Progenitors from G-CSF Mobilised Peripheral Blood of Patients with Chronic Ischaemic Heart Disease Generate Endothelial Cells In Vitro

Andrea Herbert [1], Helen Tao [1], Jason C Kovacic [2], Sandy Smith [3], Robert M Graham [2], David Ma [1]

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[2] Victor Chang Cardiac Research Institute, St Vincent's Hospital, Sydney, Australia
[3] Department of Immunology, St Vincent's Hospital, Sydney, Australia

The reported capacity of CD133+ stem cells to generate endothelial cells, together with their ability to be mobilised into the peripheral blood (PB) following administration of G-CSF, suggest this population is a candidate source of endothelial progenitor cells (EPCs) for myocardial revascularisation. Published methods for the culture and phenotypic assessment of EPCs are widely varied and ill-defined. Here we aim to develop a robust and consistent culture-system for quantifying EPCs for our clinical trial in patients with chronic ischaemic heart disease (CIHD). CD133+ cells were isolated from PB-MNCs of G-CSF-treated CIHD patients using MACS columns. We found the optimal conditions for differentiating CD133+ progenitor cells into an endothelial lineage involve culture in the presence of VEGF, IGF-1 and bFGF. As compared to MNCs, with culture of CD133+ cells, more colonies with typical endothelial morphology appeared after 3-4 weeks. Immunofluorescent staining showed cells in these colonies were positive for vWF, CD144, CD146 and VEGF-R2, took up DiI-Ac-LDL and bound UEA-1. Initially, all CD133+ cells co-expressed CD34, and in time showed increased expression of endothelial-specific markers (CD106, CD54, CD62E, CD144, CXCR4, VEGF-R2) with a concurrent decrease in CD133 and CD34 expression by flow cytometric analysis. These results suggest that under appropriate conditions, CD133+ progenitor cells from G-CSF mobilised PB-MNCs can be consistently induced to differentiate into endothelial cells. This culture system will allow the study of EPCs in our current clinical trial of CIHD patients undergoing transplantation of autologous cells (CD133+ or unselected leukocytes) for therapeutic vasculogenesis.

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The Expression of Chemokine Receptors CCR7, CCR5 and CXCR4 on Dendritic Cells Following Allogeneic Stem Cell Transplantation

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[2] Mater Medical Research Institute, South Brisbane, Australia

Aim: Dendritic cells (DCs) have been proposed to play a role in the development of graft-vs-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (alloSCT). Chemokines regulate the trafficking of DCs, and it has been shown that the chemokine receptors, CCR7, CCR5 and CXCR4, play a major role in recruiting (DC's) from the blood to tissues and lymph nodes. We therefore studied the expression of these chemokine receptors in blood DCs in patients after (alloSCT).

Method: Peripheral blood was taken from 6 patients after alloSCT and 5 healthy volunteers. DC subsets (CD11chi and CD123hi) were enumerated and assessed for expression of the chemokine receptors CCR5, CCR7 and CXCR4 by four colour flow cytometry. The chemokine receptors were expressed as a percentage of CD11chi and CD123hi positive cells.

Result: Following alloSCT, the expression profiles of CCR7, CCR5 and CXCR4 in patients was compared to healthy volunteers. Expression for CXCR4 was higher than CCR5 and CCR7 in both normals and in patients for myeloid and plasmacytoid DC's. Variable CCR7 expression occurred around day 20 post transplant in 3 of the 6 patients, mainly in plasmacytoid DC's (range 0-31% & 58%, of CD11chi and CD123hi respectively). In contrast, CCR5 expression occurred as early as day 1 post transplant and persisted to day 67 (range 0-80% & 83%, in myeloid and plasmacytoid DC's respectively). CXCR4 was expressed from day 1 to day 67 post transplant and was consistently higher than CCR7 and CCR5 in all patients. Three patients developed acute GVHD and CCR7 expression was also noted in both DC subsets compared to patients that did not develop acute GVHD.

Conclusion: Chemokine receptors are differentially expressed on blood DCs post alloSCT and may be related to pathophsiological changes occurring post transplant.

1400 to 1530 Bellarine Room 2
HSANZ Free Communications10 - Leukaemia Biology
Chair: Linda Bendall

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SDF-1 Mediates Stromal Dependent Proliferation of Pre B ALL Cells on Stroma and Interacts with IL-7 and IL-3 to Enhance Response

Julius Juarez [1], Kenneth Bradstock [2], Linda Bendall [1]

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Stromal factors are essential to the growth and survival of normal and leukemic B cell progenitors. Removal of these cells from the stromal microenvironment results in the rapid onset of apoptosis. SDF-1 is a major stromal factor known to contribute to the growth and survival both normal and malignant B cell progenitors. However the independent contribution of SDF-1 to the survival and proliferation of pre B acute lymphoblastic leukemia (ALL) cells and its interaction with other known growth factors is unclear. In this study TC14012, a potent and highly specific SDF-1 antagonist was used to determine the contribution of SDF-1 to the proliferation of pre B ALL cells in the presence of stromal support and its interaction with the growth factors IL-3 and IL-7. The average viability of ALL cells on stroma was 63.5% (range=31-83%). All cases showed proliferation on stroma and this was SDF-1 dependent, with TC14012 (50μM) inhibiting proliferation by an average of 39.3% (range 13.5%-69.5%). Six of the 11 samples responded primarily to SDF-1. Co-operative interactions between SDF-1 and either IL-3 or IL-7 were observed in 3 samples resulting in enhanced proliferation. Finally in the remaining 2 samples, IL-3 displayed a pronounced effect on proliferation. These results show that SDF-1 is an important proliferative factor, which can interact with other growth factors to enhance the proliferation of pre B ALL cells. This suggests that targeted disruption of the SDF-1 axis may result in significant inhibition of pre B ALL growth and proliferation in vivo.

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Loss-of-Function Polymorphisms in the Human P2X7 Receptor Protect Mycobacteria from ATP-Mediated Killing

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Many of our global health problems result from pathogens which invade and survive within cells of the RE system thereby evading the adaptive (T-cell) immune response and limiting the effectiveness of treatment based on vaccination. The adult incidence of tuberculosis (TB) is most commonly caused by reactivation of dormant infection that the patient may have carried for years. Twin studies indicate that genetic factors are important in reactivation of TB but the major genetic susceptibility factors remain unidentified. P2X7 is a cytolytic receptor which is activated by extracellular ATP and has the highest expression in cells of the monocyte-macrophage series. Previous studies have shown that ATP treatment of mycobacteria-infected macrophages induces apoptosis and phospholipase D activation, mediated via the P2X7 pathway leading to the death of both the host cell and internalized bacilli. Our studies have shown that genetic factors play a major role in determining the function of the P2X7 receptor with three polymorphisms (946G ->A, 1513A ->C and 1729T->A) in the P2X7 gene each causing an amino-acid change and an ATP non-responsive receptor. Interferon-g-primed, mycobacteria-infected macrophages from wild-type individuals (n=6) were incubated with ATP and this induced apoptosis and reduced mycobacterial viability by 90%. Similar treatment of macrophages from individuals homozygous for the 1513C polymorphism (n=6) failed to induce apoptosis and did not lead to mycobacterial killing via the P2X7 mediated pathway. Macrophages heterozygous for 946A or 1729A (n=5) showed intermediate level of mycobacterial killing. These data demonstrate that polymorphisms in the P2X7 gene can allow survival of mycobacteria within macrophages and may impair other aspects of innate immunity.

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Characterization of a New Myeloid Specific Antigen, 35-L3, as a Potential Leukemic Cell Target

Lubomira Jamriska, Samantha Hodgson, Derek Hart, Georgina Clark

Mater Medical Research Institute

The experience with Mylotarg, which delivers a cytotoxic drug to the CD33 surface molecule, indicates the potential for antibody-mediated therapy in the treatment of acute myeloid leukemia (AML). To evolve more effective anti-leukemic agents, there is a need to characterize other target molecules expressed by leukemic cells. We have defined and characterized a novel myeloid specific molecule, named 35-L3. This molecule is a member of the CMRF-35 immunoregulatory family. Expression studies showed that the 35-L3 mRNA is expressed by myeloid derived cell lines and cells of the myeloid lineage, but not by T or B lymphocytes. To analyze the protein expression of 35-L3, we have generated a monoclonal antibody, (MMRI-15), that binds to the recombinant 35-L3 Ig fusion protein. We have now studied the 35-L3 mRNA and protein expression on AML primary samples and AML cell lines. Using quantitative RT-PCR we showed that fifteen AML samples analyzed to date express 35-L3 mRNA. AMLs from different FAB types have been represented in the sample pool. The levels of expression varied but did not appear to correlate with FAB type. The 35-L3 protein expression was confirmed on U937 and K562 cell lines and in 6/11 AML primary samples. We are now focusing on using the reagents we have generated in the in vivo xenogeneic AML model in NOD/SCID mice to investigate the function of 35-L3 on myeloid cells in vitro and in vivo. This data has established 35-L3 as a possible target for AML immunotherapy.

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Insertion of RARA into the PML Gene Locus in Patients with Acute Promyelocytic Leukaemia Lacking the Classic t(15;17) Translocation

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Aim: Acute Promyelocytic leukaemia (APML) is typified by the t(15;17) translocation generating the PML-RARa transfusion gene. This predicts for sensitivity to all trans retinoic acid, the main stay of therapy, and the novel agent arsenic trioxide. The objective of this study was to review the incidence and potential mechanism of cases which lack the classic t(15;17) translocation.

Method: Sequential cases of APML presenting to a single institution between June 1999 and June 2004 were reviewed. All cases were analyzed on the basis of morphology and immunophenotype, conventional G banded cytogenetics, the PML localization assay, fluorescence in situ hybridization (FISH) and RT-PCR for the PML-RARA fusion gene transcript.

