

HSANZ

Improving the outcome of allogeneic stem cell transplantation from typing to conditioning

Peter Bardy

ARCBS SA

Abstract not available at time of printing

Clash of the Titans Autologous vs allogeneic transplant in myeloma

Miles Prince¹ and Jeff Szer²

1 Peter MacCallum Cancer Institute

2 Royal Melbourne Hospital

Abstract not available at time of printing

CML an overview including current therapies

Moshe Talpaz

MD Anderson Cancer Centre

Abstract not available at time of printing

The treatment of CML with STI571 experience to date

Brian Druker

Oregon Health & Science University

Abstract not available at time of printing

Haemochromatosis: Reconciling the phenotypic, genotypic and molecular features

John K Olynyk

Department of Medicine, University of Western Australia at Fremantle Hospital, Fremantle, Western Australia

Hereditary haemochromatosis is a common genetic disorder in Australia which results predominantly from a C282Y mutation in the HFE gene. One in seven individuals is heterozygous whilst 1 in 190 individuals is homozygous for the C282Y mutation in the Australian population. However the clinical and biochemical expression of iron overload varies greatly. Up to 50% of homozygotes have clinical features of disease whilst 25% develop liver disease. Sixty percent of homozygotes have biochemical evidence of progressive iron overload. There have been significant advances in recognising the evolving phenotype of iron overload syndromes and their associated genotypes over the last 5 years, although there are likely to be further candidate genes identified especially in the non-HFE iron overload syndromes. However, reconciling the mechanisms by which the various iron transport genes and proteins (such as transferrin receptors 1 and 2, DMT1, ferroportin, DcytB, hephaestin and hepcidin) contribute to normal and abnormal iron metabolism is far from complete. Candidate mutations in iron transport genes and abnormal protein functions have been described predominantly at the molecular and isolated cell level. Attempts to confirm these observations at the whole organism level have either proven difficult or not been attempted. Clearly, the key to ironing out the molecular pathogenesis of iron overload disorders will depend on the ability to correlate events at the gene, RNA and protein levels with functional outcomes in the whole organism.

Gauchers Disease in childhood

Ashok Vellodi

Great Ormond Street Hospital for Children

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Immunotherapy in leukaemia and lymphoma

John Gribben

Harvard Medical School

Abstract not available at time of printing

Activated NKT Cells: a new avenue for the treatment of malignancy

Andrew Nicol

Royal Brisbane Hospital

Abstract not available at time of printing

Analysis of the dynamics of T cell and myeloid donor engraftment in the T depleted non-myeloablative setting

DL White*, M Forte, S Heatley, Z Rudzki, P Bardy & TP Hughes
in conjunction with the ALLG

Hanson Centre for Cancer Research, IMVS, Adelaide & ARCBS, Adelaide

Allogeneic transplantation of mobilised peripheral blood cells from HLA-matched donors has gained widespread use in the treatment of haematological malignancies. The recognition of the role of immunocompetent donor T cells in the development of potentially fatal GVHD has led to the more extensive use of T-depleted grafts. Whilst this decreases the incidence of acute GVHD, it increases the risk of graft failure, and relapse. In order to assess donor cell engraftment we have developed an assay to accurately measure engraftment in total cells, T cells (CD3+) and myeloid cells (CD13+). Prior to transplantation an informative loci is identified from donor and recipient DNA by amplifying up to nine loci in a multiplex PCR setting. Post transplant analysis of the informative loci is performed on DNA isolated from ficoll separated, FACS sorted cell populations. PCR products are electrophoresed on an ABI prism DNA sequencer, and analysed using Genescan software. Percent donor chimerism is calculated using an algorithm incorporating the peak area of informative loci. Using this approach we have analysed engraftment kinetics in 16 patients transplanted with a T depleted transplant following non-myeloablative conditioning. In all patients an informative loci was readily identified. PB and/or BM were analysed from all patients at day 28, and at regular intervals post transplant. There was good correlation between total donor chimerism and CD13+ donor chimerism at day 28 (correlation 0.79). In contrast measurement of total donor chimerism did not reflect the level of T cell chimerism (correlation 0.36). Analysis at 6 months indicated that total donor chimerism correlated well with levels of both CD3+ and CD13+ chimerism (correlation 0.75, 0.99 respectively), most likely reflecting significant engraftment at this point. Measurement of either total chimerism, CD3+ or CD13 chimerism at day 28 was not predictive of donor chimerism levels at 6 months, however when high levels (>95%) of both CD3+ and CD13+ chimerism were achieved chimerism remained stable in the follow-up period (Range 6-18months: median 9 months). We have found measuring chimerism using STR-PCR on sorted cells to be an accurate, reliable and reproducible measure of donor chimerism which can be performed equally well on blood or marrow. Particularly in the T-depleted setting it is important to recognise that total cell chimerism does not reflect donor chimerism within the T cell compartment, especially at early timepoints when T cell numbers are low.

Graft Versus Host Disease and Donor Chimerism in Non-Myeloablative Bone Marrow Transplantation Following Conditioning with Fludarabine and Melphalan

David S Ritchie^{1*}, James Morton², Jeff Szer¹, Andrew Roberts¹, Peter Shuttleworth¹, Andrew P Grigg¹

¹ Department of Medical Oncology and Clinical Haematology, Royal Melbourne Hospital, Melbourne

² Bone Marrow Transplant Unit, Royal Brisbane Hospital, Brisbane

Non-myeloablative bone marrow transplantation (NMBMT) is a potential means of exploiting the graft-versus-tumour effect of allogeneic T cells, whilst mitigating the toxicities and severity of graft versus host disease (GVHD) associated with myeloablative conditioning. Comparison the rates of toxicity and GVHD in published series of NMBMT has been limited by the wide variation in conditioning regimens used and the small numbers of patients within each study. Furthermore, the absence of data on the engraftment of donor CD3 lymphocytes has prevented conclusions to be drawn on the rate and clinical consequences of T cell engraftment following NMBMT. The largest study of NMBMT published to date does not fully delineate the frequency and severity of GVHD or the kinetics of lymphoid engraftment. We report the clinical outcome of 34 patients in addition to the kinetics of T cell engraftment following non-myeloablative BMT with fludarabine and melphalan. Patients with either haematologic malignancy (31) or renal cell carcinoma (3) who were not considered appropriate candidates for full dose myeloablative allogeneic BMT, were enrolled. Average age was 53.8 years (range 20-68). One patient received a matched unrelated allograft, the remainder of patients received histocompatible sibling allografts. All transplants were T-cell replete. All patients received fludarabine in combination with melphalan. GVHD prophylaxis was with cyclosporin 6mg/kg and methotrexate (MTX). 31 patients received folinic acid rescue. Neutrophil recovery to above $0.5 \times 10^9/L$ (average=15 days). In 5 patients the neutrophil nadir did not fall below $0.5 \times 10^9/L$. Platelet engraftment to above $20 \times 10^9/L$ (average=16 days). 18/34 patients experienced grade 2 or greater toxicity. 4/34 developed grade IV toxicity (one case each of interstitial pneumonitis and multi-organ failure, and two cases of hepatic veno-occlusive disease). In addition to these acute toxic deaths, a further 5 patients died from grade IV GVHD within 100 days of BMT. Overall 20/31 developed aGVHD and 13/25 developed cGVHD. Chimerism has been analysed in 10 patients thus far, 5 by PCR, and 5 by cytogenetics. All show >95% donor engraftment by 28d. Prolonged DFS has been obtained in the majority of patients surviving beyond 100 days, with 18/25 alive in CR. Particular tumour responsiveness has been identified in patients with low-grade B cell malignancies (CLL or NHL). Our findings reveal that this approach is effective therapy but is associated with rates of GVHD in excess to those anticipated, which may be due to rapid T cell engraftment in heavily pretreated patients.

Low transplant-related mortality utilising a non-myeloablative conditioning regimen for matched and mismatched unrelated donor stem cell transplants

AK Mills*, R Chakraverty, R Chopra, D Milligan, C Craddock, A Parker, A Hunter, S Mackinnon
Haematology Departments, UK Collaborative Group, London, United Kingdom

Unrelated donor stem cell transplantation (SCT) is associated with higher rates of regimen-related toxicity, graft rejection, graft-versus-host disease (GVHD) and impaired immune reconstitution than HLA-identical sibling donor SCT. Despite improvements made by high resolution HLA molecular typing, there is a prohibitive increase in transplant-related mortality in older patients, in patients with a poor performance status and in patients with an HLA-mismatched donor. While a number of non-myeloablative regimens are being studied in an attempt to broaden the applicability of unrelated-donor SCT, many are still associated with severe acute GVHD rates of 30-60% and extensive chronic GVHD rates of up to 80%. We have investigated a non-myeloablative conditioning regimen in 48 patients with haematological malignancy receiving allogeneic progenitor cells from unrelated donors. The median age was 43 years (18-62). Transplants were performed for multiple myeloma (11), high grade NHL (8), low grade NHL (7), CML in 1st chronic phase (5), Hodgkin's disease (5), AML in CR2 (5), secondary AML (1), ALL in CR2 (3), T-PLL (1), plasma cell leukaemia (1) and CMML (1). Thirty patients (62.5%) had received a previous autograft. Twenty-one of the transplants were mismatched for HLA Class I and/or Class II alleles. Conditioning consisted of CAMPATH-1H 20 mg on days -8 to -4, fludarabine 30 mg/m² on days -7 to -3 and melphalan 140 mg/m² on day -2. GVHD prophylaxis was with cyclosporine A alone. Forty-six patients received unmanipulated bone marrow, while the remaining 2 patients received unmanipulated G-CSF-mobilised peripheral blood stem cells. Primary graft failure occurred in 2 of 44 evaluable patients (4.5%). Chimerism studies in 34 patients indicated that the majority (85.3%) attained initial full donor chimerism. Only 3 patients developed grade III-IV acute GVHD and no patients have developed chronic extensive GVHD. The estimated probability of non-relapse mortality at day 100 was 16.7% (95% CI, 6.21-27.2%). With a median follow up of 344 days (range 79-830), overall and progression-free survivals at 1 year were 73.9% (95% CI, 61.2-86.7%) and 60.2% (95% CI, 45.0-75.4%) respectively. All 5 patients with CML are negative for bcr-abl transcripts by RT-PCR. A non-myeloablative regimen incorporating in vivo CAMPATH-1H is effective in promoting durable engraftment and in reducing the risk of severe GVHD following unrelated donor transplantation. The long-term anti-tumour activity of this regimen remains unknown, though strategies utilising prophylactic or pre-emptive use of donor lymphocyte infusions may prolong remissions and improve survival in some types of haematological malignancies.

A non-myeloablative regimen utilising in-vivo CAMPATH-1H for allografting patients with Hodgkin's disease: minimal transplant-related mortality and early evidence of a graft-versus-Hodgkin's disease effect

AK Mills*, PD Kottaridis, R Chopra, DW Milligan, C Craddock, A Hunter, R Pettengell, S Mackinnon
Departments of Haematology, UK Collaborative Group, United Kingdom

The therapeutic options for patients with Hodgkin's disease who fail autologous stem cell transplantation or are not suitable candidates for this mode of therapy are currently limited. High transplant-related mortality of up to 65% has historically limited the role of allogeneic transplantation in Hodgkin's disease. We have designed a protocol for non-myeloablative allogeneic transplantation, aiming to improve survival for poor-risk patients by minimising toxicity while providing anti-tumour efficacy. Twenty-two patients, with a median age of 30 years (22-41) were transplanted. Seventeen patients had a previous failed autograft. At transplant, 4 patients were in CR, 12 in PR and 6 had refractory disease. Conditioning consisted of CAMPATH-1H 20 mg/d on days -8 to -4, fludarabine 30 mg/m² on days -7 to -3 and melphalan 140mg/m² on day -2. Sixteen recipients received unmanipulated G-CSF mobilised peripheral blood stem cells from HLA identical siblings. Six patients received unmanipulated marrow from matched unrelated donors. GVHD prophylaxis was with cyclosporin A alone in all but one sibling recipient, who also received methotrexate. All 22 patients had sustained donor engraftment. Chimerism studies, using microsatellite PCR, show most patients to be full donor chimeras in both myeloid and lymphoid lineages. Four patients developed Grade I acute GVHD and one patient developed Grade II (skin) acute GVHD. Only one of the patients died from toxicity secondary to the transplant procedure. Two patients with refractory disease have progressed and died post transplant. Five additional patients have relapsed 19, 18, 17, 9 and 6 months post transplant. Three of these patients required further chemotherapy to control rapidly progressive disease. All 5 patients subsequently received DLI and 4 achieved and maintain remission. The other patient is too early to evaluate. Two patients have died from complications of GVHD following DLI. The median follow-up is 21 months (range 6-44). The actuarial survival, progression free survival and current (in CR following DLI-chemo) disease free survival are 74%, 50% and 69% respectively. In this very high-risk group of allograft recipients there was low regimen-related toxicity, a high incidence of allogeneic engraftment and an exceptionally low incidence of GVHD. Early responses to DLI suggest the regimen may become an ideal platform for adoptive immunotherapy facilitating a graft-versus-Hodgkin's disease effect in patients who progress post-transplant. The optimal timing and cell dose of the DLI therapy remain uncertain. Longer follow up will reveal the impact of this therapeutic approach on disease control and ultimately cure.

A human blood dendritic cell purification platform for clinical applications

JA Lopez^{1*}, G Bioley¹, CSK Ho¹, CJ Turtle¹, S Vuckovic¹, G Crosbie¹, D Munster¹, S Wright², K Taylor², R Rodwell² and DNJ Hart¹

1 Mater Medical Research Institute, Brisbane

2 Mater Adult Hospital, Brisbane

Objective: To establish a blood dendritic cells (DC) isolation strategy for cancer immunotherapy. DC generated in vitro by transforming monocytes with exogenous cytokines (Mo-DC), are functionally different from blood DC. We describe a platform for blood DC isolation using the mAb CMRF-44 and CMRF-56 with the aims of produce DC that are: 1) in a defined state of differentiation, 2) able to respond to physiological stimuli with the capacity to take up and process antigen, 3) more homogeneous and, 4) not exposed to exogenously added cytokines.

Methods: After overnight culture of PBMC, biotinylated CMRF-44/56 were used to purify DC in a single step magnetic beads (Miltenyi Biotec, Germany) procedure. DC composition was evaluated by FACS in 10L and 15L COBE Spectra PBMC apheresis product obtained from 16 healthy individuals.

Results: Using buffy coats as a source of PBMC, magnetic bead separations yielded up to 99% CMRF-44⁺ cells including up to 67% CMRF-44⁺ CD14⁺ CD19⁺ DC, i.e. a 100 fold enrichment from the 0.5% starting DC population. The yield of CMRF-44⁺ cells varied with individuals (n=53) with a mean of 52% (range 19% 99%). Blood DC (CD14⁺ CD19⁺ CMRF-44⁺) averaged 17% (range 3% 67%). Similar results were obtained using CMRF-56 mAb (n=10); an average of 46% CMRF-56 cells were obtained, of which 17% (range 4% 31%) were blood DC. Overall, the procedure isolated an average of 2.2% (CMRF-44) and 1.3% (CMRF-56) of the starting PBMC, respectively. The capacity of the purified CMRF-44⁺ cell fraction to elicit an allogeneic MLR was increased in comparison to whole cultured PBMC and no allo-stimulatory activity was observed in the CMRF-44 fraction. Moreover, MLR stimulatory capacity was identical to that found with FACS sorted CMRF-44⁺ CD14⁺ CD19⁺ cells. CMRF-56⁺ isolated cells efficiently generated primary immune responses restricted by both class II (KLH) and class I (HLA A2.1 peptides) MHC molecules. We explored the yield of blood DC obtained from apheresis product in 16 healthy volunteers and established that the automated software control (6.0 Auto PBPC) yielded the better results. DC subset and activation status were not altered by the procedure, and DC numbers obtained were greater than 20 million in all cases, representing at least 20 DC immunization doses. CMRF-44⁺ cells were also successfully isolated from apheresis PBMC and showed intact allo-stimulatory capacity before and after freezing.