Result: Over a 5-year period 25 patients were diagnosed with APML by the presence of the PML-RARA fusion gene by conventional cytogenetics, FISH or RT-PCR analysis. 22/25 cases had the classic t(15;17) translocation and one a three way translocation t(1;15;17) but two consecutive cases had a normal female karyotype only on standard G-banded cytogenetics and were negative for the PML-RARA fusion gene using a single fusion FISH probe. The two patients were both female, aged 23 and 50 years of age respectively. On morphology and immunophenotype these cases were consistent with classical APML and both were positive for the PML localization assay. RT-PCR was positive in both cases with a bcr1 breakpoint and bcr3 breakpoint being demonstrated respectively. Additional FISH studies were undertaken using a new PML/RARA dual fusion and RARA breakapart probe. This analysis indicated a small insertional rearrangement in chromosome 15q22 (PML locus) with the 17q21 (RARA locus) resulting in a masked PML/RARA gene fusion.

Conclusion: Masked translocations involving the PML-RARA fusion gene appear to be relatively uncommon. In these cases the masked translocation has been shown to be due to a submicroscopic insertion, which may be missed by both conventional G banded cytogenetics and single fusion probe FISH studies. These two cases emphasize the importance of utilizing morphological, cytogenetic immunophenotypic (including the PML localization assay) and molecular analyses for the diagnosis of APML.

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Does The Panmyeloid Phenotype Define a Good Prognostic Subset of Acute Myeloid Leukaemia (AML)?

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[1] Department of Clinical Haematology and Medical Oncology, Royal Melbourne Hospital
[2] Walter and Eliza Hall Institute of Medical Research

Aim: AML expressing the panmyeloid immunophenotype - blasts or leukaemic cells expressing immature and mature myeloid antigens (CD13, CD33, CD117, CD65 and myeloperoxidase) may represent a subset of patients with improved prognosis. We sought to determine if this phenotype correlated with standard markers of favourable outcome in AML such as cytogenetics, age, WCC and lactate dehydrogenase (LD).

Result: In a single institution study, 92 consecutive patients with de-novo AML were analysed for this phenotype. 25% of patients with AML expressed the panmyeloid phenotype. These patients were also significantly younger (mean age 47y vs 61y; p=0.004), demonstrated a lower mean white cell count (20.6 vs 32.4 x 109/L; p=0.41) and had a lower LD level at diagnosis (863 x 1098 U/L; p=0.10). More patients with good prognosis cytogenetics had a pan-myeloid phenotype (p=0.01). Of patients with intermediate prognostic cytogenetics, 50% had a panmyeloid phenotype and were also younger (41 vs 63 years) (p=0.005) and had a lower white cell count (37.2 vs 13.7) (p=0.07).

Conclusion: We conclude that the panmyeloid phenotype appears to represent a subset of patients in AML with a good prognosis. Interestingly, the pan-myeloid phenotype may identify a subgroup with better prognosis within the intermediate prognostic cytogenetic group. A prospective analysis of patient outcomes with the pan-myeloid phenotype and intermediate risk cytogenetics is recommended in the context of a randomised-controlled therapeutic trial to confirm these findings.

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Acute Lymphoblastic Leukemia Cells Demonstrate a Separation Between Chemotaxis and Proliferation Signals from the CXCR4 Receptor

Rana Baraz [1,2], Julius Juarez [1,2], Sylvie Shen [2], Richard Lock [3], Kenneth Bradstock [4], Linda Bendall [1,2]

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The chemokine SDF-1 regulates motility and proliferation of acute lymphoblastic leukemia cells as well as the localization of hematopoietic stem cells within the bone marrow. Cells from the majority of ALL cases showed chemotactic and proliferative responses to SDF-1 but cells from 20% of cases examined did not respond to SDF-1 in chemotaxis assays. These cases still demonstrated dependence on SDF-1 for proliferation in stroma-supported cultures, suggesting a selective defect in signalling events leading to chemotaxis. Using inhibitors of signalling molecules we found that p38 MAPK activity was required for chemotaxis, while inhibitors of MEK and PI-3K function had limited and variable effects on SDF-1 induced motility. Western blot examination of the phosphorylation of signalling molecules in response to SDF-1 revealed activation of p38 MAPK, ERK1/2 and AKT in chemotactically responsive ALL cells. However the two assessable cases, which had not undergone chemotaxis to SDF-1, failed to phosphorylate p38 MAP kinase, and in one of these ERK1/2, following SDF-1 stimulation. AKT phosphorylation was normal in these cases. This suggests that the loss of signalling through p38 MAPK may be responsible for the defect in chemotaxis. This study reveals the separation of SDF-1 signalling through the CXCR4 receptor leading to chemotaxis or proliferation in leukemic cells from patients with ALL. The ability to specifically inhibit proliferative responses while leaving chemotactic function intact would permit the inhibition of ALL cell growth without disrupting the marrow microenvironment by the induction of unwanted stem cell mobilization.

1400 to 1530 Bellarine Room 3
HSANZ Free Communications 11 - Leukaemia Treatment
Chair: John Norman

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Low Dose Melphalan in the Treatment of Myelofibrosis
Lynette Chee, Paul Turner

Department of Haematology, Austin Health, Heidelberg, VIC

Myelofibrosis is a clonal myeloproliferative disease characterized by predominantly megakaryocytic and granulocytic proliferation with associated fibrosis in the bone marrow, extramedullary haematopoeisis, splenomegaly and a leukoerythroblastic peripheral blood film. The usual treatments include hydroxyurea and busulphan but these are associated with a leukaemogenic potential and are often not very effective in reducing splenic enlargement. Using low dose melphalan (2.5mg daily 3 times/week) in treatment of myelofibrosis, Petti et. al.(2002) achieved a 66.7% response rate (RR). We evaluated clinical and haematological responses in 9 patients who have been treated with low dose melphalan (2mg daily 3 times/week) since May 2002. Complete response (CR) was defined as normalization of all clinical and haematological parameters and partial response (PR) was defined as an improvement of >50% in spleen size, decrease in transfusional requirements or achievement of Hb>100g/L. The RR was 55.6% (11.1% CR and 44.5% PR) and 44.4% were non-responders. The median duration of melphalan administration was 10 months (range 1-25 months). Median time to at least >50% reduction in spleen size was 4.5 months (range 3-12 months) and median duration of response was 18 months. Hb normalization (>120g/L) was achieved in 2 out of 9 patients (22.2%) and median duration taken to achieve it was 12 months. Amongst the responders, the median time taken to achieve Hb>100g/L was 4 months (range 1-15 months). Amongst the non-responders, 2 out of 4 patients have only had a short follow-up period of <3 months. 3 out of 9 patients have died at time of follow-up. Reversible neutropenia was the most common complication (33%) necessitating temporary suspension of melphalan administration. We conclude that melphalan has potential in the treatment of myelofibrosis.

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Haploidentical Stem Cell Transplant as Initial Therapy for Poor Risk MDS
Mark Dawson, Andrew Spencer

Alfred Hospital Melbourne

HLA-haploidentical mismatched stem cell transplants offers some patients with leukaemia who do not have an appropriate sibling or unrelated donor the opportunity of cure. We report here a case of a 27-year-old male in complete remission 4 years following a HLA-haploidentical 4/6 matched stem cell transplant as first line treatment for poor risk myelodyplasia with monosomy 7. He presented at age 21 with perianal sepsis and was noted to be pancytopenic. Bone marrow biopsy revealed a hypercellular marrow with trilineage dysplasia and cytogenetic analysis demonstrated monosomy 7. Despite an extensive search amongst his siblings, extended family and unrelated donor registries an appropriate 6/6 HLA match was not found. As a result of symptomatic pancytopenia, an HLA-haploidentical stem cell transplant was performed one year after his original presentation. The conditioning regimen included melphalan 140mg/m2 (d -8), thiotepa 10mg/kg/day (d -7), fludarabine 40mg/m2 (d -7 to -3) and ATGAM 15mg/kg/day, (d -6 to -2). In vitro T cell depletion was performed with Isolex CD34+ selection, the graft composition included CD34+ (12.5 X 106/kg) and CD3+ (5.0 X 104/kg). The time to engraftment with a neutrophils > 1.0 X109/L was 11 days and platelets > 20 X109/L was 11 days. Time to CD4+ > 400 cells/iL was 12 months. Significant post-transplant morbidities included sinusitis with aspergillus fumigatus 8 days post transplant. Grade 1 GVHD of the colon and adenovirus related diarrhoea 20 months post transplant. This form of therapy should be considered as potential first line approach in diseases that are incurable with standard therapy in patients that lack a suitably matched donor for more orthodox transplantation.

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Can Conventional Amphotericin B (AmB) Related Nephrotoxicity In Acute Leukaemia Patients Be Predicted?

Shriram Nath,Linda Mileshkin, Monica Slavin, Karin Thursky, John Seymour

Peter MacCallum Cancer Centre

Introduction: AmB-related nephrotoxicity is common and associated with major morbidity. Lipid formulations are less nephrotoxic, but expensive. It would be useful to identify patients at greater risk of toxicity to guide selection of anti-fungal agents.

Aim: To study the risk factors for development of AmB-related nephrotoxicity in patients with acute leukaemia.

Method: Patients with ALL and AML who received chemotherapy from 1/1997-7/2000 at PMCC were analysed. Data from patient records was retrospectively collected for demographics, co-morbidities, treatment, concomitant nephrotoxins, treatment-related complications, and outcomes. The first episode of amphotericin use was analysed. Nephrotoxicity defined as doubling of the serum creatinine.

Result: 91 patients (Primary 64 AML, 27 ALL; Relapsed 10 AML, 3 ALL) received a total of 104 chemotherapy cycles. Median age 52 years (range 17-75). Conventional amphotericin was used in 57. Median duration of AmB was 8 days (range 1-44). All cases (15/57) of nephrotoxicity occurred in patients receiving AmB (26%). The overall rate of nephrotoxicity was 14.42%. 2 patients died due to nephrotoxicity in the setting of sepsis. Univariate analysis variable odds ratio p value 95% C.I. male gender 10.6 0.03 1.3-88.2 gentamycin 4.8 0.03 1.2-19.4 vancomycin 4.1 0.09 0.8-20.4 Multivariate analysis variable odds ratiop value 95% C.I. gentamycin 8.7 0.05 0.96-78.9 male gender 7.8 0.02 1.3-44.6 Patients with neither of these risks factors had a 5% likelihood of developing nephrotoxicity vs 46% in males given gentamicin.