Conclusions: We describe a clinically applicable procedure for obtaining blood DC in sufficient quantity and quality for cancer immunotherapy.

A CD4⁺ lytic clone derived from the HLA identical sibling of a patient with melanoma recognises Mage-3 in association with HLA-Dr11

DJ Gottlieb^{1*}, I Lionello², S Tanzarella², V Russo², C Traversari^{2,3}

1 Leukaemia Research Laboratory, Westmead Institute of Cancer Research, University of Sydney at Westmead Hospital, Sydney

2 Tumour Gene Therapy Laboratory, Hospital San Raffaele, Milan

3 Genera S.p.A., Milan

Objective of study: to derive anti-tumour clones from the HLA identical sibling of a patient with malignant melanoma undergoing stem cell transplantation

Methodology: A tumour cell line (WMPG) was derived from the biopsy of a metastatic melanoma lesion. A mixed lymphocyte tumour culture (MLTC) was established using irradiated WMPG cells and peripheral blood mononuclear cells from the patient's HLA identical sibling. Cultures were re-stimulated regularly with irradiated tumour and donor-derived PHA-stimulated mononuclear cells and supplemented with IL2. Cloning was performed by limiting dilution. Clones were screened by cytotoxicity against the melanoma line and were further characterised.

Results: WMPG expressed high levels of MHC class I and II. Clone PR3 obtained following limiting dilution of MLTC showed mean cytotoxicity against WMPG of 28% at an effector:target ratio of 50:1 using cold inhibition with an excess of unlabelled K562 cells. Recipient-derived PHA-stimulated blasts were not killed. PR3 typed as CD4⁺ with a minority of cells co-expressing CD8. No inhibition of cytotoxicity was observed using W6/32 (anti HLA class I) or an isotype control antibody. PR3 cells released both interferon gamma and tumour necrosis factor after overnight co-culture with the melanoma line WMPG. Neither PR3 nor WMPG alone released cytokine in overnight culture. PR3 cells appeared to recognise antigen expressed in association with HLA-Dr11 since interferon was secreted in response to a range of Dr11⁺ but not Dr11⁻ melanoma cell lines. WMPG expressed a range of recognised tumour antigens including Mage-3. PR3 did not kill HLA-Dr11⁺ EBV transformed cells, but did kill the same cells transfected with a construct encoding Mage-3. PR3 was monoclonal by T cell receptor gene rearrangement (Vb8). A series of peptides encompassing known Mage-3 antigenic epitopes were pulsed onto HLA-Dr11⁺ EBV transformed cells which were then used as targets in cytotoxicity and cytokine release assays with PR3 effectors. PR3 did not recognise any of the epitopes contained in this set of peptides.

Conclusion: PR3 recognises an as yet unidentified Mage-3 epitope expressed in association with HLA-Dr11. In a broader sense, this study confirms that the graft-versus-tumour (and graft-versus-leukaemia) effect of allogeneic transplantation can be analysed to identify specific tumour antigen components. Use of these antigens in immunotherapy strategies could improve specificity, maintain anti-tumour efficacy and reduce graft-versus-host disease following allogeneic stem cell transplantation.

Generation and expansion of cytomegalovirus-specific cytotoxic T cells by peptide-pulsed dendritic cells

AE Foster*, DJ Gottlieb, KF Bradstock

Leukaemia Research Laboratory, Westmead Institute of Cancer Research, University of Sydney at Westmead Hospital, Sydney

Cytotoxic T lymphocytes (CTL) have been implicated in the control of cytomegalovirus (CMV) reactivation and disease. In allogeneic stem cell transplants (SCT), the absence of CMV-specific CTLs can lead to CMV reactivation and disease, which may be life threatening. Infusion of CMV-specific CTLs shortly after SCT may restore immunity and protect the patient from CMV reactivation and disease.

Methodology: Peripheral blood mononuclear cells were isolated from HLA-A*0201, CMV-seropositive and seronegative donors. Monocyte-derived dendritic cells (DC) were generated by enriching for monocytes by plastic adherence and culturing cells in the presence of GM-CSF, IL-4 and TNF- α . DCs were pulsed with CMVpp65 peptide (495-503, NLVPMVATV) and co-cultured with autologous peripheral blood lymphocytes (PBL). Cultures were restimulated on a weekly basis and supplemented with IL-2 to enhance T cell proliferation. Cultures were examined for the expansion of pp65 peptide-specific CTLs by staining lymphocytes with phycoerythrin labelled HLA-A*0201-restricted CMVpp65 tetramer and anti-CD8-fluorescein isothiocyanate conjugate. Cytotoxicity towards CMV was confirmed by testing CTLs against pp65 peptide-pulsed, irrelevant peptide-pulsed and non-pulsed autologous DCs.

Results: Dendritic cells generated from the patient samples showed typical morphology and phenotype of monocyte-derived DCs (CD1a⁺, CD14⁺, CD80⁺, CD83⁺, CD86⁺, MHC I⁺ and MHC II⁺). A 42-fold expansion (range, 27 72, n=5) of CMV-specific CD8⁺ T cells was observed by HLA-A*0201 CMVpp65 tetramer staining of autologous co-cultures. The purity of CMV-specific CD8⁺ cells was greater than 43% (range, 23 61%) after two DC stimulations. Cultures stimulated with DCs over a 4 week period resulted in a 95-fold (range, 74 117, n=3) expansion of CMV-specific T cells and reached a purity of 76% (range, 59 90%). Furthermore, T cells cultured with CMV peptide-pulsed DC were only cytotoxic against target cells loaded with the pp65 peptide (>70% lysis at an effector : target ratio of 30:1) but not against unloaded (<10% lysis) or against targets bearing an irrelevant peptide (<10% lysis against PR-1, proteinase-3 peptide).

Conclusion: DCs can be successfully generated from monocytes providing potent antigen presenting cells for the selection of antigen-specific T cells. Following repeated stimulations with peptide-loaded DCs, the T cell population generated was shown to be specific for CMVpp65 by both tetramer staining and cytotoxicity assays. The use of DCs for the generation and expansion of CMV-specific CTLs is feasible and may prove useful in the development of adoptive immunotherapy for CMV following SCT.

Flow cytometric evaluation of reconstitution of cytotoxic T lymphocyte response to cytomegalovirus in allogeneic stem cell transplant recipients using HLA-A*0201 tetramers

AE Foster*, DJ Gottlieb, KF Bradstock

Leukaemia Research Laboratory, Westmead Institute for Cancer Research, University of Sydney at Westmead Hospital, Sydney

Cytomegalovirus (CMV) is a common pathogen causing significant mortality and morbidity in immune suppressed patients. Reconstitution of CMV-specific cytotoxic T lymphocytes (CTL) after allogeneic stem cell transplantation (SCT) may play an important role in controlling CMV reactivation and CMV disease. Therefore, HLA-A*0201 HCMVpp65 tetramers were used to examine the reconstitution of cellular immunity towards CMV after allogeneic stem cell transplantation in patients with haematological malignancies.

Methodology: Peripheral blood mononuclear cells were isolated from blood from patients within 90 days after allogeneic SCT. Cells were stained with phycoerythrin labelled HLA-A*0201-restricted HCMVpp65 (495 503) tetramer and anti-CD8-fluorescein conjugated monoclonal antibody. Dual positive lymphocytes were enumerated and analyzed with respect to patient/donor CMV serotypes, CMV reactivation (pp65 antigenemia and PCR detection), ganciclovir (GCV) treatment, and treatment for graft-versus-host disease (GVHD).

Results: Fluorescent HLA-A*0201-restricted HCMVpp65 tetramer successfully detected CMV-specific reconstitution in all allogeneic SCT patients within 90 days (n=14). Numbers of CMV-specific CTLs varied from 0.19% to as high as 25.9% of all CD8⁺ T cells. Differences in patient and donor CMV status played a significant role in CMV-reconstitution. CMV-specific CTL recovery was significantly greater when both patient and donor were seropositive for CMV pre-SCT, as compared to where either the patient or donor were seronegative for CMV (P=0.017).

Furthermore, CMV reactivation significantly influenced CMV-specific reconstitution. Patients who had episodes of CMV reactivation had a higher percentage of CMV-specific CTLs than those who did not have CMV reactivation (P=0.047). Finally, a trend was observed when comparing CMV-specific CTL reconstitution with post-transplant treatment variables. Recovery of CMV-specific CTLs following CMV reactivation decreased sharply after pre-emptive treatment with GCV or corticosteroid treatment for GVHD, alone or in combination. Subsequent periods of reactivation were associated with a concurrent rise in CMV-specific lymphocytes.

Conclusion: CMV-specific tetrameric complexes provide a means to examine the reconstitution of CMV-specific cellular immunity in allogeneic SCT patients. Recovery of immunity towards CMV was enhanced when patients and donors were both CMV seropositive and when an antigenic stimulus was present in the form of CMV reactivation post-transplant. Both GCV and steroids appeared to reduce the magnitude of this response. Further research will be required to determine the minimum number of CMV-specific CTLs that will provide immunological protection against CMV. These results will help evaluate CMV management and CMV-specific adoptive T cell immunotherapy applications in allogeneic SCT patients.

Strategies to Maximize the Rate of Molecular Negativity in Follicular Lymphoma (FL)

James J Biagi, M Wolf, H Januszewicz, J Seymour, T Joyce, HM Prince

Dept of Haematology, Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia

Background: Patients with advanced stage FL have disease characterized by a high clinical response rate to chemotherapy but inevitable relapse, with median survival in the range of 8-10 years. A bcl-2 gene translocation is detectable by PCR in up to 90% of cases. Conventional combination chemotherapy (CC) does not produce a complete molecular remission (CMR) even in the setting of complete clinical response. However, novel treatments that include fludarabine-based combination regimens, and the monoclonal antibody rituximab, have been associated with an increased CMR rate. Indeed, CMR is associated with improved survival outcomes. Similarly, patients infused with autologous stem cells (ASC) that are rendered bcl-2 negative by in vitro purging have better outcomes. Thus aggressive treatment strategies that maximize the CMR rate may improve clinical endpoints and change the treatment paradigm in advanced FL.

Objectives: In this case series, we sought to characterize a group of patients who received aggressive multi-step treatment for FL. The goal of therapy was to obtain a CMR prior to collection of ASC.

Methodology: At our institution newly diagnosed or relapsed patients with advanced stage FL sequentially received chemotherapy and rituximab, or rituximab alone prior to collection of ASC. At each stage of treatment peripheral blood and bone marrow were analyzed by PCR for bcl-2, as was the stem cell collection. PCR was used to detect both MBR and mcr loci to a sensitivity of at least 1×10^{-5} . ASC were to be stored on a just-in-case (JIC) basis where ASC transplantation was not planned.

Results: Eight patients (M=4/F=4) treated from August 2000 were included in this case series; median age at diagnosis was 35 years (range 31-52); the histologic diagnosis was FL in six patients and FL with transformation to diffuse large cell in two patients. Four had relapsed after previous complete response to CC or CC and radiotherapy, including the two with transformed disease; mean time from diagnosis was 37 months (range 23-67) for relapsed patients and 14 months (range 8-27) for previously untreated patients; IPI was 0 or 1 in all patients at both initial presentation and relapse; 5 of 8 patients have had successful ASC collections, with planned collection of the remaining 3. Of 3 patients with available PCR test results who were positive in peripheral blood or bone marrow for bcl-2 before treatment, all 3 reverted to CMR prior to collection of ASC.

Conclusions: The treatment paradigm for advanced stage FL is shifting to a more aggressive approach, the main aim of which is improvement in CMR rates. At our institution this approach has included a relatively young group of patients with favorable disease characteristics, in both previously untreated and relapsed settings. Further studies are required to determine if improved survival outcomes will be realized.

Evaluation of ^{18}F FDG-PET in the staging and response assessment of patients with low-grade non-Hodgkin's lymphoma (LGNHL)

R Blum¹, JF Seymour¹, A Wirth², HM Prince¹, R Hicks³

Division of Haematology and Medical Oncology¹; Division Radiation Oncology²; Dept. of Nuclear Medicine³, Peter MacCallum Cancer Institute, Melbourne.

Background: Staging and response assessment in low-grade non-Hodgkin's Lymphoma are currently heavily reliant on structural imaging. Functional imaging modalities such as Gallium have a limited role in assessment of this disease, with sensitivity ranging from 40-70%. ^{18}F FDG-PET is an alternative functional scan which has shown great promise in staging histologically aggressive NHL and Hodgkin's disease, but its role in low-grade NHL has not been established.

Aim: To retrospectively assess the sensitivity and clinical impact of FDG-PET in patients with low-grade NHL.

Method: The computerized patients record database was searched for patients with LGNHL who also had ^{18}F FDG-PET scanning from May 1997 to January 2001. Individual case records were then reviewed. At conventional staging, all patients had a minimum of history/examination, computerized tomography (CT) scans and bone marrow biopsy. ^{18}F FDG-PET was performed using a dedicated 3-D scanner. Scans were reported without blinding to prior clinical/imaging data. Disease sites were abstracted from clinical notes/scan reports.

Results: 57 patients were identified who had ^{18}F FDG-PET as part of their staging or post-treatment assessment. The records of 20 patients have currently been reviewed and a complete analysis will be presented. Currently, 10/20 pts had gallium scans with 2 having gallium negative disease. 18 pts had follicular centre cell and 2 had marginal zone histology. 12 ^{18}F FDG-PET scans were performed for staging at initial presentation or progression. 8 ^{18}F FDG-PET scans assessed treatment response. 18/20 (90%) pts had documented PET positive disease. The 2 pts with negative FDG-PET revealed a complete response to treatment that concurred with the results of conventional imaging. Both patients have not relapsed with follow-up of 15 and 18 months. On conventional staging studies there were 56 definite and 12 equivocal disease sites. By ^{18}F FDG-PET scanning 75% of the definite and 25% of the equivocal sites were positive. PET identified 21 additional sites not seen on CT, of which only two were evaluable. Both sites were proven on biopsy to be involved by lymphoma. CT detected 23 sites that were PET negative, 5 of these sites were assessable and all found to be truly negative. Therefore 7 of 44 discordant lesions were assessable and in all cases the PET was correct in its determination of the presence or absence of disease. One patient who had PET as part of the primary staging was upstaged from IA to IIIA. PET impacted on the management of 4 of the 12 patients and in all cases altered their radiotherapy fields.

Conclusion: These data suggest that ^{18}F FDG-PET has a high sensitivity for LGNHL, and appears to be superior to meticulous gallium scanning in our institution, as well as previous reports of Gallium scanning. In this series, FDG-PET influenced management in 4 cases. Examination of a larger population may provide a more precise determination of the role of FDG-PET in the assessment and management of LGNHL.

Post-transplant lymphoproliferative disorders (PTLD) after renal transplantation: management and improved outcome after chemotherapy

D Gill^{1*}, HG Juffs, Hertzog¹, AM Brown¹, CM Hawley¹, R Cobcroft¹, JJ Petrie¹, P Marlton¹, G Cull¹, R Rigby¹, D Thomson¹, SB Campbell¹, N Isbel¹, DL Nicol¹, D Norris², DW Johnson¹

*1 Departments of Renal Medicine, Haematology and Surgery, Princess Alexandra Hospital, Brisbane
2 Mater Misericordiae, Brisbane Qld*

Introduction: Post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication of organ transplantation. The data remains scanty and there is no uniform approach to the management of PTLD.

Methods. We performed a retrospective review of patients treated in the oncology/haem unit at our institution over the past 30 years.

Results. Of the 1949 kidney transplants, 27 (1.3%) patients were identified with PTLD. In 1 patient diagnosis was made at autopsy, 1 patient died during diagnostic procedure, 2 patients were treated elsewhere, 1 patient died before referral could be made. Of the 22 were referred for treatment, 13 were males and 9 females with median age 40.5 yrs (range 19-65). Median time to PTLD diagnosis was 30.5 months (range 1.5-279). Seven patients who received OKT3 for rejection had a shorter time to onset of PTLD (20.5 vs 55 months). Immunosuppressive regimens at diagnosis were variable but most patients were receiving treatment with cyclosporine, azathioprine and prednisone and OKT3 reserved for rejection. Histological review revealed PTLD were of B-cell origin in 20 (91%) and of T-cell in 2 (9%). PTLD was polymorphic in 4 (18%), and monomorphic in 18 (82%) of patients, diffuse large B-cell lymphoma 12, 1 case each of MALT lymphoma, follicular lymphoma, plasmacytoma, delta gamma proliferation, anaplastic large cell lymphoma (T-cell), and peripheral T cell lymphoma. In situ hybridisation for EBER-1 (for presence of EBV) was positive in 15 (68%) and negative in 7 (32%) of cases. Most patients had immunosuppression reduced or withdrawn, in immunosuppression reduced (ImR) alone in 1, antiviral therapy plus ImR 4, surgical excision and chemotherapy 4 (including 2 lymphomatous allograft kidneys), excision and chemotherapy plus involved field radiotherapy 3, and chemotherapy alone 9. Chemotherapy consisted of CHOP 14, HyperCVAD 1, and 1 patient had Rituximab as consolidation therapy. Immunosuppression was ceased in all patients receiving chemotherapy and introduced at about half the pre-PTLD dose after chemotherapy was completed. No patients had a rejection of their allograft kidney. Median time to follow up 2.26 years (range 0.7-9.3 yrs). Overall response rate was 73%, with CR rate 63%. Of the 15 patients treated with chemotherapy CR was achieved in 13 (86%). Overall survival was 68% and in those achieving CR 80%. Only 1 patient has relapsed (twice), however 2 other patients have died from other metastatic malignancies.

Conclusions: Patients achieving CR have durable remission with an excellent survival. No patients developed allograft rejection. Only 1 patient has relapsed with PTLD.

A pilot study of outpatient Vinorelbine and Gemcitabine with Filgrastim (VGF) support for the treatment of advanced lymphoma

Andrew Spencer^{1*}, Chris Arthur², Anthony Schwarzer¹

1 BMT Programme, Alfred Hospital, Melbourne

2 Dept of Haematology, Royal North Shore Hospital, Sydney

Both vinorelbine and gemcitabine have single agent activity in patients with advanced lymphoma. Furthermore, recent Phase II studies in breast cancer have suggested that a combination of the two can be administered in an outpatient setting. We have therefore commenced a pilot study of the tolerability and efficacy of vinorelbine and gemcitabine with filgrastim support for patients with advanced lymphoma.

Patients with relapsed or refractory non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD) were eligible. Patients with bone marrow, hepatic or renal failure not secondary to lymphomatous infiltration were not included. Four cycles of therapy at 21 day intervals were planned with response evaluation following cycles 2 and 4. Patients without evidence of response (<25% reduction in tumour bulk) following cycle 2 were withdrawn as non-responders. Each cycle comprised vinorelbine 25 mg/m² and gemcitabine 1000 mg/m² on days 1 and 8 and filgrastim 5 micrograms/kg days 2 to 7 and from day 10 until neutrophil recovery.

Since February 2001 we have accrued 16 of a planned 20 patients. Median age is 52 years (range, 29-74). Diagnoses include FCC B-NHL (n=8), DLC B-NHL (n=2), peripheral T-NHL (n=2) and HD (n=4) with a median of 2.5 prior treatment regimens (range, 1-11). Four patients had undergone prior ASCT and 7 had never achieved a prior CR. At the time of treatment, disease status was primary refractory (n=1), refractory relapse (n=1), first or second relapse (n=9) or third or subsequent relapse (n=5). At present 5 patients have received only 1 cycle of therapy. Of the remaining 11 patients 2 have been withdrawn from study; 1 non-responder and 1 patient with recurrent non-neutropenic atypical infections, both following 2 cycles of therapy. One patient died from legionella pneumoniae infection following cycle 1 despite rapid haemopoietic recovery. Treatment otherwise has been well tolerated with no admissions for febrile neutropenia, minimal GIT toxicity and no alopecia. Of the 10 presently evaluable patients 3 have completed all 4 cycles of therapy and the remainder 2 cycles. Responses have been seen in 7 patients (CR=3, PR=4). The complete responders include 2 patients who had not previously achieved a CR. No patients demonstrated disease progression whilst on treatment.

Whilst preliminary, our findings demonstrate that VGF is a promising therapeutic approach for advanced lymphoma that can be safely administered in an outpatient setting to heavily pretreated patients.

Xenobiotic polymorphisms and susceptibility to non-Hodgkin lymphoma

Ian Kerridge¹, Lisa Lincz¹, Fiona Scorgie¹, Neil Granter¹, Danica Hickey¹, Andrew Spencer^{1,2*}

¹ Hunter Haematology Unit, Mater Hospital, Newcastle, NSW

² BMT Programme, Alfred Hospital, Melbourne, Victoria

The past four decades have seen a significant increase in the incidence of non-Hodgkin's lymphoma (NHL) that may be due to increasing environmental carcinogen exposure, particularly pesticides and solvents. Furthermore, there is increasing evidence for an association between carcinogen exposure-related cancer risk and xenobiotic gene polymorphisms. Based on these factors we have undertaken a case control study of xenobiotic gene polymorphisms in individuals with a diagnosis of NHL. Polymorphisms of 6 xenobiotic genes (CYP1A1, GSTT1, GSTM1, PON1 NAT1, NAT2) were characterised in 169 individuals with NHL and 205 normal controls using PCR based methods. Polymorphic frequencies were compared using Fisher's exact tests. Odds ratios (OR) estimating the relative risk for NHL associated with each genotype were calculated. Subsequently, multivariate logistic analysis was undertaken to confirm which of the polymorphisms showed significant differences between cases and controls.

Amongst the NHL group the incidence of GSTT1 null and PON1 BB genotypes were significantly increased compared with controls, 34% versus 14%, and 24% versus 11%, respectively. Multivariate analysis demonstrated that GSTT1 null conferred a 4-fold increase in NHL risk (OR=4.27; 95% CI, 2.40 7.61, p<.001) and PON1 BB a 2.9-fold increase (OR=2.92; 95% CI, 1.49 5.72, p=.002). Furthermore, GSTT1 null combined with PON1 BB conferred an additional risk of NHL (OR=9.1; 95% CI, 2.71 30.36, p<.001) while the GSTT1/GSTM1 double null genotype conferred a 3 fold risk of NHL (OR=3.34; 95% CI, 2.7 30.3, p<0.001).

Risk Genotype	Odd Ratio	95% CI	p value
GSTT1 Null	4.29	2.41 7.63	<0.001
GSTM1 Null	0.83	0.52 1.32	0.435
NAT 1 Slow	0.68	0.42 1.11	0.124
NAT 2 Slow	1.40	0.86 2.26	0.174
PON1 BB	2.99	1.58 5.63	<0.001
CYP1A Mutant	0.86	0.46 1.60	0.639
GSTT1/GSTM1 Null	3.34	1.7 6.55	<0.001
GSTT1 Null/PON1 BB	9.10	2.7 30.3	<0.001

The two polymorphisms that we have identified, GSTT1 null and PON1 BB, are common genetic traits that pose low individual risk but may be important determinants of overall population NHL-risk, particularly amongst groups exposed to NHL-related carcinogens. Further evaluation of the GSTT1 null and PON1 BB polymorphisms in populations with a history of relevant environmental exposures and NHL, and the role of the organophosphate-metabolising enzyme PON1 in the aetiology of NHL are justified.

Primary Non-Hodgkin's Lymphoma of the Female Breast

G Ryan¹, D Roos², R Fisher¹, JF Seymour^{1*}

¹ Peter MacCallum Cancer Institute, Melbourne

² Royal Adelaide Hospital, Adelaide

Primary non-Hodgkin's lymphoma (NHL) of the female breast constitutes ~0.5% of breast tumours and a similar percentage of cases of NHL. Its natural history, patterns of failure, and optimal treatment have not been defined.

Objectives: To review patterns of failure, prognostic factors and survival data for patients with primary breast NHL.

Methodology: Eligible patients presenting between 1980 and 1998 inclusive were identified via computerized databases at the participating institutions. Patients were included in the analysis only if this was their initial presentation with histologically-proven NHL restricted to the breast (may be bilateral) and regional lymph nodes.

Results: Twenty eligible patients were identified. Median age was 62.5 years (range 24-83). Distribution of tumours were 55% right, 30% left, and 15% bilateral. Stage was IE in 14 cases, IIE in 5, and uncertain in 1. Only one patient had B symptoms, median performance status was 0 (range 0-2) and median IPI score was 1 (0-2). Predominant histology was diffuse large B-cell lymphoma (n=15); other histologies were low-grade (n=3), high-grade (n=1), and undifferentiated (n=1). The LDH was normal in all cases where recorded (n=11). Treatment received was: surgical excision only (n=2); systemic chemotherapy alone (n=5), radiotherapy alone (n=2), combined chemo-radiotherapy (n=11). CHOP chemotherapy was the most frequently used regimen (duration 3-6 cycles). Radiation fields generally incorporated the breast only for Stage I disease, and the breast, axilla and supraclavicular nodes for Stage II disease. IT prophylaxis was not used in any case, nor was prophylactic irradiation to the contralateral breast unless both breasts were initially involved. Median follow-up of surviving patients is 28 months (range 4-165). Thirteen patients have relapsed with median time to recurrence of 13 months. Two patients relapsed in the ipsilateral breast (post-XRT), 4 in the contralateral breast, 5 in the central nervous system (3 leptomeningeal, 2 parenchymal), and 7 patients relapsed systemically (2 only without other sites of recurrence). Ten patients have died of progressive lymphoma. Estimated median survival is 46 months and five-year actuarial survival is 35%.

Conclusions: The prognosis of patients with primary breast NHL in our series is poor and appears worse than that anticipated for patients with low-risk early stage large-cell NHL. The risk of recurrence in the CNS and contralateral breast is high and suggests merit for the prophylactic treatment of both areas. This pilot study forms the basis of an international retrospective study under the auspices of the IELSG.

Fludarabine & Cyclophosphamide; Highly Effective Therapy For Patients With Low-Grade Lymphoproliferative Disorders

B Ma, MM Wolf, EH Januszewicz, HM Prince, D Westerman, JF Seymour*

Department of Haematology, Peter MacCallum Cancer Institute, East Melbourne

Fludarabine (FAMP) has a single-agent response rate of 50-60% in previously treated patients (pts) with CLL or low-grade non-Hodgkin's lymphoma (NHL). The cellular repair of cyclophosphamide (Cyclo)-induced DNA damage is potently inhibited by Famp. Based on this synergy, from 10/96 to 6/01 we have treated 44 pts with relapsed or refractory chronic lymphoproliferative disorders with Famp & Cyclo (15 follicular NHL, 15 CLL, 3 PLL, 8 Waldenström's, 3 mantle-cell). Median prior therapies is 2 (range 0-6); 6 previously untreated, 24 relapsed (median duration last remission 13 Mo.), and 14 refractory. Prior chemotherapy included; alkylating-agents in 35, anthracyclines 20, and Famp 14. Median age is 59 yrs (37-80), 64% male, median International Prognostic Factors Index (IPI) score 2 (0-4), raised LDH in 19%, raised β 2-microglobulin in 77%, PS \geq 2 in 16%. 40 pts had Rai III/IV or Ann-Arbor IV disease. FAMP 25mg/m² & CYCLO 250mg/m² were given IV daily for 3d every 28d with 5-HT₃ antagonists and no corticosteroids. 154 cycles were delivered (median 3/pt; range 1-6) with only 17% delayed >7d, doses reduced in 2.6%, and G-CSF used in 4.5%. 35 of 42 evaluable pts responded (83%; 95% CI 69-93%) with 10 CR (24%) and 25 PR (60% - 2 still on therapy). Response rates were; previously refractory disease 93%, relapsing pts 77%, previously untreated 83%, and prior FAMP treatment 73%. IPI score, no. prior therapies, and raised LDH/ β 2-M were not predictive of response (P \geq 0.4). Pts aged \geq 65 yrs had a lower response rate (64% vs 90%; P=0.06). The median remission duration is 11.5 Mo. (max 26+) The median follow-up of survivors is 23 Mo. (1.5-40), median survival is 32 Mo., and actuarial 3yr survival rate 36-13%. There was one treatment-related death (aspergillus pneumonia). Haematologic toxicity was non-cumulative; Neutropenia \geq Gr. 3 occurred in 29% of evaluable cycles, Gr. 4 in 22% and thrombocytopenia of Gr. \geq 3 in 13%. There were no bleeding complications and no platelet transfusions were used. Infections occurred in 11% of cycles (30% of pts.), including 3 pneumonia (1 fungal), 3 febrile neutropenia and 1 possible hepatic candidiasis, but no cases of PCP (no prophylaxis in 30 pts). Pts with \geq 3 prior therapies had increased infection rates (20% vs 4% of cycles; P = 0.003). There were two cases of transient Gr. 3 hepatotoxicity, and 1 of bladder irritation responsive to mesna. Nausea/vomiting were moderate. Two pts developed Hodgkin's variant Richter's transformation, and one had MDS. Efficacy and toxicity are comparable to a less-heavily pre-treated cohort who received Famp & mitozantrone (90% response, median remission duration of 11.9 Mo., 16% Gr. 4 neutropenia, 10% infection/cycle; P \geq 0.4). In conclusion, The FAMP/CYCLO combination is a moderately myelosuppressive, but well-tolerated and very effective salvage regimen. Despite a high frequency of prior treatment with FAMP and alkylating agents, pts attained a higher response rate than reported for single-agent FAMP.

Outpatient use of Ifosfamide, Carboplatin, and Etoposide (ICE) for Both Salvage and Stem Cell Mobilisation in Relapsed Lymphomas

MS Hertzberg^{1*}, W Benson¹, P Castaldi¹, P Stavros¹, C Crombie², J Taper² and KF Bradstock¹

¹ Department of Haematology, Westmead Hospital, Westmead NSW

² Nepean Cancer Care Centre, Nepean Hospital, NSW

We have treated 19 individuals with relapsed lymphoma using an outpatient-based regimen of Ifosfamide, Carboplatin, and Etoposide (ICE) for both salvage and peripheral blood stem cell (PBSC) mobilisation. Patients included relapsed or refractory diffuse large cell lymphoma (8), Hodgkin's disease (5), follicular lymphoma (4), NK/T cell (1) and mantle cell lymphoma (1). Chemotherapy cycles were administered every 21 days as an outpatient and included: Ifosfamide 5,000 mg/m² iv divided over 3 days, Carboplatin (mg dose=5x AUC) iv on day 1, and Etoposide 100 mg/m² iv daily for 3 days. Subsequently, G-CSF 5 g/kg sc was administered daily while patients usually received two or three cycles of ICE. The median age of patients was 49 years and patients received a mean of 2.3 cycles of ICE. There were no toxic deaths and no significant non-haematological toxicities, while the major haematological toxicity was grade III/IV thrombocytopenia in 53% of patients. The median time to PBSC harvest was 14 days (range=10-20), while the median CD34 cell yield was 5.2x10⁶/kg (range=2.5 to 27.2x10⁶/kg). None of the chemotherapy responders failed to mobilise stem cells, with the vast majority requiring one pheresis only. A total of 11 patients achieved a complete response (58%) and five achieved a partial response (26%). One of the three chemotherapy induction failures responded completely to ICE but relapsed prior to autograft, while the other two patients remained chemorefractory. Fifteen patients proceeded to autologous transplantation while another received a non-myeloablative allogeneic stem cell transplant. Of the transplanted patients all but one are alive in CR or PR at a median of 145 days. Taken together, these features would confirm the efficacy and tolerability of ICE as both a salvage and mobilisation regimen that can be readily delivered as an outpatient.