Conclusion: We have identified risk factors for AmB nephrotoxicity that allow rational selection and cost-effective utilisation of the available anti-fungal drugs.

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Invasive Mould Infections in Patients Undergoing Treatment for Acute Leukemia at Royal Melbourne Hospital: 1999-2003

Monica Slavin [1], Andrew Grigg [2], Jean Cameron [2], Karin Thursky [1], Janette Vincent [3], Jeff Szer [2]

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[2] Department of Haematology and Medical Oncology, Royal Melbourne Hospital
[3] Department of Radiology, The Royal Melbourne Hospital, Parkville Vic

Aim: Treatment for acute leukemia is often complicated by fungal infection. No trials of antifungal prophylaxis have demonstrated a reduction in invasive mould infection (IMI) in this population. This review was undertaken to determine epidemiology of and risk factors for IMI in patients treated at RMH.

Method: The files of all patients treated for acute leukemia at RMH between 1999-2003 were reviewed. Antifungal prophylaxis, underlying disease, chemotherapy regimen, duration of neutropenia and occurrence of proven or probable IMI (according to internationally accepted criteria) were recorded.

Result: 154 patients were treated. Antifungal prophylaxis regimens changed over this time, although chemotherapy treatment regimens did not. In 1999-2000, 44 pts were treated and fluconazole prophylaxis was used. There were 9 IMIs (5 Aspergillus). In 2001-2002, 62 pts were treated and itraconazole prophylaxis was used. There were 9 IMIs (2 aspergillus). In 2003, 36 pts were treated and voriconazole prophylaxis was used. There were 3 IMIs (no aspergillus). Risk factors for infection will be further analysed.

Conclusion: Antifungal prophylaxis has impacted on the occurrence of and organisms responsible for IMI in patients receiving treatment for acute leukemia. There may be a shift away from infection with organisms such as Aspergillus when broader spectrum azoles are used. Although retrospective, this data suggests that the incidence of IMI can be reduced in this population with antifungal prophylaxis active against Aspergillus. A randomised trial of itraconazole or voriconazole versus fluconazole is indicated.

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A Matched Pair Retrospective Comparison of Adult Unrelated and Sibling Donor Allogeneic Stem Cell Transplantation (SCT) for AML

John Moore [1], Ken Bradstock [2], Jeff Szer [3], Ian Nivison-Smith [4], David Ma [1], Kim Goh [5]

[1] St Vincent's Hospital Sydney
[2] Westmead Hospital
[3] Royal Melbourne Hospital
[4] Australasian Bone Marrow Transplant Recipient Registry (ABMTRR)
[5] Kuala Lumpur Hospital

Aim: Recent studies have shown nearly equivalent survival outcomes for unrelated donor (URD) SCT for CML compared to sibling donors. This study compared outcomes for a set of URD transplants for AML and a strictly selected matching set of sibling donor transplants.

Method: 100 adult histocompatible URD transplant recipients with AML were selected from the ABMTRR database. 100 adult first 6 HLA-identical sibling transplants were selected, matched with the subjects for disease stage, sex and age as a control group.

Result: Follow-up data was available on 162 cases: 22 for good-risk (CR1 - 15 siblings, 7 unrelated) and 140 poor-risk (beyond CR1 - 77 siblings, 63 unrelated). Disease free survival (DFS) was identical for good-risk sibling and unrelated donor recipients (p=0.2). However the 5-year DFS of poor-risk URD was worse than sibling recipients (14.1% vs 29%, p=0.01). In a multivariate Cox regression model, the independent risk factors for DFS were URD (p=0.003), disease stage beyond CR1 (p=0.04), recipient male (p=0.04), age 40 (p=0.04) and recipient CMV positivity (p=0.03).

Conclusion: Adult URD allograft recipients for poor risk AML have poorer DFS probability than sibling recipients. In this study donor type was not a significant DFS risk factor in good risk AML, however this would need confirmation with larger patient numbers. This study provides a rationale for a larger prospectively tracking study of risk factors in allotransplantation for AML. The ABMTRR is an important national data resource providing a large and readily accessible sample frame for retrospective studies on specific transplant indications.

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Targeting Chemoresistance: Swinging the Balance from Cancer Towards Cure?

Andrew Wei, Lin Chen, Simon Willis, Andrew Roberts, David Huang

Walter and Eliza Hall Institute of Medical Research

Aim: Despite some stunning therapeutic advances, the majority of advanced solid and haemopoeitic malignancies remain incurable. A common cause of treatment failure is tumour resistance to standard therapy, often due to defective apoptosis caused by p53 loss or overexpression of pro-survival proteins like Bcl-2. Bcl-2 and its functional homologues promote cell survival until antagonised by BH3-only proteins such as Bim. A potential way of overcoming tumour resistance is to engineer BH3-only mimetics to promote cell death. To assess the feasibility of this approach, we asked if the physiological Bcl-2 antagonists, the BH3-only proteins, act promiscuously or selectively. Selective targeting of the specific pro-survival molecules overexpressed in a tumour (e.g. Bcl-2 in lymphomas or Mcl-1 in plasma cell neoplasia) may well be better tolerated without compromising efficacy.

Result: We assessed the binding of eight mammalian pro-apoptotic BH3-only proteins (Bim, Puma, Bmf, Bad, Bik, Hrk, Bid, Noxa) to their pro-survival Bcl-2-like targets (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1). Based on the results of these experiments, we evaluated the killing activity of selected BH3-only proteins overexpressed by a retrovirus. We also tested the ability of diverse BH3-only proteins to sensitise Bcl-2 overexpressing cells to commonly used cytotoxic therapies. The targeting of pro-survival Bcl-2-like proteins has been considered to be promiscuous, but unexpected selectivity emerged from our analysis of the binding affinity of eight BH3 peptides for five Bcl-2-like proteins. The interactions varied over 10,000 fold in affinity and, accordingly, certain protein pairs associated selectively inside cells. Some death ligands (Bim, Puma) that bind promiscuously are potent killers, whereas Bad and Noxa, which showed a more selective binding profile, were weaker killers. Interestingly, even though Noxa overexpression induced apoptosis poorly, its expression was able to potently co-operate with standard cytotoxic drugs to overcome chemoresistance imposed by Bcl-2 overexpression.

Conclusion: Our results suggest that selective interactions between particular subsets of these family members may have prominent biological roles and that it should be feasible to discover BH3 mimetic drugs that inactivate specific pro-survival targets. Although drugs that mimic the action of the BH3-only proteins may have a useful role in overcoming chemoresistance, a serious concern is that such compounds may have deleterious effects on normal cells. Importantly, our studies demonstrating selective targeting of Bcl-2 and its homologues by their physiological ligands suggest that the discovery and design small molecules that are more selective in their action, and hence possess better therapeutic profiles, is feasible.

1400 to 1530 Bellarine Room 4
HSANZ Free Communications 12 - Lymphoma / CLL/Myeloma
Chair: Susan Moreton

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A Predictive Model for Infectious Episodes during Fludarabine Combination Chemotherapy without Corticosteroids

Constantine Tam [1, 2], Max Wolf [1], E. Henry Januszewicz [1], Andrew Grigg [3], H Miles Prince [1], David Westerman [1], John F Seymour [1]

[1] Haematology Department, Peter MacCallum Cancer Centre, East Melbourne Vic Australia.
[2] Haematology Department, The Alfred Hospital, Prahran Vic Australia.
[3] Department of Clinical Haematology and Medical Oncology, The Royal Melbourne Hospital, Parkville Vic Australia.

Aim: Infection is the principal toxicity of fludarabine combination chemotherapy (FAMP combo) in the management of patients with indolent lymphoid malignancies. We sought to construct a predictive model for infections during FAMP combo.

Method: Baseline variables in 92 patients treated with fludarabine-mitoxantrone (FM, n=29) or fludarabine-cyclophosphamide (FC, n=63) were examined for their relationship to risk of infection during FAMP combo therapy.

Result: Patient characteristics : median age 59 yrs (range 34 - 80), stage III or IV disease 88%, previously treated 88%, #prior therapy 2 (0 - 10), previous FAMP 21%, time from diagnosis to treatment (TTT) 40 months (0 - 324), performance status (PS) 1 (0-3), baseline neutrophil 3.1 x 109/L (0.6-26.0). By histology: CLL/SLL/PLL 46%, follicular lymphoma 37%, other 17%. In total 323 cycles were evaluable; infection (any grade) and grade 4 neutropenia complicated 14% and 17% cycles respectively (FC vs FM, p>0.49). In univariate analysis six risk factors were associated with increased rate of infections: Age > 60, 3+ prior therapies, prior FAMP , TTT >3 years, PS 2+, and baseline neutrophil <2.0 x 109/L. Compared to patients with <3 risk factors, patients with >3 risk factors had a higher rate of infections (26% vs 7% per cycle, p<0.0001), grade 4 neutropenia (41% vs 8% per cycle, p<0.0001) and neutropenic sepsis (15% vs 1% per cycle, p<0.0001).

Conclusion: We have constructed a predictive index for infection risk during FAMP combo that identifies patients at high risk for infections. These patients are an appropriate group for consideration of prophylactic strategies.

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Transgenic Mouse Model for Evaluation of Oncogenesis and Therapeutics of Protein Kinase C Beta Over-expression in Lymphoma

Kritika Chaiwatanatorn [1,2], Carly Selan [2], Harshal Nandurkar [1,2], Frank Firkin [1,2]

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[2] Department of Medicine, St. Vincent's Hospital, Melbourne

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm in adults. Majority of patients succumb to the disease despite chemotherapy. The differences in outcome likely reflect the disease's molecular heterogeneity. Over-expression of the gene protein kinase C betaII (PKCbetaII) identified by microarray in DLBCL has recently been suggested to correlate with fatal/refractory disease. PKC is a family of key enzymes involved in signal transduction pathways which regulate cell growth and differentiation. PKCbeta plays an important role in B-cell receptor signalling and inactivation of the gene leads to B-lymphoid deficiency in mice.

Aim: To create a transgenic mouse model over-expressing constitutively active PKCbetaII gene in lympho-haemopoietic tissue.

Methods and Result: A transgenic construct consisting of a constitutively active bovine PKCbetaII gene coupled to the vav promoter to limit expression to haemopoietic tissue was assembled and introduced into fertilised eggs from C57BL/6 x CBA mice by standard pronuclear microinjection. Transgenic pups were identified for genomic integration by Southern blot analysis of tail DNA, RNA expression by RT-PCR of peripheral blood leucocytes (PBL) and protein expression by flow cytometric analysis of PBL. Of 40 pups generated, 4 are transgenics with evidence of RNA expression and 2 transgenics have protein expression. Breeding is currently in progress to generate sufficient transgenic mice for analysis.

Conclusion: Our transgenic mouse model will contribute to the understanding of the signalling pathways implicated in the development of lymphoma. It will form an excellent in vivo model for pre-clinical testing of novel forms of targeted cancer treatment such as PKCbeta-selective inhibitors.

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Short Report: The Role of Rituximab for Chronic Lymphocytic Leukaemia Associated with Autoimmune Haemolysis or Thrombocytopenia

Philip Saunders [2], Julian Cooney [1], Michael Leahy [1], Frank Cordingley [1], Lynette Tytherliegh [1]

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We review four cases of chronic lymphocytic leukaemia complicated by autoimmune haemolytic anaemia or immune thrombocytopenia (ITP/AIHA) treated with rituximab, with the aim of assessing the role of anti CD 20 therapy in this setting. In three cases, single agent rituximab achieved a durable response against early/moderate stage CLL and ITP/AIHA. In a patient with more advanced CLL and refractory AIHA, combination therapy with cyclophosphamide, rituximab and methylprednisolone proved effective. We conclude that rituximab has a valuable role to play in the management of patients with CLL and ITP/AIHA. Clinical benefits include efficacy against early or moderate stage CLL, rapid and durable control of AIHA/ITP, reduced steroid requirements, avoidance of splenectomy and minimal complications from therapy. Evidence based guidelines on the use of rituximab for CLL and AIHA/ITP are currently lacking. Incorporating recent evidence supporting dose escalation and maintenance therapy into clinical practice may improve outcomes, but a cost-benefit analysis of such strategies will be needed. While AIHA/ITP is not believed to alter survival in CLL, an observed increase in morbidity and clinical interventions amongst this patient group should provide a focus for improvements in therapy.

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Does the Addition of Rituximab to Fludarabine and Cyclophosphamide (FC) Increase the Risk of Early or Late Infectious Complications?

Constantine Tam [1, 2], Michael Brown [3], John Seymour [1], Philip Campbell [4], John Scarlett [5], Craig Underhill [6], David Ritchie [7], Rodney Bond [8], Andrew P Grigg [3],

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[3] The Royal Melbourne Hospital, Parkville Vic Australia.
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[6] Border Medical Oncology
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[8] Ballarat Oncology

Aim: The anti-CD20 antibody Rituximab(R) effectively depletes B-cells and may impair antibody responses. It is known that FC is associated with a moderate risk of infection, but the impact of the addition of Rituximab on early and late infection rates is currently unknown.

Method: 91 patients from seven centers were treated with FC-rituximab (FCR; F 25mg/m2 x3, C 250mg/m2 x3, R 375mg/m2 x1 q28 days) from 12/00 -8/03. Infections during chemotherapy and in the initial 12 months of remission were compared with a historical cohort of 64 patients treated with FC at PMCC from 10/96-1/03.

Result: Patients characteristics (FCR): median age 61 yrs (range 30-89), 63% male, 24% previously untreated, CLL/follicular lymphoma/other 42%/33%/25%, time from diagnosis 36 months (0-196), performance status 1 (0-3), PCP prophylaxis 29%, antiviral prophylaxis 15%, antifungal prophylaxis 12%, G-CSF support 34%, baseline neutrophils 2.8 x 109/L (0.6-11.0); these were similar to the FC cohort. A total of 326 cycles of FCR were evaluable: infections complicated 20% of cycles (9%/cycle Grade 3+ infection); this was not significantly different from FC (15% and 8%/cycle respectively, $p>0.17$). Over a total of 567 patient-months (PM) of follow-up, rates of infection (1/13 PM), severe infection (1/35 PM) and herpes-virus infection (1/94 PM) were not significantly different from that of FC (1/13, 1/84 and 1/48 PM respectively, $p>0.16$).

Conclusion: The addition of R to FC does not result in an increased risk of infection either during or within 12 months after treatment over that seen with FC alone.

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Single Dose Per Cycle Pegfilgrastim Supports Full Dose Intensity CHOP-14 in Patients Over 60 Years with Non-Hodgkin's Lymphoma (NHL) and Mobilises Peripheral Blood Progenitor Cells (PBPC)

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[4] Department Of Haematology, Royal Perth Hospital, Perth
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[6] Department Of Haematology, Royal Brisbane Hospital, Brisbane
[7] Amgen Australia Pty Ltd, Melbourne
[8] Division Of Haematology & Medical Oncology, Peter MacCallum Cancer Centre, Melbourne.

CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) administered at 21 day intervals for 6 to 8 cycles has been the standard treatment for patients with aggressive NHL. CHOP given every 14 days (CHOP-14) may produce better outcomes. Preliminary efficacy and safety of a CHOP-14 regimen was assessed for patients over the age of 60 years supported by a single dose of pegfilgrastim (6mg SC) on day 2 of each cycle. The potential for pegfilgrastim to mobilise peripheral blood progenitor cells (PBPC) in these patients was investigated by recording circulating CD34+ cells at baseline and days 5, and 7 to 12 in cycle 1. Thirty patients received 159 treatment cycles (129 cycles 2-6, with 106/129 (82%) delivered on schedule at day 15). Delay occurred specific to haematological toxicity (febrile neutropenia (1), thrombocytopenia (1), neutropenia/thrombocytopenia (1) in 3 patients. Febrile neutropenia (6 grade 3, 1 grade 4) occurred in 7 patients, thrombocytopenia (grade 3) in two patients. One patient experienced grade 3 thrombocytopenia in 4 cycles. There were 17 dose reductions for febrile neutropenia (7) and thrombocytopenia (1) and four episodes of bone pain. Overall response rate was 77% (10 CR/13 PR). No deaths or unexpected toxicity occurred on study. Six patients (67%) achieved peripheral CD34+ counts >20 cells x 106/L during days 8 to 12. CD34+ counts were maximal on day 12 (median 37.4; mean 38.4; range 2-107 cells x 106/L). These results demonstrate the safety and efficacy of CHOP-14 with pegfilgrastim in patients >60 years and suggest potential for PBPC mobilisation.

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Outcome of Mini-Autologous Transplantation for Multiple Myeloma and Amyloidosis in a Single Institution

Alhossain Abdallah [1,2], Muhanna Al-Muslahi [1], Karen Olsen [1], Noemi Horvath [1,2]

[1] The Royal Adelaide Hospital (RAH)
[2] Institute of Medicine and Veterinary Science (IMVS) South Australia

Aim: Tandem dose-reduced autologous stem cell transplantation (SCT) has been used to treat multiple myeloma (MM) with the goal of prolonging disease-free survival (DFS) in elderly patients or patients with reduced performance status and renal impairment to minimize the morbidity and mortality associated with the conditioning regimen.

Patients and Method: We analysed retrospectively the outcome and prognostic factors of 12 patients (11 MM and 1 amyloidosis) treated up-front with mini-autologous SCT (100mg/m2 Melphalan) during the period from August 2002 to May 2004 at the Royal Adelaide Hospital. The male-to-female ratio was 50:50, and the median age was 70.5 years (range; 53-74). Median time from diagnosis to the first transplant was 7.5 months (range; 5-13). Three patients with MM had an unfavorable cytogenetic risk (13q-, p53 deletion) and five patients had high $ß_2$ microglobulin levels. Nine of 12 patients underwent double transplant while 3 had only one transplant, due to delayed recovery in two cases and septicemia with subsequent renal failure in one case. All patients received three cycles of induction chemotherapy (VAD), ten patients underwent stem cell mobilisation with cyclophosphamide and G-CSF, while the remaining two patients (1 amyloidosis and 1 MM) underwent mobilisation with G-CSF alone.

Result and Conclusion: After a median follow up period of 11.5 months (range; 6-26), all 12 patients were alive and 11 remained in remission (4 CR, 7 PR), while one MM patient showed disease progression. Our retrospective analysis shows that elderly or poor risk MM patients can be safely treated with tandem dose-reduced autologous SCT.