Use of the signal transduction inhibitor, STI 571 (Glivec), in an expanded access program for treatment of chronic myeloid leukaemia (CML) in chronic phase for patients resistant to or intolerant of alpha interferon. Results of the Australian experience.

Chris Arthur^{1*}, Kerry Taylor², Jeff Szer³, Richard Hermann⁵, Tim Hughes⁴, Keith Fay¹, Naomi Mackinlay¹, Luke Coyle¹, Chris Ward¹, Andrew Grigg³, Mark Bentley², Peter Browett⁶, Kevin Lynch⁷, Patricia Plenge¹, Sonya Walsh⁴, Rosie Hoyt³, April Fitzsimmons², Sonja Downey⁵

1 Royal North Shore Hospital, Sydney

2 Mater Hospital, Brisbane

3 Royal Melbourne Hospital, Melbourne

4 Royal Adelaide Hospital, Adelaide

5 Royal Perth Hospital, Perth

6 Auckland Hospital, Auckland

7 Novartis Pharmaceuticals, Sydney

STI 571 (Glivec[®]) is a specific tyrosine kinase inhibitor of the Bcr-Abl protein present in CML. Inhibition of the Bcr-Abl protein blocks the overactive signal transduction pathways that cause the leukaemia. Results from a recent phase II study indicate that 90 percent of CML patients who are either resistant to, or intolerant of interferon, will achieve haematological remission with STI 571. An expanded access program/clinical trial was initiated for Australia and New Zealand in October 2000. At the end of June 2001, 119 patients have been entered onto this program. Median follow-up is 15 weeks (1-36 weeks). Reasons for entry into the program were: interferon intolerance-62, haematological resistance or refractoriness-15, cytogenetic resistance or refractoriness-42. Patients received STI 571 at 400mg once daily. Treatment has been generally well tolerated and side-effects reflected those seen in the previous phase II studies. A minority of patients had temporary cessation of treatment due to cytopenia but one patient had prolonged severe neutropenia and died from infection. One patient withdrew due to severe fatigue and one withdrew with skin toxicity. Efficacy analysis is currently available on 104 patients with at least 4 weeks follow-up and shows a complete haematological response in 92% at 3 months. Cytogenetic analysis on 56 patients at 3 months shows complete cytogenetic remission in 10/56 (18%) and partial cytogenetic remission (<35% Ph-ve) in another 16/56 (29%). At 6 months the complete cytogenetic response increased to 35% (9 out of 26 patients). Progression of the disease has occurred in 8 patients and dose escalation is currently being attempted. In conclusion these results reflect the experience from other phase II studies and confirm that STI 571 represents a major advance in the management of CML. There is a small proportion of patients however who either respond poorly or progress on this treatment and mechanisms of resistance are currently under investigation. The long-term benefits of STI 571 will require long-term follow-up of this patient cohort.

T(8;22) Myeloproliferative disease a new entity resembling CML characterised by fusion of BCR to FGFR1

A Demiroglu¹, J Steer¹, C Heath¹, K Taylor^{2*}, M Bentley², S Allen³, P Koduru³, G Hawson⁴, R Rodwell², M-L Doody², F Carnicero¹, A Reiter⁵, J Goldman¹, J Melo¹ and N Cross¹

1 Hammersmith Hospital, London

2 Mater Hospital, Brisbane

3 North Shore University Hospital, Manhasset

4 Nambour General Hospital, Nambour

5 Medizinische Universitätsklinik, Mannheim

Two patients presented with clinical and morphologic features of Chronic Myeloid Leukaemia (CML). Cytogenetically they were demonstrated to have an acquired t(8;22)(p11;q11). FISH and RT-PCR analysis in both was BCR-ABL negative, but indicated disruption of the BCR gene. Further studies with FISH revealed a breakpoint within fibroblast growth factor receptor 1 (FGFR1), a receptor tyrosine kinase disrupted in a distinct disorder known as the 8p11 myeloproliferative syndrome, most notably by fusion to ZNF198. RT-PCR was able to confirm the presence of an inframe mRNA fusion between BCR and FGFR1 in both cases. Expression of BCR-FFR1 in the factor-dependent cell line Ba/F3 resulted in IL-3 dependant clones. Growth of these transformed cells was able to be inhibited by the P13 kinase inhibitor LY294002, the Ras farnesyl transferase inhibitors L.744,832 and manumycin A, the p38 inhibitor SB202190, but not the MEK inhibitor PD98059. Growth was not significantly inhibited by treatment with STI571 but was inhibited by SU5402, a compound with known inhibitory activity against FGFR1. Inhibition with SU5402 was associated with decreased phosphorylation of ERK1/2 and BCR-FGFR1 in a dose dependant manner.

This distinct myeloproliferative disease with BCR-FGFR1 fusion may be amenable to treatment with specific FGFR1 inhibitors.

Comparative safety and efficacy of rh-Thrombopoietin-derived autologous cryopreserved platelets to support high-dose chemotherapy and autologous peripheral blood progenitor cell transplantation

Mark Bentley^{1*}, Kerry Taylor¹, Bruce Dawkins², Cathryn Kelly¹, Kim Jardim-Surman³, Silvana Lanzalone³, Adele Morganti³, Debra Taylor¹, Paula Favot¹, Dianne Baldry¹, Anne-Marie Kerwick¹, Katrina Williams¹, Robyn Rodwell¹

¹ Mater Hospitals, South Brisbane, Australia

² Australian Red Cross Blood Service (ARCBS), Brisbane, Australia

³ Pharmacia, Sydney, Australia and Milan, Italy

Autologous platelet cryopreservation is feasible utilising the thrombopoietic stimulatory properties of recombinant human thrombopoietin (rhTPO; Pharmacia). This product can be used safely to support both carboplatin-associated severe thrombocytopenia (Vadhan-Raj et al, 2001) and high-dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT) (Bentley et al, 2000). The ultimate proof of functionality for platelet transfusion support is in vivo survival as reflected in the corrected count increment (CCI) and the prevention of bleeding complications. We have enrolled 27 patients on a Phase I/II study (TPO009; Pharmacia) utilising rhTPO to derive autologous platelets for cryopreservation. Although not designed to provide comparative data, in the absence of randomised controlled trials comparative analysis with historical controls may provide preliminary evidence of the relative efficacy of this approach. Twenty-four patients have undergone platelet collection and 23 HDCT/ASCT. Data from the first 10 patients (9 NHL, 1 myeloma) is compared with 10 historical controls (6 NHL, 3 myeloma, 1 germ cell tumour) randomly selected from transplants performed in the 12 months immediately prior to study commencement. One hour post-transfusion platelet counts were evaluable for 39/45 homologous (87%) and 42/42 (100%) autologous transfusions. Homologous platelet transfusion dose was taken as 2×10^{11} per transfusion, which represents the minimum acceptable dose issued by ARCBS. Median average autologous platelet dose was 2.75×10^{11} (range; 0.7-6.2); 5 patients had received rhTPO 1.2mcg/kg in two doses 4 days apart and 5 patients 2.4mcg/kg in a single intravenous dose.

Parameter; median (range)	rhTPO-platelet support	homologous platelet support
Age	56.5 (50-69)	54 (49-70)
Male/Female	6/4	7/3
CD34 reinfusion dose	5.91 (2.92-11.3)	6.58 (4.86-14.45)
Time to myeloid recovery	10.5 (10-12)	10 (9-18)
Time to platelet recovery	12 (10-17)	13.5 (10-29)
Days platelets <20	3 (2-9)	4 (1-20)
Number of transfusions	3 (2-9)	3.5 (1-12)
Average CCI at 1 hour	14.31 (10.45-24.66)	15.15 (9.1-41)

No toxicity or significant (>grade II) haemorrhagic complications have been observed with rh-TPO-derived autologous cryopreserved platelets. This comparative analysis supports the safety and in vivo functionality of rhTPO-derived cryopreserved platelets and suggests this product is efficacious in the prevention of haemorrhagic complications following stem cell transplantation.

Non-HLA genetic determinants of outcome following allogeneic stem cell transplantation: TNF, IL-10 and Fas and mannose-binding lectin polymorphisms predict graft-versus-host disease and infection

Charles G Mullighan^{1,2*}, Sue Heatley¹, Kathleen Doherty¹, Ferenc Szabo², Andrew Grigg³, Anthony Schwazer⁴, Jeff Szer³, Timothy P. Hughes², L. Bik To² and Peter G. Bardy^{1,2}

1 Research and Development, Australian Red Cross Blood Service, Adelaide

2 Haematology, Institute of Medical and Veterinary Science, Adelaide

3 Medical Oncology and Clinical Haematology, Royal Melbourne Hospital, Melbourne

4 Bone Marrow Transplant Program, The Alfred Hospital, Melbourne

Aim: Complications such as severe acute graft-versus-host disease (aGVHD) and infection remain major barriers to the success of allogeneic haemopoietic stem cell transplantation. There is preliminary evidence that polymorphisms in the genes encoding cytokines and inflammatory mediators influence the risk of aGVHD. The aim of this study was to examine polymorphisms in non-HLA immunoregulatory genes, and identify genetic factors that determine risk of aGVHD and infection.

Method: Thirteen single nucleotide polymorphisms in the genes encoding tumour necrosis factor (TNF), interleukin 10 (IL-10), the apoptosis gene Fas and the complement component mannose-binding lectin (MBL) were genotyped using the polymerase chain reaction and sequence specific primers in 160 HLA-matched sibling donor-recipient pairs in two cohorts from Adelaide (Royal Adelaide Hospital) and Melbourne (Alfred and Royal Melbourne Hospitals). Comprehensive clinical data were collected by case note review. Genotype frequencies were examined in each cohort independently. Associations detected in the Adelaide cohort were deemed significant if confirmed in the Melbourne cohort.

Results: An intronic polymorphism in the TNF gene was strongly associated with both acute and chronic GVHD. All 17 recipients carrying the TNF +488A allele developed aGVHD, compared with 70% of patients without this allele ($p=0.0009$, OR 16.1). Acute GVHD in TNF 488A+ patients was mostly of high grade (77% of TNF 488A+ patients had grade 2-4 aGVHD v. 44% of TNF 488A- patients; $p=0.01$, OR 3.5). All TNF 488A+ recipients also developed chronic GVHD, v. 31% of TNF 488A- individuals ($p=0.002$, OR 24). The Fas 1377G/670G promoter haplotype was also associated with aGVHD risk: 86% of Fas GG+ recipients developed aGVHD v. 63% Fas GG- recipients ($p=0.002$, OR 3.6). Recipient IL-10 promoter polymorphisms were associated with the risk of cGVHD. 80% of recipients carrying the IL-10 -1082A/-819T/-592A haplotype developed cGVHD v. 39% of recipients without this haplotype ($p=0.002$, OR 3.9).

Conclusions: We have identified several associations between non-HLA genetic polymorphisms and acute and chronic GVHD. TNF and IL-10 are key regulators of the inflammatory response, and apoptosis is an important effector mechanism in the pathogenesis of aGVHD. We anticipate several important clinical benefits of this work: (1) the ability to refine donor selection; (2) the potential to identify those at highest risk of GVHD and tailor immunosuppressive therapy, and (3) to offer novel treatments such as anti-cytokine therapy to those predicted to be at high risk by their non-HLA genetic profile. Further work completing genotyping of other immunoregulatory polymorphisms in sibling and matched unrelated donor cohorts is underway.

A Switch in Time: BCLF Represses Foetal γ -globin Gene Expression

Jeremy Turner^{1*}, Melissa Holmes², Robert Czolij¹, Stuart Orkin³, Beng Chong², Merlin Crossley¹

1 Department of Biochemistry, University of Sydney, NSW 2006, Australia

2 Centre for Thrombosis and Vascular Research, University of New South Wales, NSW 2052, Australia

3 Harvard Medical School, Boston, USA

Aim of Study: Promoter-specific repression is believed to play a key role in silencing human γ -globin expression at the onset of adult life. However, the factors mediating this effect are yet to be identified. Basic Krppel-like factor (BCLF) is a transcriptional repressor highly expressed in the erythroid lineage. Our objective was to analyse whether BCLF plays a role in foetal γ -globin gene repression.

Methodology: Globin gene expression was analysed in BCLF knock-out mice carrying a human β -globin locus 230kb YAC (yeast artificial chromosome) transgene. In vitro binding and transrepression assays were used to examine BCLF's direct effect on the γ -globin promoter. Chromatin immunoprecipitation (ChIP) analysis was used to examine BCLF binding at the γ -globin promoter in vivo.

Results: RNase protection analysis demonstrates BCLF knock-out mice are unable to silence foetal γ -globin gene expression. DNase I footprinting and gel shift assays show that BCLF binds at multiple sites in the γ -globin promoter, and transfection studies show BCLF can potently repress GATA-1 mediated activation of the γ -globin promoter. ChIP analysis from the foetal liver of mice shows BCLF is present at the γ -globin promoter in vivo at the time of the foetal to adult globin switch.

Conclusions: These results indicate BCLF plays a role in γ -globin silencing in vivo through direct binding and repression of the γ -globin promoter. Thus, by specifically antagonising BCLF function we may be able to develop novel strategies for therapeutic reactivation of the normally silenced foetal γ -globin genes in some β -thalassaemia patients.

BMT in low grade lymphoma

John Gribben

Harvard Medical School

Abstract not available at time of printing

Translational development of vaccines against B-cell malignancies

Larry W Kwak

Experimental Transplantation and Immunology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

The central hypothesis of active immunotherapy of cancer is that either the tumor cell itself or antigens derived from the tumor cell which are specific, or at least selective, for the tumor cell can be modified and injected back into the patient as a therapeutic vaccine. The desired result is activation of both major arms of the immune response, the host antibody response and potentially a host T-cell response. Recently, in support of cancer vaccine development efforts, a large number of potential tumor antigen candidates have been identified for melanomas and solid tumors. Increasingly, a number of potential tumor antigens have also been identified for hematologic malignancies, including minor histocompatibility antigens, HA-1 and HA-2, proteinase-3, and WT1. However, one of the limitations of the cancer vaccine hypothesis is that this experiment has already failed in nature. That is, by virtue of the tumor's clinical appearance, the host immune system has already failed to recognize this growing tumor. Thus, the primary question facing researchers at this point is whether it is even possible to immunize against an inherently weak, self tumor antigen. Therefore, cancer vaccine development, in general, must be focused on answering two independent questions in the proper sequence. The first question is a scientific one, whether one can even immunize against a tumor antigen. Answering this scientific question is the goal of most phase I and II cancer vaccine clinical trials.