1400 to 1530 Bellarine Room 5
HSANZ Free Communications 13 - Stem Cell Transplantation - III
Chair: David Ritchie

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Autiologous Stem Cell Transplantation for Severe Systemic Sclerosis - Feasibility and Outcome of Stem Cell Mobilisation

Steven Austin [1], Helen Englert [2], L Schreiber [3], John Moore [1]

[1] Haematology Department, St Vincent's Hospital, Sydney
[2] Department of Rheumatololgy, Westmead Hospital, Westmead
[3] Department of Rheumatology, Royal North Shore Hospital, Sydney

Haemopoietic stem cell transplantation (HSCT) has in recent years been suggested as a therapeutic modality in systemic sclerosis (SSc) patients who have failed other therapies. We have recently initiated a pilot study of HSCT in SSc patients who have failed monthly cyclophosphamide in order to evaluate the safety and efficacy of this procedure. Four female patients, median age 34 yrs (range 22-45 yrs) have been entered into the program and at the time of writing 1 has been transplanted. All patients had previously received prednisone, D-penicillamine, cyclosporin and monthly cyclophosphamide with only marginal benefit. Mobilisation was achieved with cyclophosphamide 2g/m2 with methylprednisone 1 g and G-CSF 10mcg/kg from day 2 until collection. All patients had a Vascath inserted on the day of cell collection and a median of 12 litres was processed in a single apheresis collection. Median stem cell yield was 43.2 million CD34+ cells/kg on day 10. There were no complications from the apheresis or the cyclophosphamide and 3 out of the 4 patients noticed a softening of their skin tightness. One patient has undergone HSCT with cyclophosphamide (50mg/kg x 4 days) and ATG (10 mg/kg x 4) conditioning. This patient experienced a hypertensive crisis on day 20 and underwent dialysis for 2 months before regaining normal renal function. She is now 18 months post HSCT and has experienced a marked improvement in her disease parameters with a reduction in her Rodnan skin score from 27/51 to 8/51 and a marked improvement in her HAQ score (Health Assesment Questionaire) having ceased all immunosupression. In summary, stem cell mobilization and HSCT is a feasible and promising treatment for SSc. An update on the progress of 2 further patients to be transplanted this month will be added.

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Real Time PCR Monitoring of Cytokines as a Predictor of Acute Graft Versus Host Disease

David Ritchie, Janet Seconi , Julie Walton

Malaghan Institute of Medical Research, Wellington, New Zealand

Graft versus host disease (GVHD) is a debilitating complication of allogeneic stem cell transplantation (SCT), potentially limiting the application of this curative treatment modality. Cytokine production, in particular TNF-a and IFN-g, by alloagressive donor T cells is central in the pathogenesis of GVHD in both the clinical and experimental setting. Previous studies have measured the production of cytokines during established GVHD and therefore have not been useful in the design of interventional strategies to prevent or minimise the impact of GVHD. We have hypothesised that early detection of cytokine production may allow prediction of GVHD onset and thereby allow early therapeutic intervention. In this pilot study we have examined cytokine RNA production for TNF-a, IFN-g, IL-10, IL-4 and GM-CSF in donor T cells of 18 patients (median age =43, unrelated donor = 5) undergoing either myeloablative or non-myeloablative allogeneic stem cell transplantation. Serial measurements indicate that two patterns of cytokine production are predictive for the onset of acute GVHD. Firstly 'spikes' of TNF-a production predate the onset of GVHD (particularly gut GVHD) by 5-7 days in 5 patients. Secondly failure of early suppression of either TNF-a or INF-g within he first 50 days post-SCT also predicted for the onset of acute GVHD in 2 patients. These initial findings allow the design of intervention studies to manage immunosuppression post-allogeneic SCT.

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Therapeutic Antibody Mediated Depletion of Activated Dendritic Cells and the Prevention of Graft Versus Host Disease

John Wilson, Alison Rice, Derek Hart, David Munster

Mater Medical Research Institute, South Brisbane, Australia

Graft versus host disease (GVHD) is the most serious complication of haemopoietic stem cell transplantation. Current GVHD prevention targets donor T lymphocytes but this strategy is associated with long term immunosuppression, loss of the graft versus leukaemia effect and graft failure. Dendritic cells (DC) are a population of antigen presenting cells (APC) which, when activated, stimulate the donor T lymphocyte attack in GVHD and as such, represent a possible alternative therapeutic target. CMRF-44, a monoclonal antibody specific for activated DC, has been shown to deplete activated DC in-vitro. To investigate the effect of DC depletion on GVHD, we used an established mouse-human chimeric model of GVHD in which human T lymphocytes are known to be effector cells. Severe combined immunodeficient mice were injected with whole or depleted human peripheral blood mononuclear cells (PBMC), causing a GVHD-like syndrome that was measured by clinical symptoms, mouse survival and human cell engraftment. To validate the model, we have shown that human dendritic cells are the critical APC in stimulating the disease process in the model. Contrary to a previous report, human B-lymphocytes do not appear to contribute to GVHD in this model. To characterize the human effector cells, we have shown that CD4+ T lymphocytes are required to proliferate in order to cause mouse mortality. We found that mice injected with human PBMC depleted of CMRF-44+ cells in vitro, survived longer than undepleted controls (p=0.064). Work is currently underway assessing in vivo depletion of human DC in this model.

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A Pilot Study to Explore the Tolerability and Efficacy of Thalidomide Containing Regimens to Reduce Tumour Cell Load Prior to HSCT in Multiple Myeloma and the Feasibility of Harvesting HSC Following Thalidomide Containing Regimens

Noemi Horvath [1], Luen Bik To [1], Douglas Joshua [3], John Gibson [3], Andrew Roberts [2]

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Aim: To explore the role of thalidomide as part of first line treatment in multiple myeloma.

Patients and Method: 27 patients with advanced de-novo multiple myeloma, median age 55, median beta-2-microglobulin 4.2. Ten of 17 patients with FISH or metaphase cytogenetics had del 13q. The regimen included TDx3 (thalidomide 400mg/dx21, pulse dexamethasone 32mg TDSx5), followed by DT-PACEx2 (thalidomide 400mg/dx28, dexamethasone 40mg/dx4,and 4 day infusion of cisplatin 10mg/m2/d, doxorubicin 10mg/m2/d, cyclophosphamide 400mg/m2/d, etoposide 40mg/m2/d).

Result: 14/27 patients completed study treatment, 12 had successful stem cell harvests, 8 were withdrawn, 1 died of sepsis and 4 were still to complete planned therapy. 2 failed stem cell harvest. Median tumour bulk at end of TDx3 and DT-PACEx2 was 13% and 5% respectively. A historical group of 52 patients, treated with VAD and HD cyclophosphamide,had median tumour bulk at end of VAD and cyclophosphamide 5G/m2 of 32% and 25% respectively(p=0.025 at end of induction/harvest). The median stem cell harvest was 4.3x106/kg CD34+ cells, after a median of 3 aphereses. After VAD/cyclophosphamide, median harvest and median aphereses were 11.6x106/kg and 2 respectively (p=0.000 and 0.004). The commonest adverse events were: fever 11, infection 6 (1 fatal) rash and neutropenia 4 each.

Conclusion: 1. Thalidomide containing combination appears to be as efficatious as VAD/HD cyclophosphamide as first line therapy in advanced myeloma. 2. Thalidomide containing combinations may reduce stem cell harvests. Conclusions need to be tested in a randomised study.

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Treatment of Steroid-Refractory Acute Graft versus Host Disease with Combination Monoclonal Antibody Therapy: A Pilot Study

Amanda Johnston, Anthony Dodds, Sam Milliken, David Ma, John Moore

St Vincent's Hospital, Sydney

Severe (Grade III-IV) acute graft versus host disease (aGVHD) is a serious complication of allogeneic haemopoietic stem cell transplantation. Patients not responding to high dose corticosteroids have a very poor prognosis (60-90% mortality). Previous second line agents, such as anti-thymocyte globulin, have had limited success in controlling steroid-refractory aGVHD. Recently monoclonal antibodies that target cytokines (TNF and IL-2) have been trialled, with several case series reporting responses to high dose infliximab (10mg/kg, x1-9 doses) and daclizumab (1mg/kg, x5-8 doses), alone or in combination. Here we report the results of a pilot study using reduced-dose infliximab and daclizumab, along with strict antimicrobial prophylaxis, for refractory aGVHD. The aim was to maintain the efficacy of monoclonal antibody therapy whilst reducing the infectious complications seen in previous series. Since January 2003 we have treated 8 allogeneic transplant patients with one or both of the monoclonal antibodies. All had failed a minimum of 3 days methylprednisolone 2mg/kg/day, started a median of 35 days post-transplant. The first four patients received infliximab 10mg/kg, x2-3 doses. Two of four had a complete response with one patient alive 15 months after antibody treatment. The remaining three died of opportunistic infections. Following this, a second cohort of patients were given low dose, combination therapy (infliximab 5mg/kg, x2 doses and daclizumab 1mg/kg, x4 doses). All four had complete resolution of aGVHD and three of four are alive at 58 to 95 days post-treatment. In conclusion, low dose combination monoclonal antibody therapy appears to be a promising treatment for refractory aGVHD.

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TGFb is a Differential Regulator of Acute and Chronic GVHD After G-CSF Mobilised Allogeneic Stem Cell Transplantation

Tatjana Banovic [1], Kelli MacDonald [1], Edward Morris [1,2], Vanessa Rowe [1], Rachel Kuns [1], Alastair Don [1], Steve Ledbetter [3], Andrew Clouston [4], Geoffrey Hill [1,2]

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[3] Genzyme Corporation, Framingham USA
[4] Department of Pathology, University of Queensland, Australia

Aim: Donor treatment with G-CSF augments donor regulatory responses and attenuates acute GVHD (aGVHD) after experimental allogeneic stem cell transplantation (SCT) although paradoxically increases the severity of chronic GVHD (cGVHD). We investigated the role of the regulatory cytokine transforming growth factor beta (TGFb) in this paradox.

Method: Two well-established murine models of acute and chronic GVHD where donors are pretreated with G-CSF and recipients administered control or TGFb neutralising antibody.

Result: Donor treatment with G-CSF prevented aGVHD in a TGFb dependent fashion (survival: 83% v 17% in control v anti-TGFb Ab, $P<0.01$). Neutralisation of TGFb after SCT augmented the proliferation, Th1 differentiation and expansion of donor T cells ($P<0.05$) in allogeneic but not syngeneic recipients. Neutralisation of TGFb following transplantation of IL-10-/- allogeneic grafts significantly accelerated mortality (median survival 17 days) compared to allogeneic recipients of wild-type grafts and anti-TGFb (median survival 30 days, $P<0.01$) confirming that TGFb and IL-10 act synergistically in regulating aGVHD after G-CSF mobilised SCT. Donor T cells from G-CSF treated donors regulated aGVHD induced by T cells from control treated donors in a TGFb dependent fashion (survival: 30% v 5%, $P<0.05$). In cGVHD, delayed neutralisation of TGFb attenuated the severity of GVHD as determined by semi-quantitative histopathology ($P<0.05$). In contrast to the protective T cell derived TGFb in aGVHD, pathogenic TGFb during cGVHD appeared to be derived from cells of the monocyte/macrophage lineage.