As one example, lymphomas express a tumor-specific antigen which can be targeted by cancer vaccination. The ability of a new idiotype vaccine formulation to elicit T-cell immunity in 20 patients in a homogeneous, chemotherapy-induced first clinical complete remission (CR) was recently studied. Nineteen of the 20 patients tested showed tumor-specific CD8+ T-cell responses using autologous tumor targets as the read-out for these assays. In addition, eleven patients had detectable t(14;18) translocations and were PCR+ in the blood both at diagnosis and after chemotherapy, despite being in CR. However, 8 of 11 patients converted to PCR negative after vaccination. Taken together, these results definitively answer the scientific question of whether one can immunize against this tumor antigen. An NCI-sponsored, multicenter, controlled, randomized Phase III clinical trial with the clinical endpoint of disease-free survival, was opened in January 2000 to provide the definitive answer to the second major question facing the cancer vaccine field; namely, can immunization produce clinical benefit? In addition, the analysis of T-cell responses against autologous tumor targets and vaccine administration in a minimal residual disease setting provide general principles relevant to the design of future clinical trials of cancer vaccines in other tumor types.

CD38 expression in chronic lymphocytic leukaemia predicts for earlier progression and requirement for treatment

Glen A Kennedy*, Devinder Gill, Paula Marlton, Ralph Cobcroft, Karen Grimmett and Gavin Cull

Department of Haematology, Princess Alexandra Hospital, Brisbane

Introduction: Some patients with CLL survive for prolonged periods without requiring definitive therapy, while others die rapidly despite aggressive treatment. The Binet and Rai staging systems provide useful information regarding survival but do not predict the patients with early stage disease who will progress rapidly from those with indolent disease. Recently, two new prognostic markers have been reported which appear to be very accurate in predicting the clinical course of patients with CLL: surface expression of CD38 and somatic mutational status of the IgH variable gene region.

Aim: To correlate expression of CD38 on leukaemic B-cells of CLL patients with clinical parameters and assess for prognostic significance.

Method: Sixty six patients with B-cell CLL attending our institution were analysed by three-colour flow cytometry for surface expression of CD38. CD5+/CD19+/CD38+ cells were expressed as a percentage of total CD5+/CD19+ cells. Patients were considered positive if >30% of cells expressed CD38. Treatment free interval (TFI) was calculated as time from diagnosis to first treatment.

Results: TFI was significantly shorter for CD38-positive patients [10 months (0-93)(n=45)] compared with CD38-negative patients [34 months (0-288)(n=21), p=0.01]. Analysis of only early stage (Binet A) patients confirmed a significantly shorter TFI for CD38-positive patients [18 months (0-93)(n=33)] compared with CD38-negative patients [42 months (0-288)(n=15), p=0.01]. Of 33 early stage patients who were CD38-negative, 1 (3%) required treatment within 12 months and 2 (6%) required treatment within 24 months. Of 15 early stage patients who were CD38-positive, 4 (27%) required treatment within 12 months and 6 (40%) required treatment within 24 months. There was no association between CD38 expression and Binet/Rai stage or LDH. CD38-positive patients were more likely to have a high β_2 -microglobulin (57% vs 32%).

Conclusions: CLL patients who express >30% CD38 on their leukaemic B-cells are more likely to progress early and require treatment. Of particular significance, patients with early stage disease who are CD38-negative are less likely to progress than CD38-positive patients. While this marker clearly has prognostic value, there is considerable clinical overlap between CD38-positive and CD38-negative patients. Therefore, it should be used in conjunction with staging systems and other prognostic markers in the individual patient. Longer follow-up to assess the impact of CD38 expression on survival is required.

Levels of a circulating soluble form of CD86 are elevated in a number of acute myeloid leukaemia patients

BD Hock¹, WN Patton², S Budhia¹, D Mannari² and JL McKenzie¹

1 Haematology Research Group, Christchurch Hospital, Christchurch, NZ

2 Haematology Department, Christchurch Hospital, Christchurch, NZ

Cell surface expression of B7 molecules (CD80, CD86) provides a critical costimulatory signal for T cell activation. Although it is clear that these signals play an important role in the development of anti tumour responses, the progression of a number of haematological malignancies can occur despite constitutive expression of B7 by the malignant population. Recent studies have shown that CD86 expression by malignant cells is in fact associated with a poor prognosis in both acute myeloid leukaemia (AML) and multiple myeloma (MM).

The release of soluble forms of membrane molecules provides a powerful mechanism by which the function of their membrane bound counterparts can be either enhanced or inhibited. Soluble forms of CD86 (sCD86), if generated *in vivo*, would provide a potentially powerful mechanism by which the immune system and/or malignant cells could modulate the costimulatory signals delivered through cell membrane CD86.

We therefore investigated the potential presence of a circulating soluble form of CD86 (sCD86) in normal donors and patients with acute myeloid leukaemia (AML) or B cell chronic lymphocytic leukaemia (B-CLL).

Plasma levels of sCD86 were analysed by ELISA and specificity confirmed by western blotting and immunoabsorption.

Circulating sCD86 was detected in the plasma of all normal individuals (1.10 – 0.31 ng/ml) and patients analysed. Plasma collected from AML patients in remission contained only low levels of sCD86 but significantly elevated levels (≥ 5 ng/ml, $p < 0.0001$) were detected in 6/14 AML patients analysed at the time of presentation or relapse. Significantly elevated levels of sCD86 were also detected in 2/17 B-CLL patients. There was no correlation between sCD86 levels and other clinical parameters. Analysis, during treatment, of three of the AML patients with elevated sCD86 levels at presentation demonstrated that sCD86 steadily declined during chemotherapy, becoming normal by the time of first remission. RT-PCR analysis demonstrated that normal monocytes and dendritic cells, as well as isolated AML and B-CLL cells, expressed an alternatively spliced transcript of CD86 which encoded a soluble form absent from normal T, B and NK cells.

The results of this study suggest that sCD86 may have a *in vivo* role in modulating the costimulatory signals delivered via mCD86. The finding that at least some leukaemia patients contain elevated levels of sCD86 further suggests it may provide a useful marker of disease activity/prognosis and may also have a role in modulating mCD86 signalling during the malignant process .

CA15-3 level as a measure of response, and potential prognostic marker in multiple myeloma (MM)

HM Prince, JJ Biagi, L Mileshkin*, JF Seymour, D Westerman, N Crinis

Departments of Haematology and 2Pathology, Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia

Introduction: MUC1 is a glycosylated transmembrane protein, the function of which remains incompletely understood. A soluble form of MUC1 can be readily measured by serum CA15-3 assay and is elevated in some patients (pts) with adenocarcinomas, correlating with disease burden. Indeed, in selected pts with metastatic breast cancer it provides a useful means of monitoring disease. In this pilot study we examined CA15-3 levels in pts with multiple myeloma (MM).

Materials & Methods: Serum CA15-3 levels were routinely analyzed in MM pts referred to our Institute over the last 1% years, with a Chiron Diagnostics automated mouse monoclonal B27.29 antibody immunoassay. The upper limit of normal is 31 U/ml. Sections of bone marrow trephine from the same patients were stained to determine overexpression of MUC 1 by plasma cells with immunohistochemistry.

Results: 60 pts with MM were tested. 47% had an elevated CA15-3; within this elevated group the mean=50 U/ml, median=43 U/ml (range: 32-158). In a subgroup of pts at our Institute enrolled in an ongoing Phase II trial of thalidomide for relapsed or refractory MM (n=36), CA15-3 was elevated in 52% at study entry. At the point of maximum response, there was significant decrease in CA15-3 in partial responders (PR) (n=9); mean pre-thalidomide=35 (range: 5-80), mean at time of maximum response=23 (range: 5-37) (p=0.039); no such trend was observed in pts with stable disease (SD) (n=20), with pre-thalidomide mean=30 (3-55) compared to 32 (7-80) at the time of best response (p=0.49). There is insufficient data at this time regarding therapy-related changes of CA15-3 in pts with progressive disease. However, of the 15 pts who have progressed on thalidomide (including those with initial PR/SD), 11 pts (73%) had an elevated CA15-3 at study entry.

There was a weak positive correlation between baseline CA15-3 and β 2M (r=0.42, p=0.01) and LDH (r=0.34, p=0.04). There was no correlation with marrow plasmacytosis, CRP, urine/serum M band level or creatinine. Despite the correlation with the known prognostic factors of β 2M and LDH, there was no difference in time-to-disease-progression (TTP) between patients with normal or elevated (defined as greater than the mean) CA15-3 at study entry (p=0.67). However, median follow-up is short at 105 days (range: 4-440 days). Results of the MUC-1 immunohistochemistry will be available at the time of the meeting to determine if there is a correlation with serum levels of CA-153.

Conclusions: In this pilot study, we have demonstrated that:

- 1 serum CA15-3 level is elevated in the majority of patients with active MM;
- 2 CA15-3 levels fall in pts responding to treatment;
- 3 There may be a correlation between CA15-3 and β 2M/LDH which warrants further investigation;
- 4 Although our pt numbers are small, elevated CA15-3 levels may predict disease progression. A prospective study of CA15-3 in newly diagnosed MM patients will be required to better elucidate its prognostic significance.

A simple test to predict outcome in childhood B-lineage acute lymphoblastic leukaemia, by quantifying leukaemia in marrow at day 14 of induction treatment

S Latham, M Brisco,* E Hughes, P Sykes, A Morley

Department of Haematology & Genetic Pathology, Flinders University and Flinders Medical Centre, Adelaide, South Australia

During induction treatment, the levels of leukaemia in marrow indicate the risk of marrow relapse, and we have previously shown that at day 14, patients with leukaemia $>10^2$ are at high risk. However, the quantification techniques used were complicated and difficult, because for every patient, a marker gene had to be identified, sequenced, and a unique clone-specific PCR test designed. We have therefore studied a combination of simpler techniques, to see if they could quantify leukaemia more easily.

We studied 44 children, diagnosed from 12/1991-4/97 in S Australia, treated on ANZCCSG Study VI. All achieved remission, and could be followed either until first relapse, or until 6/2001. First, we used a pair of consensus V and J primers to amplify the rearranged immunoglobulin heavy chain genes in all diagnosis marrows. Monoclonal PCR products were obtained from the leukaemic clone in 33 patients (76%), and in each patient, the PCR products had characteristic sizes. For 27 of those patients, we then amplified the immunoglobulin genes in day 14 marrow (there were no samples for the other 6 patients). Fourteen patients gave polyclonal PCR products, and from previous studies, this implied leukaemia $<10^3$. Thirteen patients gave monoclonal PCR products, the same size as the leukaemic products seen at diagnosis, implying leukaemia $>10^3$. For those 13 patients, leukaemia was then quantified by a simple competitive PCR method, whose results correlate well with results from the other methods ($r^2=0.87$; $n=7$). A known amount of control DNA (from leukaemic cells from a reference case) was added to each patient's DNA sample, immunoglobulin genes were amplified with the consensus primers, and products analysed by Genescan. The reference leukaemic product and the patient's leukaemic product were identified by size, and their relative amounts showed the levels of leukaemia.

Leukaemia At day 14	Relapse, involving marrow	OUTCOME	
		Relapse, extramedullary	Remission to June 2001
10^0-10^1	5		1
10^1-10^2	2	1	3
10^2-10^3		1	
$<10^3$	3	2	9

The simpler methods used here quantify leukaemia at day 14 and indicate relapse risk. 7/12 patients (58%) with leukaemia $>10^2$ relapsed in marrow, compared to 3/15 patients (20%) with leukaemia $<10^2$ ($p<0.05$). The simplicity of these methods may make them clinically useful.

Runx3/Aml2 is required for zebrafish haematopoiesis and neurogenesis

ML Kalev*, JA Horsfield, MV Flores, PS Crosier, KE Crosier

Division of Molecular Medicine, The University of Auckland, Auckland, New Zealand

Members of the runt family of transcription factors play critical roles in both normal developmental processes and disease. In mammals, RUNX1/AML1 is required for definitive haematopoiesis, and chromosomal translocations involving RUNX1 contribute to several human leukaemias. Mammalian RUNX2/AML3 regulates bone development, and mutations resulting in haploinsufficiency of RUNX2 underlie autosomal dominant human disorder of skeleton formation, cleidocranial dysplasia. The function of RUNX3/AML2 has been only partially defined. Expression of RUNX3 in haematopoietic cells has been demonstrated, and involvement in their differentiation suggested. The AML1-ETO and TEL-AML1 fusion proteins have been shown to interfere with RUNX3-dependent transcription, raising the possibility of a role for RUNX3 in leukaemogenesis. In addition, there is evidence that RUNX3 interacts with Smad3 in the TGF- β signaling pathway.

To analyse a potential role for RUNX3 in development, we isolated the zebrafish orthologue and examined its expression and function during embryogenesis. Zebrafish runx3 shares a high degree of amino acid similarity with its mouse and human counterparts. Whole-mount in situ hybridisation revealed runx3 expression in trigeminal ganglia and Rohon-Beard cells (sensory neurons related to dorsal root ganglia) from 14 hours post-fertilisation (hpf). At 24 hpf, expression was present in the intermediate cell mass, a site of haematopoiesis.

Antisense morpholino-modified oligonucleotides (morpholinos) were used to effect targeted inhibition of runx3 translation and generate embryos with loss of Runx3 function. The most prominent abnormality observed in embryos injected with runx3-morpholinos was a reduction in the number of circulating blood cells. Dilatation of ventricular spaces within the central nervous system was also observed. To further investigate defects associated with abrogation of Runx3 function, in situ hybridizations were performed on embryos that had been injected with runx3-morpholinos. A marked reduction in expression of c-myb and pu.1 in these embryos suggests that Runx3 is required for definitive haematopoiesis. The expression pattern of the neuronal marker HNK-1 demonstrated atypical positioning of Rohon-Beard cells.

Together, our results provide the first direct evidence of a role for Runx3 in early haematopoiesis, and indicate a requirement for Runx3 in zebrafish blood and neural development. This raises the possibility that like RUNX1, RUNX3 may contribute to disorders of the blood.

Assessment of the potential of PR1, a proteinase-3 peptide, as a target of immunotherapy in myeloid leukaemia

M Marangolo, A Bartlett, AE Foster, LJ Bendall, KF Bradstock, DJ Gottlieb*

Leukaemia Research Laboratory, Westmead Institute of Cancer Research, University of Sydney at Westmead Hospital, Sydney

Proteinase-3, a myeloid granule protein, can be an autoantigen in Wegeners granulomatosis and may also be over expressed in patients with myeloid leukaemia. PR1, a peptide derived from proteinase-3, appears to be antigenic and may represent an immunotherapy target.

Objective of study: to assess the antigenicity of the nonapeptide VLQELNVTV (PR1) in myeloid leukaemia and the potential of an immunotherapy strategy in this patient group using PR1-pulsed autologous dendritic cells to raise specific anti-PR1 cytotoxic T lymphocytes.

Methodology: The presence of PR1 specific CD8+ lymphocytes in the circulation was assessed using a phycoerythrin labelled HLA-A0201 PR1 specific tetramer together with a fluorescein labelled anti-CD8 antibody. Peripheral blood mononuclear cells from patients with acute myeloid leukaemia in complete remission were cultured with GM-CSF, IL4 and TNF α and analysed by flow cytometry for expression of markers associated with dendritic cell differentiation and for their capacity to stimulate alloreactivity. PR1 pulsed dendritic cells were used to stimulate autologous T lymphocytes and assessed for the generation of cytolytic activity against PR1 by chromium release assay.