Conclusion: The therapeutic neutralisation of TGFb late after allogeneic SCT may attenuate the severity of cGVHD following transplantation of G-CSF mobilised stem cell allografts whilst maintaining the beneficial early regulatory effects of TGFb in aGVHD.

0800 to 1015 La Trobe Theatre
Research Plenary Session 1 - Don Metcalf Jubilee
Chair: Andrew Roberts, Nicos Nicola

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Molecular Mechanisms of Cytokine Signalling Suppression by SOCS Proteins
Nicos Nicola

The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC

The Suppressor of Cytokine Signalling (SOCS) proteins were first discovered in a screen to detect proteins that could inhibit interleukin-6 signalling in M1 myeloid leukaemic cells. The family now contains eight members (Cis and SOCS1-7) each of which contains a central SH2 domain and a C-terminal homology domain called the SOCS box. The transcription of SOCS genes is induced by cytokine signalling and the SOCS proteins then act as feedback inhibitors that terminate signalling through the JAK/STAT pathway. Deletion of SOCS1 in mice leads to a hyper-inflammatory syndrome as a result of excessive signalling by interferon- and other T-cell-activating cytokines. Deletion of SOCS2 leads to gigantism due to excessive responses to growth hormone. Selective deletion of SOCS3 in liver or haemopoietic cells revealed excessive signalling by G-CSF and IL-6. The cytokine specificities of different SOCS proteins are at least in part determined by the specificity of their SH2 domains with SOCS1 binding selectively to JAK kinases, SOCS2 binding to phosphotyrosines on the growth hormone receptor and SOCS3 binding to phosphotyrosines on the G-CSF receptor and gp130 component of the IL-6 receptor. Inhibition of signalling pathways is achieved in part by a kinase inhibitory region present in SOCS1 and SOCS3 that inhibits JAK activity, in part by competition for the same binding sites on the receptor (eg by SOCS3 and SHP2 on gp130), and in part by the SOCS box that acts as an E3 ubiquitin ligase resulting in polyubiquitination and proteasomal degradation of attached substrates.

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G-CSF and Beyond: The New Generation of Protein Therapeutics
Glenn Begley

Global Hematology and Oncology Reseach, Amgen Inc. USA

The discovery and clinical development of G-CSF opened a new horizon in protein therapeutics. Its discovery reflected a sustained investment in fundamental biology. Its clinical development heralded new therapeutic opportunities in oncology. As a result of the outstanding safety and efficacy profile of this molecule, G-CSF has achieved widespread clinical use in the context of chemotherapy induced neutropenia and blood stem cell transplantation. Further understanding of the mechanism of elimination of G-CSF formed the basis of a strategy to further improve on the pharmacological properties of this molecule. Pegylation of G-CSF resulted in a molecule, Neulasta, with increased plasma half-life and retention of desired safety and efficacy characteristics of G-CSF. This molecule has driven additional advances in clinical application of hematopoietic growth factor therapy.

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Zebrafish Models of Haemopoietic Disease

Graham J. Lieschke, Judith E. Layton, Meredith O. Crowhurst, Benjamin M. Hogan, John Hayman, Fiona Connell, Sony Varma, Dora McPhee, Luke Kapitany, Ryan Longland, Andrew Trotter, Andrew Hughes

Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Melbourne, VIC

Zebrafish have emerged as a biologically relevant, genetically tractable model organism for studying haemopoietic development and disease. In particular, forward genetic approaches can be efficiently and economically undertaken, supported by flexible reverse genetic techniques for studying specific gene function. Although initial general chemical mutagenesis screens in zebrafish generated a group of anaemic mutants, few mutants affecting myeloid development were identified. To generate a collection of zebrafish mutants with genetically-based myeloid-failure syndromes which would model congenital myeloid-failure diseases, we undertook a gynogenetic haploid screen of ethylnitrosurea-mutagenised zebrafish genomes. From this we have to date recovered 11 mutants with defects affecting the developmental pathway from mesoderm to a mature myeloid cell, with 19 other putative mutants at various stages of pedigree recovery. Mutants such as taminga fail to express the early myeloid transcription factor spi1(pu.1) and have loss of several haemopoietic lineages. Several mutants were identified for failure of myeloperoxidase (mpx) expression, a marker of zebrafish granulocytes: in shiraz, the absence of mpx-expressing cells occurs in a morphologically normal non-anaemic embryo, and in tarrango, loss of mpx-expressing cells is accompanied by loss of cells marking as macrophages and thymocytes, along with later developmental abnormalities of other organs. These examples illustrate the diversity of mutant phenotypes with myeloid failure generated. We anticipate that the further biological characterisation of this collection of mutants, and their genetic definition by positional cloning, will ultimately lead to unexpected new insights about the molecular regulation of myeloid development, and the genetic basis of congenital myeloid disease.

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Suppressor of Cytokine Signaling-3 (SOCS3) is Critical for both Negative Regulation and Maintenance of Specificity of G-CSF and IL-6 Signalling in Haemopoietic Cells.

Andrew Roberts, Ben A Croker, Don Metcalf, Sam Wormald, Doug Hilton, Nicos Nicola, Warren Alexander

The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC

We have investigated the physiological importance of SOCS3 in blood cell development and function by creating conditionally-targeted mice with SOCS3-deficient hematopoiesis. These mice develop a fatal inflammatory disease in adulthood characterised by tissue infiltration with neutrophils and macrophages. Investigations using both unfractionated and highly purified bone marrow cells reveal hyper-responsiveness to G-CSF and IL-6 in vitro, associated with increased and prolonged duration of STAT3 phosphorylation, but minimal changes in MAPKinase activation. Microarray experiments reveal not only quantitative changes in transcription, but changes in the profile of genes activated and suppressed. In haemopoietic progenitors, the consequences of this distorted signalling are reflected in aberrant proliferation and maturation with a bias towards macrophage development, rather than the neutrophil lineage. How this altered signalling relates to the development of inflammation in vivo is being investigated through the use of mice where SOCS3 is selectively deleted in T and B lymphocytes, myeloid cells and combinations of these. Results to date suggest SOCS3 is a major negative regulator of both G-CSF and IL-6 signalling, and is required to prevent spontaneous overactivity of the innate immune system in vivo.

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Myb Mutations Cause a Multilineage Dysplastic State

Donald Metcalf

The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC

Thrombocytopenic male mice lacking the thrombopoietin receptor, c mpl, were treated with the mutagen ENU and used to produce 1200 progeny mice that were screened for elevated platelet levels. Two such mice were identified and the genotype shown to be semi-dominant. From these founder mice, two lines of homozygous mice were developed, Plt3 and Plt4. Both were shown to have an identical point mutation in the myb gene although this was located in different regions in the two lines. Plt3 and 4 mice showed identical haematological abnormalities. Platelet levels were four times normal and 40-fold elevated above those in mpl / mice. The mice had excess numbers of megakaryocyte progenitor cells and megakaryocytes in the marrow and spleen, exhibited aberrant megakaryocyte colony formation when stimulated by GM CSF and loss of responsiveness to IL 6. They also showed severe depletion of B lymphocytes and a mild anaemia. The mice have a striking structural abnormality in the red pulp of the enlarged spleen which also exhibits some fibrosis. The abnormalities involving multiple haematopoietic lineages suggested that stem cell function might be abnormal and this has been confirmed by transplantation studies and the analysis of the recloning capacity of blast colony-forming cells in vitro. Myb was originally identified as a viral-incorporated oncogene but myb function could not be studied in vivo because / mice died in utero. The present partial loss of function mutations result in a dysplastic state that is being monitored longterm to determine whether leukaemia ultimately supervenes.

1045 to 1200 La Trobe Theatre
Research Plenary Session 2 - Don Metcalf Jubilee
Chair: Don Metcalf

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Ephs and Ephrins: From Leukaemia to the Egg and Back
Andrew Boyd

The discovery of a group of novel receptor tyrosine kinase protein in the late 1980s opened the path to the identification of the Eph family, the largest group of receptor tyrosine kinases. Several of the Ephs were identified as highly expressed genes during embryonic development, others were identified because of their over-expression in tumours. We isolated EphA3 as a protein expressed in a subset of pre B ALL and T cell leukaemia/lymphoma. The tumour specific nature of the high level of expression in these leukaemic processes was evident from studies of the normal counterparts of these tumours; bone marrow pre B cells and thymocytes. In these normal cells EphA3 expression was found to be vanishingly low. The ligands of the Eph family, the ephrins, were identified in the mid-90s. The ephrins were also membrane bound proteins with the capacity to signal. The Eph/ephrin system is now known to be capable of bi-directional signalling, the dominant effect being modulation of cell adhesion and cell movement. These effects underpin many critical events in embryogenesis, the elucidation of these processes continues to dominate this field of research. However, the role of these proteins in adult tissues and in disease is now gaining greater prominence. A role in normal haemopoietic tissues is evident from recent studies of platelet function and from evidence of a role for specific members of the family in individual haemopoietic lineages. In cancer, over-expression of Eph and ephrin proteins is implicated in tumour progression rather than initiation. Whilst the effects of Eph/ephrin signalling on cancers remains unclear, the well documented effects on adhesion and movement make it likely that over-expression enhances tumour spread and metastasis.

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The Search for Activators of Fetal Globin Gene Expression
Stephen Jane

Bone Marrow Research Laboratories, VIC

The human globin genes (ε, $^{G}\gamma$, $^{A}\gamma$, δ, β) are expressed at high level throughout ontogeny in a stringently regulated developmental and tissue-specific pattern. Two switches in β-like globin chain synthesis occur during development, the ε- to γ -globin switch at 5 weeks of gestation and the fetal (γ) to adult (β) at birth. The second of these two switches heralds the onset of β-thalassemia and sickle cell disease. However, in patients who co-inherit a mutation manifesting with persistently high fetal hemoglobin levels (Hereditary Persistence of Fetal Hemoglobin or HPFH), the clinical course of β-thalassemia or SCD is significantly ameliorated. This observation suggests that treatment strategies capable of prolonging or re-activating fetal hemoglobin expression after birth should be explored. To achieve this goal we must first understand the mechanisms underlying the developmental regulation of the β-globin locus, particularly the factors that can modulate γ-globin gene expression.

My laboratory has utilized two approaches to identify fetal globin regulatory molecules. The first focused on defining regulatory elements in the gamma globin promoter and the factors which bound them. This led to the cloning and characterization of the human transcription factor NF-E4, which plays a role in the preferential expression of the gamma globin genes during the fetal stage of erythropoiesis. The second approach, still in its early stages, involves genome wide mutagenesis in mice to identify modifiers of fetal globin gene expression. The ultimate aim of both these approaches is the development of genetic therapy strategies for the treatment of the hemoglobinopathies.

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Regulation of Phagocytosis by Insitol Polyphosphate 5 - Phosphatases

C A Mitchell [1], K Horan [1], R D Huysmans [1], A Tan [1], A Sriratana [1], F Wiradjaja [1], J Dyson [1], Gurung R Ooms [1], C Bailey [2], J Rasko [2], A Kong

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[2] Gene Therapy Research Unit, Centenary Institute for Cancer Medicine and Cell Biology, Royal Prince Alfred Hospital, NSW

Phosphoinositides are ubiquitous signaling molecules that regulate vesicular trafficking, proliferation, apoptosis, and cytoskeletal reorganization. Phosphoinositides generated by the phosphoinositide 3-kinase (PI3-kinase), include PtdIns(3,4,5)P3 and PtdIns(3,4)P2 that inhibit cell death and promote cell survival by activation of the Akt signaling pathway. In post-mitotic cells phosphoinositides such as PtdIns(3,4,5)P3 and PtdIns(4,5)P2 direct actin polymerization via regulation of Rac and actin binding proteins and thereby regulate cell adhesion and cell migration. Phosphoinositide kinases and phosphatases are targeted to specific subcellular membranes and thereby regulate the duration and spatial distribution of phosphoinositide signals, which in turn leads to the reversible localized membrane recruitment of critical effectors. The inositol polyphosphate 5-phosphatases (5-phosphatase) dephosphorylate the 5-position phosphate PtdIns(4,5)P2 and PtdIns(3,4,5)P3 forming PtdIns(4)P and PtdIns(3,4)P2 respectively. Ten mammalian 5-phosphatases have been identified, which have been implicated in regulating white cell proliferation/activation, human leukemia, osteoclast function, and glucose homeostasis. We have investigated the expression and subcellular localization of 5-phosphatases in macrophages and have shown a recently identified family member, the 72 kDa PtdIns(3,4,5)P3 5-phosphatase is highly expressed. In macrophages this 5-phosphatase localizes to the plasma membrane, co-localizing with submembraneous actin. Overexpression of the wild type 72 kDa 5-phosphatase in macrophages inhibited receptor-mediated phagocytosis whilst overexpression of a dominant-negative 72 kDa 5-phosphatase significantly enhanced actin accumulation in the phagocytic cup. These studies have identified a functional role for a recently identified signal terminating enzyme in regulating macrophage phagocytosis.

WEDNESDAY 20/10/04

0900 to 1030 John Batman Theatre
HSANZ Plenary Session 8 - ALLG Advances in Management of Acute Leukaemia
Chair: Max Wolf

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Acute Promyelocytic Leukaemia
Pierre Fenaux

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BC - ?Q-PCR in AML Follow Up
Cheryl L Willman

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Australasian Trials in Acute Promyelocytic Leukaemia (APL)

Harry Iland

Royal Prince Alfred Hospital, NSW

The last two decades have witnessed remarkable advances in the management of APL, manifest primarily by dramatic improvements in relapse rate and overall survival since the introduction of ATRA and its combination with anthracycline-based chemotherapy. At the same time, an increased understanding of APL pathobiology has enabled the use of sensitive molecular monitoring techniques for the detection of minimal residual disease and the recognition of early relapse. These advances have been mirrored in the Australasian context, with a series of APL trials that have facilitated the introduction of modern treatment and laboratory protocols that are now available for patients with APL. The ALSG's APML1 trial was the first APL-specific study in Australia, and involved three successive cohorts. The first consisted of poor prognosis patients who were treated with ATRA monotherapy, whereas the second and third cohorts explored different chemotherapy- and corticosteroid-based strategies for the prevention of ATRA syndrome. The ALLG's APML3 trial then used ATRA and intensive idarubicin for both induction and consolidation, and helped establish molecular monitoring as standard practice in Australasia. The recently activated APML4 trial has ushered in a new phase of APL therapy by (1) adding arsenic trioxide to standard ATRA and idarubicin for induction, (2) omitting conventional chemotherapy from consolidation, and (3) prospectively incorporating real-time quantitation of PML-RARA transcripts. The enthusiastic support of ALLG members for these trials and for protocol-driven sample acquisition has also generated an invaluable resource that is fuelling the search for novel independent determinants of prognosis in Australasian APL patients.

1100 to 1300 John Batman Theatre
HSANZ/ANZSBT/ASTH Presidential Symposium
Chair: Mark Hertzberg, Ken Davis, Hatem Salem
session sponsored by Pall Medical

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Mutation Screening in Imatinib-Treated Chronic Myeloid Leukaemia (CML) Patients is Indicated in Primary Refractory Patients and Those with Greater than Two Fold Rises in the BCR-ABL Level

Susan Branford [1], Rebecca Lawrence [1], Chani Field [1], Zbigniew Rudzki [1], Andrew Grigg [2], Kerry Taylor [2], John F Seymor [2], Simon Durrant [2], Peter Browett [2], Anthony P Schwarer [2], Chris Arthur [2], John Catalano [2], Michael F Leahy [2], Robin Filshie [2], Kenneth Bradstock [2], Richard Hermann [2], David Joske [2], Kevin Lynch [3], Tim Hughes [1]

[1] Institute of Medical and Veterinary Science, Adelaide
[2] Australasian Leukaemia and Lymphoma Group
[3] Novartis

Imatinib provides significant therapeutic benefit for patients with CML. However, resistance can occur in any disease phase. Somatic mutation within the BCR-ABL kinase domain is the main mechanism of acquired resistance and monitoring patients for mutations provides an important guide for clinical management. It is unclear how frequently patients should be monitored. We evaluated 214 patients receiving stable doses of imatinib for up to 39 months (median 15 months) to determine if molecular monitoring of BCR-ABL transcript levels could guide the frequency and timing of mutation analysis. We hypothesised that the emergence of a mutation would lead to a rise in the BCR-ABL level. Of the 214 patients, 5 were in blast crisis at the start of imatinib therapy, 27 in accelerated phase, 48 in late-chronic phase and 134 in early-chronic phase (defined as less than 12 months since diagnosis). BCR-ABL transcript levels were measured using real-time quantitative PCR at 1 to 6 month intervals (total 1,533 samples, median 5 per patient). The patients were screened for mutations at least every 6 months by PCR isolation of the BCR-ABL allele and direct sequencing (804 samples). Fifty-seven patients had a greater than 2-fold rise in BCR-ABL in consecutive samples (median rise 3.0-fold). Mutations were detected in 35 (61%) of these patients. In 37%, the mutations were retrospectively detectable prior to the rise (median 4 months). Only 1 of 157 patients (0.6%) with stable or decreasing BCR-ABL had a mutation detected ($P<0.0001$). Irrespective of the disease phase, failure to achieve a 1-log reduction in BCR-ABL by 6 months of imatinib therapy, which occurred in 17% of all patients, was associated with a significantly higher probability of having a mutation detected by 24 months ($P<0.0001$). We conclude that the highest incidence of mutations occurs in patients with a rise in BCR-ABL of more than 2-fold and in those who fail to achieve a 1-log reduction by 6 months of imatinib therapy. Mutation screening can be reliably and cost-effectively restricted to these patients.

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An Essential Role for Trp570 and Phe568 in the Cytoplasmic Domain of Glycoprotein Iba for filamin A Binding

Susan L Cranmer [1], Inna Pikovski [1], Pierre Mangin [1], Philip E Thompson [2], Teresa Domagala [1], Mark Frazzetto [1], Hatem H Salem [1], Shaun P Jackson [1]

[1] Australian Centre For Blood Diseases, Monash University
[2] Department Of Medicinal Chemistry, Victorian College Of Pharmacy, Monash University

Binding of the platelet glycoprotein (GP) Ib/V/IX receptor to von Willebrand factor (vWf) is critical for platelet adhesion and aggregation under conditions of rapid blood flow. The adhesive function of GPIb a is regulated by its anchorage to the membrane skeleton through a specific interaction with filamin A. In this study, we examined the amino acid residues within the cytoplasmic tail of GPIb a that are critical for association with filamin A, using a series of 25-mer synthetic peptides that mimic the cytoplasmic tail sequences of wild-type and mutant forms of GPIb a . Peptide binding studies to purified human filamin A have demonstrated a major role for the conserved hydrophobic stretch L567FLWV571 in mediating this interaction. Progressive alanine substitutions of triple, double, and single amino acid residues within the Pro561-Arg572 region suggested an important role for Trp570 and Phe568 in promoting GPIb a binding to filamin A. The importance of these two residues in promoting filamin A binding to GPIb a in vivo was confirmed from the study of Chinese Hamster Ovary (CHO) cells expressing GPIb a Trp570Ala and Phe568Ala substitutions. Phenotypic analysis of these cell lines in flow-based adhesion studies revealed a critical role for these residues in maintaining receptor anchorage to the membrane skeleton and in maintaining cell adhesion to a vWf matrix under high shear conditions. These studies demonstrate a novel filamin A binding motif in the cytoplasmic tail of GPIb a that is critically dependent on both Trp570 and Phe568.