Results: The percentage of CD8+ lymphocytes stained by the HLA-A0201 PR1 specific tetramer was as follows:

	HLA-A2- normal	HLA-A2+ normal	HLA-A2+ ALL post allotransplant	HLA-A2+ AML post chemotherapy	HLA-A2+ CML	HLA-A2+ CML post allotransplant
Mean	0.17	0.37	0.25	0.49	1.42	3.71
SD	0.23	0.41	0.32	0.42	0.97	3.53
Range	0 0.33	0 1.07	0 0.68	0.14 1.00	0.11 2.41	0.37 11.86
N =	2	6	4	5	4	20

Dual positive cells were seen as early as 30 days and beyond 1 year after allotransplant for myeloid malignancies. Monocyte derived dendritic cells derived from patients with acute myeloid leukaemia in remission expressed high levels of CD1a, CD80, CD83, CD86, CD11c and CD40 and MHC class I and II. They were able to stimulate proliferation in an allogeneic mixed lymphocyte reaction (3H uptake 46,171+3,666 v. 12,319+4054 cpm for DCs v. PBMCs as stimulators). Dendritic cells derived from normal HLA-A0201 positive peripheral blood monocytes and pulsed with PR1 were able to stimulate autologous peripheral blood lymphocytes to kill PR1 coated targets.

Conclusion: PR1 specific CD8+ cells circulate in high numbers in some HLA-A0201+ patients with chronic myeloid leukaemia especially those post allogeneic transplant. Monocyte derived dendritic cells from patients with myeloid leukaemia are functionally active and may be able to induce a specific autologous T cell anti-PR1 response that could be of therapeutic value in HLA-A0201 patients with myeloid leukaemia.

An inherited mutation of the transcription factor AML1 causes thrombocytopenia with a predisposition for acute myeloid leukaemia

Logan Walker¹, Jane Stevens², Hamish Campbell¹, Rob Corbett², Ruth Spearing², David Heaton², Donald McDonald³, Christine Morris^{1*} and Peter Ganly¹

¹ Department of Pathology, Christchurch School of Medicine, Christchurch

² Haematology Unit, Canterbury Health Laboratories, Christchurch

³ Haematology Unit, Hammersmith Hospital, London

We have treated a woman of 32 years for acute myeloid leukaemia (AML). Four years prior to this diagnosis she had thrombocytopenia and myelodysplasia. Her mother and two sisters also have thrombocytopenia, (platelets 100-150x10⁹/L) as does a nephew. Her father and her only remaining relative, another nephew, have normal blood counts. Our patient is in continuing complete remission 18 months after the diagnosis of AML, but remains severely thrombocytopenic, having had an autologous bone marrow transplant as consolidation therapy.

Other families with a similar phenotype (autosomal dominant disorder characterised by familial thrombocytopenia with a predisposition to AML) have been shown to have germ line mutations in one allele of AML1 (also known as CBFA2 or Runx-1). AML1 is also disrupted by common recurring translocations in both acute myeloid and lymphoblastic leukaemia. AML1 is a member of the runt transcription factor family and is responsible for DNA binding and heterodimerization with CBF β . AML1 plays an important part in regulating haematopoiesis, a complex process through which undifferentiated immature blood stem cells mature giving rise to functional blood cells. Our aim was to ascertain whether this family carried a germ line AML1 mutation.

Exons 3 to 6 of AML1 (which code for the runt domain, where mutations have been previously found in familial AML) were sequenced from our patient with leukaemia. One AML1 allele was wild type whereas the other had a transversion at nucleotide 844 from G \rightarrow C. This mutation introduces a BstN1 restriction site (CCWGG) not found in the wild type sequence, and had the effect of changing the amino acid at residue 107 of the AML1 protein from alanine (GCT) to proline (CCT). The BstN1 restriction digest of DNA from family members showed that only those with thrombocytopenia were heterozygous for this missense mutation.

Alanine at position 107 participates in the heterodimerization of AML1 with CBF β . This interaction is essential for the transcription factor complex to bind DNA. Heterodimerization is likely to be lost by the substitution of a small non-polar alanine with a large cyclic proline, so AML1 would not bind DNA. Thus the AML1 gene is haploinsufficient in this family. In mice, haploinsufficiency of AML1 affects the temporal and spatial generation of haemopoietic stem cells. Haploinsufficiency of AML1 in this family causes thrombocytopenia and an increased likelihood of progression to AML.

Preliminary Results of a Randomised Trial of Amifostine Cytoprotection in Myeloma Patients Undergoing Autologous Stem Cell Transplantation

Andrew Spencer^{1*}, Noemi Horvath², John Gibson³, Miles Prince⁴, Richard Hermann⁵ on behalf of the Australasian BMT Co-operative Study Group

1 BMT Programme, Alfred Hospital, Melbourne

2 Division of Haematology, IMVS, Adelaide

3 Haematology Department, Royal Prince Alfred Hospital, Sydney

4 Department of Haematology, Peter MacCallum Cancer Institute, Melbourne

5 Department of Haematology, Royal Perth Hospital, Perth

Between May 1999 and November 2000 90 patients aged between 31 and 69 years undergoing an initial autologous stem cell transplant (ASCT) for multiple myeloma (MM) were randomised to receive melphalan 200mg/m², with (n=43) (Treatment Group/TG) or without (n=47) (Control Group/CG), prior amifostine (910 mg/m² as a rapid IV infusion). All received filgrastim 5 g/kg daily from day +1 following stem cell reinfusion. Thrice weekly maintenance therapy with Intron-A was planned upon haemopoietic recovery.

The 2 groups were matched for disease, prior treatment and response 63% (TG) and 50% (CG) PR or CR with prior chemotherapy. 89 patients are evaluable for ASCT toxicity and outcome. Minor adverse reactions secondary to the amifostine (hypotension, nausea, vomiting and facial flushing) were seen in 63% of cases. There was a non-significant trend to higher CD34 inoculums within the CG compared with the TG (5.5x10⁶/kg vs 4.6x10⁶/kg, respectively). Median times to neutropenia ($\leq 0.5 \times 10^9$ /litre) (both 5 days), thrombocytopenia ($\leq 20 \times 10^9$ /litre) (8 days vs 7 days, respectively, for the CG and TG) and neutrophil engraftment ($> 0.5 \times 10^9$ /litre) (both 10 days) were not different. Platelets recovered sooner within the CG compared with the TG 11 days vs 14 days and 14 days vs 17 days for platelets of $> 20 \times 10^9$ /litre and $> 50 \times 10^9$ /litre, respectively, but this did not achieve statistical significance. There were no significant differences in transfusion, parenteral nutrition, parenteral analgesic and non-prophylactic anti-microbial requirements. Fewer TG patients experienced grades 3 or 4 mucositis (12% vs 33%, p=.02). At the time of ASCT 7 patients were already in CR and 2 further patients within the control group were inadequately staged pre or post ASCT, therefore 81 patients were evaluable for response to ASCT, 41 within the control group and 40 within the treatment group. Further disease responsiveness (PR or CR) was seen overall in 63% of patients with no differences between the two groups, both 63%. However, more patients within the amifostine group went on to achieve a CR than within the control group, 12 of 40 (30%) vs 5 of 41 (12%), p=.05.

Amifostine significantly reduces mucositis in the setting of high-dose melphalan administration for patients with MM undergoing an initial ASCT. Further follow-up is required to determine if there is any effect on the duration of post-ASCT disease responsiveness and overall survival.

Transplantation of ex vivo expanded mobilised blood CD34+ cells reduces cytopenia following high dose chemotherapy

DN Haylock*, HM Prince, D Wall, L Barber, L Mints-Kotowska, L Teoh, PJ Simmons

Centre for Blood Cell Therapies, Division of Haematology and Medical Oncology, Peter MacCallum Cancer Institute (PMCI), Melbourne, VIC

Background: Neutropenia and thrombocytopenia remain as significant clinical problems following high-dose chemoradiotherapy (HDT). One possible means of abrogating cytopenia is by infusion of ex vivo generated neutrophil and megakaryocytic precursor cells either alone or with unmanipulated mobilised blood progenitor cells. Herein we report that this approach has been successfully used for haemopoietic support following HDT.

Methods: Patients with metastatic breast cancer were entered into this Phase I clinical trial of repetitive cycles of HDT with cyclophosphamide, thiotepa and paclitaxel (CTP). Ex vivo expansion of Isolex 300i selected-CD34⁺ cells was performed by culture in X-VIVO-10 media supplemented with 0.5% human serum albumin (Buminate, Baxter Hyland) and G-CSF (Filgrastim), SCF (Stemgen) and MGDF (provided by AMGEN). Cultures were initiated in 3 litre Stericell culture flasks (1 litre/flask) and incubated for 12 days within the cleanrooms of the GMP facility at the PMCI. At the completion of culture the cell suspension was washed (Haemonetics Haemolite-2) and concentrated to 300ml prior to infusion (d0).

Results: These conditions promoted a 40-fold expansion of total cell numbers and development of both neutrophil and platelet precursor cells, as defined by multiparameter immunophenotyping. Approximately 90% of cells expressed cell surface markers associated with neutrophil maturation (CD15, CD11b), 5% expressed CD34 without lineage markers and the remaining 5% were megakaryocytic, as evidenced by co-expression of CD61 and CD42a. The first patient received 7.8x10⁹ ex vivo generated nucleated cells together with unmanipulated mobilised PB cells to support a second cycle of high dose CTP therapy. The same patient was previously transplanted 35 days earlier with unmanipulated mobilised peripheral blood stem cells (PBSC) to support the first CTP cycle. Infusion of the ex vivo expanded cells resulted in a briefer period of neutropenia ($< 0.5 \times 10^9$ ANC/L; 3d vs 7d) and a faster rate of neutrophil recovery than that observed following transplantation with unmanipulated PBSC. In addition to an increased rate of neutrophil recovery, this patient exhibited a more rapid recovery of platelet count ($> 20 \times 10^9$ /L=9d vs 13d), a faster time to platelet independence (8d vs 12d) and reduction in platelet transfusions post transplant (3 vs 5 transfusions).

Conclusion: This data demonstrates the feasibility, safety and efficacy of transplantation with ex vivo generated haemopoietic cells. Notably, we have demonstrated that such cells enhance platelet recovery following high-dose therapy. The trial continues to accrue.

Telomere Length Predicts Neutrophil Recovery After Autologous Peripheral Blood Stem Cell Transplantation

Lisa F Lincz^{1*}, Fiona E Scorgie¹, Jennette A Sakoff², Kerry A Fagan³, Stephen P Ackland², Arno Enno^{1,4}

1 Hunter Haematology Research Group, Mater Misericordiae Hospital, Newcastle, NSW

2 Medical Oncology Department, Mater Misericordiae Hospital, Newcastle, NSW

3 Cytogenetics Unit, Hunter Area Pathology Service, Newcastle, NSW

4 Hunter Haematology Unit, Hunter Area Pathology Service, Newcastle, NSW

Autologous peripheral blood stem cell (PBSC) rescue after high dose chemotherapy is becoming an increasingly common treatment for haematological malignancies. Most patients recover in 9–11 days, but 5–35% experience delayed engraftment, which can require weeks to months of hospitalisation. This high variation in patient recovery time occurs despite receiving what are believed to be adequate numbers of CD34⁺ progenitor cells to allow for marrow regeneration. This may reflect the ability of the cells to proliferate, a process which is believed to be due in part to the progressive shortening of chromosome ends (telomeres) with each cell division. We have conducted a pilot study to determine if telomere length in PBSCs could be used to predict bone marrow recovery time following transplantation. Southern blotting was used to calculate the average telomere length of cells in apheresis collections from 20 patients (median age 51 years, range 18–64 years) undergoing PBSC transplantation as part of treatment for NHL (n=14), MM (n=3), AML (n=1), Breast Cancer (n=1), or Hodgkin's disease (n=1). All patients were in remission at the time of transplant, received an average of 7.8×10^6 CD34⁺ cells/kg (range 2.2×10^6 – 36.1×10^6), were treated with G-CSF from day 1 following transplant, and suffered no complications or disease progression during recovery. Bone marrow recovery was measured in days to reach PB neutrophil levels of $0.5 \times 10^9/L$, and recovery was considered normal if these levels were achieved in <10 days and slow if recovery took longer. The difference in mean recovery times was statistically different between the normal versus slow groups and all data was normalised for statistical calculations. Forward multiple stepwise regression was performed using neutrophil recovery versus the variables of age, gender, telomere length and number of CD34⁺ cells reinfused. Of these, the combination of telomere length, number of CD34⁺ cells reinfused, and gender were found to be predictive of neutrophil recovery ($R^2=0.43$, $P=0.03$). However, telomere length was the only variable which provided a significant unique contribution to neutrophil recovery ($\beta=0.731$, $P=0.005$). This preliminary data suggests that for patients receiving $>2.2 \times 10^6/kg$ of CD34⁺ cells, neutrophil recovery is dependent on telomere length, with recovery being more efficient in patients receiving infusions of cells with long telomeres.

Enhanced Survival of Human Haematopoietic Cells Following Transduction of CD34⁺ Cells with hTERT.

N Elwood^{1*}, X Jiang², C Chiu², S Lichtsteiner², J Lebkowski² and C Smith¹

1 CGCT, Duke University Medical Center, Durham, NC, USA

2 Geron Corporation, Menlo Park, CA, USA

The proliferative life-span of primary human epithelial cells and fibroblasts can be extended by expression of the catalytic subunit of human telomerase (hTERT). The aim of this study was to investigate whether hTERT transduction of CD34⁺ cells could extend the life-span of primitive human haematopoietic cells. CD34⁺ cells, isolated from human cord blood, were transduced with a Moloney-based retrovirus which expressed hTERT. Control cells were transduced with an analogous vector lacking the hTERT gene. At the time of transduction, 90–10% of cells expressed CD34. Following retroviral transduction, cells were maintained under serum-free conditions in the presence of IL-3 and Flt3 ligand. We hypothesized that expression of hTERT would confer a selective growth advantage to cells so cultures were grown in the absence of drug selection. hTERT activity was initially detected in both Control and hTERT-transduced cells. At 6 weeks post-transduction (PT) hTERT activity was detected in 5 of 7 hTERT-transduced samples tested, but not in Control samples (5 tested). At 8 weeks PT, neither Control nor hTERT-transduced samples displayed hTERT activity (4 of each tested), suggesting down-regulation of retroviral hTERT expression.

Cell proliferation was monitored for up to 1 year. During the first 20 weeks of culture no significant differences in cell number were observed between Control and hTERT-transduced samples; maximum proliferation was achieved by 12 weeks. A gradual decline in cell number was observed for both groups after 16 weeks. Cells in Control cultures died or ceased proliferation at a faster rate than those in the hTERT-transduced cultures. By week 40 PT, 4-fold more cells remained in the hTERT-transduced cultures than Control cultures ($p<0.01$, $n=8$). Since retroviral hTERT may only be expressed for the first two months in-vitro, an early effect of hTERT is implied in extending the life-span of more primitive cells and thus generating increased numbers of mature progeny observed at later time points. Normal differentiation along the myeloid and erythroid lineages was observed in the hTERT-transduced cultures. We did not observe immortalisation of progenitor cells. Expression of the hTERT gene, even if transient, may prove useful for enhancing the availability of more primitive cells for genetic therapies and transplantation and for increasing the numbers of mature progeny during ex-vivo expansion.

Staged Autologous Peripheral Blood Stem Cell Transplantation for Primitive Neuroectodermal Tumours (PNET)

David S Ritchie*, Andrew P Grigg, Andrew Roberts, Mark Rosenthal, Richard Fox, Jeff Szer
Department of Medical Oncology and Clinical Haematology, Royal Melbourne Hospital, Melbourne Australia

Chemotherapy regimens for PNET result in disease-free survival (DFS) of 60%. Adverse prognostic features include soft tissue metastases (DFS of 20 to 30%) and metastases to bone or bone marrow (DFS=0%). Current treatment protocols result in high complete response (CR) or partial response (PR) rates of 69% to 94%. However, these protocols are prolonged and produce significant toxicity. The addition of single consolidation stem cell transplant (SCT) for advanced disease has not shown improvement in overall survival (OS). In order to limit the duration and toxicity of therapy, and improve the outcome in this patient group, we undertook a study of four, staged autologous SCT for PNET in 10 adult patients (age 16-30 years), six Ewings sarcoma (ES), and four primitive rhabdomyosarcoma. All patients with ES proceeded to SCT within 4 weeks of diagnosis, and received no prior chemotherapy other than cyclophosphamide 2g/m² and filgrastim 5 g/kg for the purposes of stem cell mobilisation. For ES four cycles of intensive combination chemotherapy were given, alternating between epirubicin and cyclophosphamide (cycle 1 and 3) and ifosfamide and etoposide (cycle 2 and 4). Patients with rhabdomyosarcoma had all previously received adriamycin-based chemotherapy and had proceeded to SCT either due to primary refractory disease or relapse. For rhabdomyosarcoma alternating cycles of epirubicin and cyclophosphamide (cycle 1 and 3) and carboplatin and etoposide (cycle 2 and 4) were used. Two patients with refractory disease were removed from study after two SCT. All others received four SCT, resulting in a total of 36 procedures. Median follow up is 34 months (range 7-78 months). There was no treatment-related mortality. Only one protocol delay occurred. Non-hematological toxicities included mucositis and sepsis (14 episodes). Neutrophil and platelet recovery was rapid (median 12 days). Three of five patients with ES completing four SCT remain alive and in remission more than four years after diagnosis. Of the four patients treated for rhabdomyosarcoma one is alive and in remission. A further patient relapsed with local disease at 6 months despite absence of disease on assessment at 4 months. The failure to achieve prolonged disease free survival in three of the four patients with rhabdomyosarcoma indicates that SCT may be more appropriately offered as initial therapy. This study demonstrates that staged SCT can be undertaken safely, with minimal treatment-related toxicity, completed without protocol delay and results in prolonged disease-free survival in a proportion of cases.

Sequential High-Dose Chemotherapy (HDC) and Autologous Stem Cell Transplantation (ASCT) for Metastatic Breast Cancer – A Retrospective Analysis of 44 patients

Jeremy Wellwood^{1*}, Kerry Taylor¹, Susan Wright¹, John Bashford², Mark Bentley¹, Katrina Williams¹, Cathrine Kelly¹, Rob Hitchins³, John Macintosh¹, Chris Pyke¹, Paul Eliadis², Michael Slancar³, Andrea Rentoul¹, Diana Moore¹, Donna Ryan¹

¹ Mater Adult Hospital, South Brisbane

² Wesley Medical Centre, Brisbane

³ Medical Oncologist, Gold Coast

The role for HDC with ASCT in metastatic breast cancer remains unclear. We performed a retrospective analysis of 44 patients receiving sequential HDC and ASCT from 1996-1999 for metastatic breast cancer. Growing enthusiasm for this therapy emerged in the context of reported benefits in the first randomised trial published at that time. This is one of the largest reported series in an Australian population.

Referral was from surgical and medical oncology units. Median age at time of transplant was 44 years (range 33-62). Median interval from initial diagnosis to transplant was 30 months. At the time of HDC 13/44 (30%) of the patients had evidence of visceral metastases, 19/44 (43%) had metastases confined to the bones and 12/44 (27%) had involvement of distant nodal groups only. Oestrogen receptor status of original tumour was known for 28 patients with 61% (17 of 28) oestrogen receptor positive. Number of patients treated with anthracyclines or taxanes prior to HDC was 50% and 12% respectively.

Choice of HDC regimen depended on past history and avoided agents previously used. Regimens included i/ carboplatin, cyclophosphamide, thiotepa [CCT (38%)]; ii/ high dose melphalan (28%); iii/ epirubicin and cyclophosphamide (18%); iv/ taxol, carboplatin (14%); and v/ cyclophosphamide, mitozantrone, etoposide (2%). The majority of patients received two or three cycles of HDC (58% and 24% respectively) with a range of one to four. The median stem cell dose infused per transplant was 6.5x10⁶ CD34/kg (range 2.5-28).

43 of 44 patients were evaluable for progression free survival (PFS) and 42 of 44 for overall survival (OS). Median follow-up is 28.5 months (range 4 to 64). Twenty-five deaths have occurred. There was one early death in the first 100 days. 17/42 are alive with 11/43 alive and free of disease progression. Projected 5 year overall survival and progression free survival is 33% and 21% respectively. Median survival is 28.3 months and median progression free survival is 15.6 months.

Median PFS for patients with visceral, bony or distant nodal metastases at the time of transplant is 8.4, 21.3 and 15.6 months respectively. Median OS for these groups is 15.4, 30 and 23.4 months respectively.

These results suggest that HDC with ASCT is feasible in this patient population with outcomes comparing favourably with other similar series. The question of whether this provides greater benefit than standard dose therapy awaits evaluation of mature data from several ongoing phase III randomised trials.

Fluconazole Versus Itraconazole Prophylaxis in Neutropenic Patients

MJ Greenwood¹, AJ Dodds^{1*}, DDF Ma¹, ST Milliken¹, A Concannon¹, P Presgrave¹, S Tomlinson¹, I Nivison-Smith¹, J Harkness²

1 Department of Haematology and Stem Cell Transplantation, St Vincent's Hospital, Sydney

2 Department of Microbiology, St Vincent's Hospital, Sydney

Fungal infections are a major contributor to mortality and morbidity in neutropenic patients. The risk of fungal infection is related to multiple factors, the most important of which include duration and severity of neutropenia, degree of immunosuppression and rates of environmental fungal contamination. Increases in environmental contamination are associated with local construction and demolition work. Fluconazole and Itraconazole are both effective antifungal agents, though both have significant disadvantages. Few studies have directly compared their effectiveness as prophylactic agents.

Aims: to compare the efficacy of oral (po) Fluconazole and Itraconazole in the prevention of fungal infection in neutropenic Haematology/stem cell transplant (SCT) patients. Rates of fungal infection were correlated with numbers of airborne *Aspergillus* colonies as assessed by MAS 100 air sampling during a period of hospital demolition and construction.

Methods: a prospective comparison study with historical controls was used to assess rates of proven and suspected invasive fungal infection and mucosal infection between two consecutive cohorts. The prospective group was those treated with Itraconazole (200mg po bd) between 5/99-5/00 and the controls were those treated with Fluconazole (200mg po daily) between 5/98-5/99. Patients with previously documented invasive fungal infection were excluded. Infection rates were then correlated with the results of routine air sampling performed from 4/99-5/00.

Results: 165 patients representing 225 neutropenic episodes were examined. Rates of proven fungal infection in the Fluconazole and Itraconazole cohorts were 9% (10/80) and 4% (5/85) respectively ($p=0.07$). Environmental contamination was minimal with no airborne *Aspergillus* detected in 71% (22/31) samplings. While there was no overall difference in the rates of proven and suspected invasive fungal infection or mortality between the two groups, a planned subgroup analysis revealed a lower rate of proven fungal infection in allogeneic SCT patients ($p=0.05$) and reduced mucosal fungal infection ($p=0.05$) in those receiving Itraconazole. Median Itraconazole levels were lower (120 g/L) in those patients developing fungal infection than in those that did not (419 /L). No correlation could be found between rates of airborne fungal contamination and invasive fungal infection.

Conclusions: Fluconazole and Itraconazole are both effective in preventing fungal infection in neutropenic patients, though Itraconazole may be more effective in allogeneic SCT and in the prevention of mucosal infection.

In childhood B-lineage acute lymphoblastic leukaemia, the relapse clone is often present at diagnosis

N Paech^{1,3}, M Brisco^{1*}, P Sykes¹, S Neoh¹, G Marshall², A Morley¹

1 Department of Haematology & Genetic Pathology, Flinders University and Medical Centre, Adelaide, South Australia

2 Division of Haematology & Oncology, Children's Cancer Institute, Sydney Children's Hospital, Sydney, New South Wales

3 Current address: CSIRO Division of Plant Industry, Adelaide, South Australia

In this disease, relapse happens when some leukaemic cells become resistant to drugs, perhaps through acquiring mutations in genes which control drug metabolism or cell growth. We have previously shown that in many cases, a small population of resistant cells can be detected just 3-5 weeks after diagnosis. However, it is not clear whether resistant cells are already present at diagnosis, or arise during treatment, perhaps as a side-effect of mutagenic drugs. To investigate this further, we identified patients whose leukaemia, at relapse, showed a new DNA marker (a unique rearranged immunoglobulin heavy chain (IgH) gene). Using this marker, we then examined whether the relapse clone was present at diagnosis, and at what levels.

We first performed polymerase chain reaction (PCR) with consensus V and J primers, to compare rearranged IgH genes found in leukaemic cells at diagnosis, and at relapse. Six patients were identified who had a new leukaemic clone at relapse, as marked by the presence of an IgH gene which was absent at diagnosis. The gene was sequenced, PCR primers were designed to its CDR3 region, and a PCR test specific for the relapse clone was developed. To confirm that the test worked, we tested the relapse marrow, and in all six cases detected the target clone. We then tested marrow taken at diagnosis. In four of the six patients, the relapse clone was detected, at levels from 5×10^{-1} to 2×10^{-3} (1 cell in 2, to 1 in 630). In another two patients, the relapse clone was not detected, and from the size of sample studied, its levels were $< 6 \times 10^{-5}$ and $< 3 \times 10^{-6}$. In two patients, the relapse clone seemed large enough to have been detected by the consensus primers, but these amplify some genes better than others, and this selectivity presumably explained why other genes were seen instead. Thus in 4/6 patients, the clone that causes relapse was already present at diagnosis, and mutations causing resistance must have occurred before diagnosis. In the other two patients, the data suggest the clone arose later, but do not rule out the possibility that the relapse clone was present at diagnosis, but as an extremely small population.

Durable Molecular Remissions Following Treatment Of Relapsed Acute Promyelocytic Leukaemia with Arsenic Trioxide and All Trans Retinoic Acid

HJB Goodman^{1*}, P Harper², SJ Palmer¹, AR Varcoe¹, TE Hawkins¹, NJ Van der Water^{1,3}, PJ Browett^{1,3}

¹ Department of Haematology, Auckland Hospital, Auckland, New Zealand

² Haematology Department, Palmerston North Hospital

³ Division of Molecular Medicine, University of Auckland

Several investigators have shown that arsenic trioxide (As₂O₃) is effective at inducing haematological remissions in patients with relapsed or refractory APML, although molecular remission rates vary substantially. Reports from cell line and murine models of APML suggest that ATRA and As₂O₃ act synergistically to eradicate leukaemic cells that express the PML-RAR α fusion protein. We have previously reported the early efficacy and safety of combination therapy with As₂O₃ and ATRA, and now highlight the durability of molecular remissions in six patients with relapsed APML.

Five of the six patients relapsed following initial induction therapy with ATRA and idarubicin, and one was in second relapse 12 months following an autologous peripheral blood stem cell transplant. The median age was 29 (20-69), and median duration of CR1 was 9 months (6-17). All patients had the standard t(15;17) translocation at diagnosis, with additional changes seen in 2 patients at relapse.

The reinduction protocol consisted of 2-4 cycles of As₂O₃ 10mg/day IV and ATRA 45mg/m²/day PO (maximum 80mg) for 28 days. Six patients received a total of 18 cycles of As₂O₃/ATRA (table 1), including concurrent chemotherapy for 1 cycle in each of 4 patients (2 for hyperleucocytosis, 1 for persistent cytogenetic changes after the first cycle, and 1 for extramedullary disease). Treatment was well tolerated, with admission to hospital necessary in 6/18 cycles only. Transient elevations in liver transaminases were seen in 5 cycles (median toxicity Miller grade 2), neutropenia in 10 cycles (median grade 3), and infective episodes in 6 cycles (median grade 2).

After the first cycle of As₂O₃ and ATRA, all 6 patients attained morphologic remission, 5 were in cytogenetic remission, and 4 in molecular remission with the bone marrow negative for the PML-RAR α transcript by RT-PCR. After 2 cycles, all patients had morphologic, cytogenetic and molecular remission.

One patient had no further therapy after As₂O₃/ATRA, two remain on oral maintenance (per the APL93 trial), including one following consolidation chemotherapy, and 2 received autologous stem cell transplants (table 1). One patient has died after a further relapse, and five remain in molecular remission. The median molecular CR is 16 months (range 5-19).

Table 1. Exposure to arsenic/ATRA, subsequent therapy, and outcome

Case	Total cycles of As ₂ O ₃ /ATRA	Cycles to molecular CR	Subsequent Therapy	Current Status	Duration of remission
1	3	1	maintenance	well	19mo
2	4	2	nil (patient choice)	well	17mo
3	3	1	maintenance 1mo (poorly tolerated)	relapsed, died	5mo
4	2	2	2 cycles of CT, then ASCT	well	14mo
5	3	1	1 cycle of CT, then ASCT	well	15mo
6	2	1	1 cycle of CT, then maintenance	well	18mo

CT (intravenous) chemotherapy; maintenance see text; ASCT autologous stem cell transplantation; CR complete remission

The combination of As₂O₃ and ATRA has been effective, well tolerated, and encouragingly durable in this group of 6 patients. The issues of optimal consolidation and continuing therapy require further study.

A new non-random unbalanced 17;20 translocation in myeloid malignancies

Lynda J Campbell*, Cris Patsouris, Kathleen C Rayeroux and Patricia M Michael

Victorian Cancer Cytogenetics Service, St. Vincent's Hospital Melbourne

We present four cases of a novel 17;20 translocation in patients with myeloid malignancies resulting in partial deletions of the short arm of chromosome 17 (17p) and the long arm of chromosome 20 (20q). Deletions of both 17p and 20q are well-described abnormalities in myeloid disorders. Deletion of 17p has been observed in approximately 4% of acute myeloid leukaemias (AML) and myelodysplastic syndromes (MDS) and has been strongly associated with deletion and mutation of p53. Deletion of 20q is usually interstitial whilst 17p deletion may be caused by an interstitial deletion or an unbalanced translocation, most commonly with chromosomes 5 or 7 as translocation partners.

In the last 2% years, 3/723 (0.4%) new cases of MDS and 1/374 (0.3%) AMLs karyotyped by the Victorian Cancer Cytogenetics Service were found to have a dic(17;20). The four patients were all male with a median age of 67.5 years (range: 64-87). The MDS cases had hypodiploid karyotypes and the patient with AML had two cell lines, one containing only a deletion of 20q and the second with the unbalanced 17;20 translocation, suggesting the del(20q) may have occurred first. The patient with AML failed to respond to combination chemotherapy and died one month after diagnosis. Three of the patients had de novo disease but one patient appeared to have developed therapy-related MDS 12 years after successful therapy for AML.

Translocations involving 20q are rare in myeloid disorders. A balanced t(11;20)(p15;q11) involving a NUP98-TOP1 fusion has been described in therapy-related MDS but only one other unbalanced 17;20 translocation has been reported to date, in a 66 year old male with AML who achieved CR but relapsed 5 months after diagnosis with resistant disease. Hence, this report identifies a rare but recurrent abnormality in MDS and AML with an elderly male predominance, involving deletions of regions of 17p and 20q known or suspected of harbouring tumour suppressor genes of importance in the development of haematological malignancies.

Internal tandem duplication (ITD) mutations of FLT3 in acute promyelocytic leukaemia (APL).

Juliet Ayling^{2*}, Harry Iland^{1,2}, Chong Li² and Albert Catalano²

1 Australasian Leukaemia and Lymphoma Group (ALLG)

2 Kanematsu Laboratories, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

FLT3, a type III receptor tyrosine kinase, plays a fundamental role in the proliferation and differentiation of hematopoietic cells. FLT3 is highly expressed in most cases of AML of all sub-types, and may play an important role in leukemogenesis. Recently discovered internal tandem duplications of the FLT3 gene result in elongation of the receptor's juxtamembrane (JM) domain and constitutively activate its tyrosine kinase activity in a ligand-independent manner. ITDs have been described in ~20% of all AML subtypes, and are associated with a significantly worse overall survival, independent of cytogenetic risk group. In APL, FLT3 ITDs are associated with hyperleukocytosis, but not necessarily with adverse outcome.