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Adhesion of Stored Red Blood Cells to Vascular Endothelium Increases with Duration of Product Storage and Leucocyte Burden

Angela Anniss, Kath Patton, Rosemary Sparrow

Australian Red Cross Blood Service

Adherence of red blood cells (RBCs) to vascular endothelium impairs blood flow, decreases oxygen delivery and leads to vaso-occulusion. RBCs are relatively non-adherent however little is known of how changes to RBCs for transfusion during storage may affect their adherence properties.

The aim of this study was to monitor adherence of stored RBCs to vascular endothelium under conditions of continuous flow in vitro. Specifically the influence of RBC storage time and leucocyte burden of stored red cell preparations was investigated. Human umbilical vein endothelial cells (ECs) were grown to confluence on fibronectin-coated coverslips. Non-leucocyte-reduced, buffy-coat-reduced and leucocyte-filtered RBC products were prepared according to standard blood bank procedures. RBC samples were collected at multiple time points until product expiry and perfused across an EC monolayer using a parallel flow chamber mounted to an inverted microscope. Perfusion of RBCs was controlled for shear stress and temperature. RBC-EC interactions were recorded using a digital camera attached to the microscope. The number of RBCs adhering to the EC layer progressively increased with product storage time. RBCs from products stored for 28 and 42 days were significantly more adherent than fresher cells. RBCs from products containing leucocytes were also significantly more adherent to the EC layer on days 28 and 42 of storage than RBCs from leucocyte-reduced products.

Our findings indicate that product storage time and leucocyte burden increase the adhesion of RBCs to an EC layer. These results may lead to greater understanding of the interaction of transfused RBCs with recipient endothelium and the biological consequences of this adherence.

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Development of a Platform in Zebrafish for Studies of Immunity

Maria Flores, Scott Mead, Chris Hall, Enid Lam, Kathryn Crosier, Philip Crosier

The University of Auckland, Auckland, New Zealand

The zebrafish immune system has considerable structural equivalence with that of mammals. The utility of this model system for genetic and embryological studies led us to develop a platform in zebrafish for studies of immunity.

This has involved identification of zebrafish orthologues of genes that might regulate B and T lymphocyte development, analysis of their expression patterns and identification of promoter regions for linkage to EGFP to mark lymphocyte compartments. Initial work has involved the Src family kinase Blk and the Runx family member Runx3.

Zebrafish *blk* was identified, mapped and shown to have conserved syntenic relationships when compared with mammalian genomes. It is first expressed in individual cells throughout the developing pancreas and later in aggregations of cells close to the gut, suggestive of developing gut-associated lymphoid tissue (GALT). This expression parallels that of *Igμ* and *rag1*, supporting the identification of *blk* as a B cell-specific marker in zebrafish. To further characterise zebrafish GALT, we are generating transgenic fish that express EGFP under control of the zebrafish *blk* promoter.

Zebrafish runx3 is expressed in the thymic rudiment during early development. This expression is present in the haematopoietic mutant *cloche* but absent in the endodermal mutant *casanova*, suggesting that Runx 3 is initially derived from thymic epithelial cells and not thymocytes. Runx3 is a regulator of mammalian gastric epithelial cells and a tumour suppressor gene in this environment. Our results raise the possibility of a similar role in development of the thymic epithelium.

Together, these studies have provided additional tools for studying the immune system.

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Suppressor of Cytokine Signalling-3 (SOCS3) negatively regulates G-CSF-driven Emergency Granulopoiesis

Ben Croker, Donald Metcalf, Nicos Nicola, Warren Alexander, Andrew Roberts

The Walter and Eliza Hall Institute of Medical Research

Aim: G-CSF drives emergency granulopoiesis during the body's response to infection. When used clinically to accelerate neutrophil production or to mobilise stem cells, some patients develop unexpected inflammation or marked splenomegaly. We aimed to determine how G-CSF signalling is switched off to prevent development of toxicity. We hypothesised that the intracellular protein, SOCS3, was responsible for inhibiting G-CSF signalling.

Method: Socs3 knockout mice die early in utero precluding analysis. We therefore generated mice with a conditional deletion of SOCS3 specifically in haemopoietic cells. Bone marrow (BM) cells were assayed in vitro for G-CSF responsiveness. Neutrophil activation and function were also assayed. Young mice were injected with G-CSF 5μg/day, or placebo, for 4 days, then analysed.

Result: When stimulated with G-CSF in vitro, SOCS3-deficient BM cells exhibited enhanced and prolonged STAT3 activation, and increased proliferation and survival. Progenitor cell proliferation was also specifically increased in response to G-CSF. Mice developed neutrophilia and a spectrum of acute inflammatory pathologies with age. Prior to the development of inflammation, young mice, and age-matched controls were injected with G-CSF. The experiment was terminated after 4 days because of hindleg paresis in the SOCS3-deficient animals injected with G-CSF. No similar toxicity has ever been observed in wild-type mice injected with G-CSF. Mutant mice displayed enhanced neutrophilia, progenitor cell mobilisation, and splenomegaly, and histology revealed inflammatory neutrophil infiltration into multiple tissues, including the spinal cord.

Conclusion: SOCS3 plays a critical role during G-CSF-driven emergency granulopoiesis to switch off the response and limit tissue damage by activated neutrophils.

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Oestrogen Regulation of the Anti-coagulant Protein S

Quintin Hughes [1,2], Janelle Staton [2], Mark Watson [2], Ross Baker [2]

[1] University of Western Australia, Perth WA
[2] Royal Perth Hospital, Perth WA

Aim: The anticoagulant Protein S (PS) is coded for by the PROS1 gene and serves as a co-factor to APC inactivation of FVa and FVIIIa. Previous Studies have shown a reduction in circulating PS levels with increasing oestrogen (E2) levels resulting in an increased thrombotic risk.

Method: We have identified a potential oestrogen response element (ERE) spanning -350° -367 within the 5` UTR of PROS1. Using an EGFP expression vector, clones containing this ERE, the entire 5' UTR (948bp) and the Sp1 binding site (-66° -75) have been transfected into HepG2 cells and expression measured by flow cytometry.

Result: The ERE sequence alone was able to independently drive transcription. Surprisingly, the PROS1 5` UTR vector increased expression with increasing E2 levels.

Conclusion: These results suggest that the ERE is responsible for part, but not all, of the regulation of PS levels by E2. Further regulation is likely to be exerted by E2 regulated binding proteins. The discovery of an ERE in the PROS1 promotor and identification of previously undescribed (or novel) interacting proteins is a significant step forward in explaining the in vivo observation of E2s influence on circulating PS levels.

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Cytokine Expanded Myeloid Precursors Function as Regulatory Antigen Presenting Cells and Induce Tolerance Through IL-10 Producing Regulatory T Cells

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Stem cell mobilisation with the synthetic G-CSF and Flt-3 receptor agonist Progenipoietin-1 (ProGP-1) is superior to G-CSF for the prevention of graft-versus-host disease (GVHD). In this study we evaluated the contribution of ProGP-1 and G-CSF expanded donor antigen presenting cells (APC) to the amelioration of GVHD after allogeneic stem cell transplantation (SCT) in a well described murine SCT model (B6 into B6D2F1). The role of indirect antigen presentation in allogeneic responses was examined by adding populations of cytokine-expanded donor APC to haematopoietic grafts that would otherwise induce lethal GVHD. ProGP-1 and G-CSF expanded myeloid DC, plasmacytoid DC and a novel granulocyte-monocyte precursor population (GM)that differentiate into class IIpos, CD80/CD86pos, CD40neg APC during GVHD. Whereas addition of plasmacytoid and myeloid donor DC augmented GVHD, GM cells induced transplant tolerance. When purified GM cells from ProGP-1 treated B6 (H2b), DBA/1 (H2q) or B6 class II-/- (H2b) mice were added to control allogeneic grafts (H2b), only the B6 (H2b) GM cells provided long-term protection, confirming the protection was MHC class II restricted. GM cells added to T-cell depleted control grafts supplemented with IL-10-/- T cells failed to rescue animals from GVHD, demonstrating a requirement for donor T cell IL-10 production for GM mediated protection. Thus, G-CSF and ProGP-1 expand granulocyte-monocyte precursors that function as regulatory APC which induce transplant tolerance via the class II restricted generation of IL-10 secreting antigen specific regulatory donor T cells. The data suggest that G-CSF derivatives may have an application outside the context of GVHD, including disorders characterised by a loss of self-tolerance.

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Testing for Homologous Blood Transfusion in Elite Athletes - RPAH AT ATHENS 2004.

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Following the 2000 Sydney Olympics and the introduction of testing for erythropoietin in elite athletes, it became apparent that many athletes had reverted to blood doping using homologous and autologous transfusions. The Science and Industry Against Blood doping research consortium approached Dr Nelson at RPAH to determine if a test could be developed to detect recent blood transfusions. A grant was provided by the US Anti-Doping Agency to develop a flow cytometric assay which detects the expression of a panel of minor blood group antigens. Most blood bank reagents are IgM and are not satisfactory as they cause aggregation. A series of IgG polyclonal antibodies were sourced and the optimal titre of each antibody to detect minor negative and positive red cell populations in in vitro mixtures was determined. The test was validated by four Sydney flow cytometry laboratories who tested unknown samples in a proficiency exercise. In April 2004, IOC, WADA and ATHOC agreed to introduce the test into the Athens laboratory in time for the 2004 Olympics with assistance from RPAH. From May till August, RPAH sourced suitable antibodies, performed antibody titrations, provided a standard operating procedure, consulted daily with both the Athens lab and the Anti-doping lab in Lausanne and conducted a series of 3 Proficiency Testing exercises for both labs. No false positives were found in 254 antibody/sample test combinations. The Athens lab used a panel which included antibodies to C, c, E, Jka, Jkb, Fyb , Fyb , M, N and S. Reagents for anti s, K and e were also provided by RPAH. Two weeks before the Games, the lab personnel moved into the IOC Athens Testing lab. A total of 350 endurance athletes were tested. This included placegetters plus randomly selected competitors.

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