Aim: To determine the incidence and prognostic significance of internal tandem duplications of the FLT-3 gene in APL.

Methodology: We have examined FLT3 transcripts in 40 APL patients treated in the ALLG's APML3 trial. After reverse transcription and a single round of PCR, fluorescent-labelled FLT3 products were visualized in high resolution denaturing acrylamide gels on an ABI 373A DNA sequencer with Genescan 672 software.

Results and Conclusions: PCR products, 456bp in length, representing wild type FLT3 transcripts were amplified from all samples at diagnosis. Sixteen patients (40%) had additional in-frame products ranging from 471-558bp. Their relationship to FLT3 was demonstrated by DraI digestion, which cleaved the predicted 100bp from both wild type and abnormal PCR products. Sequence analysis in selected patients confirmed the presence of ITD involving exon 11, -intron 11, -exon 12 (encompassing the JM domain of FLT3). Two patients appeared to have multiple FLT3 ITDs at diagnosis. Splice variants, which have not been reported previously, were also identified, but would not be expected to elongate the JM domain.

There was no correlation between FLT3 ITD and age, white cell count or platelet count at diagnosis. However, the presence of FLT3 ITD correlated strongly with PML intron 3 breakpoints (bcr-3) as summarised in the table, suggesting differential interactions exist between FLT3 or its downstream effectors and various isoforms of PML-RAR α fusion proteins.

p<0.0001	bcr-1	bcr-2	bcr-3	Total
Wild type FLT3 only	18	4	2	24 (60%)
FLT3 ITD	3	1	12	16 (40%)

Peptide Analogues Derived From Polyphemus II Inhibit SDF-1 Binding To CXCR4 And SDF-1 Driven Responses In Acute Lymphoblastic Leukemia Cells

J Juarez¹, KF Bradstock², DJ Gottlieb³ and LJ Bendall^{1*}

1 Westmead Millennium Institute, University of Sydney, Sydney

2 Dept. Hematology, Westmead Hospital, Sydney

3 Dept Medicine, University of Sydney at Westmead Hospital, Sydney

Background: Although acute lymphoblastic leukemia (ALL) can often be successfully treated, a significant proportion of patients (approximately 25% of children and 65% of adults) fail current treatment protocols and die of leukemia. New approaches to the treatment of ALL are necessary. Recently SDF-1 has emerged as a key regulator of the behaviour of normal and leukaemic pre-B cells both in vitro and in vivo. CXCR4, the G protein coupled receptor for SDF-1, also acts as a co-receptor for the human immunodeficiency virus (HIV) and SDF-1 and HIV directly compete for binding to CXCR4. Blockade of SDF-1 mediated signalling significantly impairs the growth and survival of pre-B ALL cells in vitro. In addition we have demonstrated that SDF-1 stimulates the function of integrins involved in pre-B ALL cell adhesion to bone marrow stromal components, and that SDF-1 and CXCR4 are important for the transmigration of human leukaemic pre-B cells through bone marrow stromal layers. Most importantly, downregulation of CXCR4 expression results in a 70% inhibition of the homing of human leukaemic pre-B cells to the bone marrow and a 50% inhibition of engraftment using a NOD/SCID mouse model of human leukemia.

Methods: We examined a number of peptide based inhibitors of SDF-1 or HIV binding to CXCR4 in ligand binding, migration and chemotaxis assays. These peptides are derived from SDF-1 itself or peptide analogues derived from the horseshoe crab self-defence peptide polyphemus II.

Results: We found two peptides, T-140 and T-134, derived from polyphemus II, to be potent inhibitors of the binding of the CXCR4 antibody 12G5 with IC50s of 2.0 nM and 2.5 nM respectively. The ability of these peptides to block SDF-1 mediated chemotaxis in pre-B ALL cells was demonstrated using double chamber chemotaxis assay. These peptides completely blocked the chemotactic response of pre-B ALL cells to SDF-1 at concentrations as low as 0.1 μ M. At 1 μ M they were as effective as 12G5 antibody at inhibiting the migration of NALM6 cells through human stromal layers.

Conclusions: Considering the effect of CXCR4 downregulation on the engraftment of pre-B ALL in animal models it is likely that these peptides or related inhibitors of SDF-1 binding to CXCR4 be useful in studying the biology of ALL and should be explored further for in vivo effects in a murine model.

The immunophenotype of the microgranular variant of acute promyelocytic leukaemia: frequent expression of CD34 and HLA-DR antigens in comparison to classical (hypergranular) forms

Glen A Kennedy¹, Jennifer L Curnow^{1*}, Peter Wood¹, John Rowell¹, Gavin Cull², Devinder Gill², Paula Marlton², Ralph Cobcroft², Karen Grimmett², Bronwyn Williams¹

¹ Department of Haematology, Royal Brisbane Hospital, Brisbane

² Department of Haematology, Princess Alexandra Hospital, Brisbane

Introduction: Recent reports have suggested that the classic immunophenotype (CD13/CD33 positive; CD34/HLA-DR negative) associated with acute promyelocytic leukaemia (APML) is infrequently expressed in the microgranular variant (M3v).

Aim: To determine whether M3v is associated with an aberrant phenotype with respect to classical (hypergranular) APML.

Methods: All APML cases with adequate morphological, flow cytometric, cytogenetic and / or molecular data seen at our institutions were retrospectively reviewed. Diagnosis of M3v was made if the predominate blast population consisted of agranular or hypogranular blasts with bilobed or reniform nuclei. Antigen expression was considered positive if at least 20% of the defined cell population expressed the antigen of interest.

Results: Overall 6 cases of M3v and 31 cases of classical APML were identified (see Table). All but one case had cytogenetic and/or molecular evidence of a t(15;17) or variant RAR α translocation. Median age was similar between the 2 groups (43 vs 38 years, p=0.4). CD34 and HLA-DR expression were significantly associated with M3v morphology vs classical APML (5/6 and 3/6 cases vs 2/30 and 1/31, p<0.01 and p<0.01 respectively). Co-expression of both CD34 and HLA-DR was also significantly associated with M3v morphology (3/6 vs 0/30 cases, p<0.01). Conversely, although expression of CD15 was more common in classical APML, this did not reach significance (7/23 vs 0/6 cases, p=0.31). There were no other differences in expression of other myeloid or lymphoid antigens (CD11b, CD14, CD41, CD42b, CD61, GPA, CD71, CD56, CD2, CD7, CD10) between the 2 groups.

Morphology	No	CD13	CD33	CD34	HLA-DR	CD15
Classic APML	31	30/31	28/31	2/30	1/31	7/23
M3v	6	5/6	6/6	5/6	3/6	0/6

Conclusion: Our data confirms that M3v frequently expresses CD34 and HLA-DR, uncommonly displaying the typical immunophenotype associated with classical (hypergranular) APML. As such, immunophenotype alone should not be relied upon for differentiation of M3v from other acute myeloid leukaemias.

Optimal therapy of CLL

Michael Keating

Abstract not available at time of printing

Significance of MRD in B cell malignancies

John Gribben

Harvard Medical School

Abstract not available at time of printing

Bone Marrow Transplantation for CLL

Michael Keating

Abstract not available at time of printing

Bone marrow transplantation for lysosomal storage disorders

Ashok Vellodi

Great Ormond Street Hospital for Children

Abstract not available at time of printing

New agents in malignant haematology

William Plunkett

Abstract not available at time of printing

Radioimmunotherapy of haematological malignancy

J Harvey Turner

Department of Nuclear Medicine and the University Department of Medicine, Fremantle Hospital, Western Australia

Radiolabelled monoclonal antibodies offer a new dimension to targeted treatment of haematological malignancies.

In this review of work-in-progress, the principles of radiopharmaceutical therapy will be outlined and the radiobiological effects on tumours compared and contrasted with those of conventional external beam radiotherapy. In addition, the relative merits of high linear energy transfer (LET) alpha particle radiation will be compared with those of low LET beta radiotherapy.

Radioimmunotherapy of non-Hodgkins lymphoma has been performed using murine (^{131}I -tositumomab Bexxar[®], ^{90}Y -ibritumomab Zevalin[®]) and chimeric (^{131}I -rituximab) anti CD-20 and human (^{90}Y -epratuzumab) anti CD-22 monoclonal antibodies. The results of these clinical trials of radiolabeled antibodies will be compared with those of immunotherapy of NHL with rituximab (Mabthera[®]).

The indications for use of radioimmunotherapy using iodine-131 and yttrium-90 radiolabelled anti CD-20 monoclonal antibodies alone as first line treatment, or in combination with chemotherapy, or as treatment for relapsed or refractory NHL will be evaluated by reference to ongoing clinical trials.

The relative merits of myeloablative regimens with autologous stem cell rescue will be compared with those of non-myeloablative radioimmunotherapy in both aggressive, refractory high grade and relapsed low grade non-Hodgkins lymphoma. The response and toxicity of US and European trials of radioimmunotherapy of low/intermediate grade NHL will be detailed. Preliminary results of the current Australian physician-sponsored Phase II clinical trial of ^{131}I -rituximab in relapsed/refractory NHL will be reported.

The Origins of the Hematopoietic and Endothelial Lineages

Gordon Keller¹, Marion Kennedy¹ and Jim Palis²

1 Carl C. Ican Institute for Gene Therapy and Molecular Medicine, Mount Sinai School of Medicine, New York, NY

2 Dept. of Pediatrics and Cancer Center, University of Rochester, Rochester, New York

The hematopoietic and endothelial lineages are thought to arise from a common precursor, a cell known as the hemangioblast. To search for the hemangioblast, we have utilized a model system based on the in vitro differentiation of embryonic stem (ES) cells. When removed from conditions that maintain them in an undifferentiated state, ES cells differentiate and form colonies known as embryoid bodies (EBs) which contain precursors from many lineages including those of the hematopoietic and vascular systems. Using this model, we have identified a precursor with characteristics of the hemangioblast within EBs, at a stage prior to the onset of primitive hematopoiesis and vasculogenesis. These precursors generate blast cell colonies with primitive and definitive hematopoietic and endothelial potential when cultured in the presence of vascular endothelial growth factor (VEGF) in methylcellulose cultures. To determine whether or not similar precursors are also present in vivo, we analyzed embryos ranging in developmental stage from mid-primitive streak to late neural plate (E7.0-E8.0), preceding the establishment of the yolk sac blood islands. Precursors able to generate blast colonies similar to those identified in the EBs were detected in late primitive streak and neural plate stage embryos. Analyses of these colonies demonstrated that they contained primitive and definitive hematopoietic precursors as well as precursors that generated adherent cells. Preliminary studies have shown that these adherent cells express PECAM-1, suggesting that they are of the endothelial lineage. Taken together, these findings suggest that precursors with hemangioblast potential do exist in both the EBs and the early embryo, and that this population represents the earliest stage of hematopoietic and endothelial development.

Cell adhesion molecules as regulators of haemopoietic cell homing and development

Paul Simmons

Peter MacCallum Cancer Institute

Abstract not available at time of printing

Rac2, a haemopoietic-specific small GTPase, controls mature and primitive blood cell movement

Andrew W Roberts^{1*}, Ben A Croker¹, Fengchun Yang², Simon Atkinson², David A Williams²

1 The Walter and Eliza Hall Institute of Medical Research, Melbourne

2 Howard Hughes Medical Institute and Indiana University School of Medicine, Indianapolis

The Rac family of small GTPases are intracellular molecular switches that critically regulate the rearrangement of the actin cytoskeleton as well as a broad range of cellular responses to extracellular signals, such as transcriptional activation, proliferation, and apoptosis. One member of this family, Rac2, is only expressed by haemopoietic cells. However, like all mammalian cells, haemopoietic cells also express the closely related protein Rac1, suggesting that Rac2 is required for the specialised functions unique to blood cells. To investigate this hypothesis, we created a mutant mouse absolutely deficient in Rac2, and have studied the consequences of this deficiency for mature and immature blood cells. As expected, all haemopoietic lineages investigated have shown abnormalities in actin cytoskeleton rearrangement. Neutrophils, mast cells, T and B lymphocytes demonstrate severe defects in chemotaxis along chemoattractant gradients and diminished L-selectin-mediated rolling on endothelial ligands *in vitro*, and more subtle defects *in vivo*. Actin polymerisation in response to chemoattractants is deficient in these mature cells. Unexpectedly, lack of Rac2 renders haemopoietic stem cells hypermotile and hyperresponsive to chemoattractants such as SDF-1 α *in vitro*. Further, stem cells display defective adhesion via VLA-4 to fibronectin in the absence of Rac2. *In vivo*, this results in an increased number of circulating stem cells in the blood and augmented mobilisation in response to G-CSF. The primary phenotype of Rac2-deficiency is a functional neutrophil immunodeficiency. Such a deficiency has now been identified in several paediatric patients with previously unclassifiable disorders. Most recently, we have identified important abnormalities in B lymphocyte generation and function that extend the breadth of immunodeficiency due to the absence of normal cytoskeletal rearrangements regulated by Rac2.

Use of the signal transduction inhibitor, STI 571 (Glivec[®]), in an expanded access program for treatment of chronic myeloid leukaemia (CML) in blastic phase and Ph+ ALL. Results of the Australian experience.

Chris Arthur¹, Kerry Taylor², Jeff Szer^{3*}, Richard Hermann⁵, Tim Hughes⁴, Keith Fay¹, Naomi Mackinlay¹, Luke Coyle¹, Andrew Grigg³, Mark Bentley², Peter Browett⁶, Kevin Lynch⁷, Clare Inglis¹, Sonya Walsh⁴, Rosie Hoyt³, April Fitzsimmons², Sonja Downey⁵

1 Royal North Shore Hospital, Sydney

2 Mater Hospital, Brisbane

3 Royal Melbourne Hospital, Melbourne

4 Royal Adelaide Hospital, Adelaide

5 Royal Perth Hospital, Perth

6 Auckland Hospital, Auckland

7 Novartis Pharmaceuticals, Sydney

STI 571 (Glivec[®]) is a specific tyrosine kinase inhibitor of the Bcr-Abl protein present in CML.

Glivec is highly effective in the chronic phase of CML and surprisingly at least 50-60% of patients in the blastic phase also respond but generally the response is short lived. An expanded access program/clinical trial of STI 571 for CML in blastic phase was initiated for Australia and New Zealand in October 2000. This report also includes patients with Ph+ve ALL who were treated on an identical schedule. At the end of June 2001, 43 patients have been entered. Reasons for entry were myeloid blast crisis in 28, lymphoid blast crisis in 6 and Ph+ve ALL in 9. Patients received 600 mg of STI571 once daily. Treatment has been generally well tolerated but there was one withdrawal due to pericarditis/myocarditis. Nausea, fluid retention, skin rashes and cytopenia occurred and were similar to results from other phase II studies. Analysis is currently available on 41 patients with at least 4 weeks follow-up. Most patients had initial improvement in blast counts or leukocytosis but at 5 weeks only 15/41 (37%) had a normal white cell and platelet count. Cytogenetic analysis at 3 months showed complete cytogenetic remission in 4/16 (25%) and at 6 months 2/9 (22%). The duration of response is up to 9 months+. Progression of the disease is common and 16 patients have had disease progression with death in 7 patients and another 6 have proceeded to transplantation. One patient with Ph+ve ALL was primarily refractory to three months of intensive chemotherapy but is in complete remission after six months of STI 571 with no detectable Bcr-Abl transcripts. In conclusion these results reflect the experience from other phase II studies. Although good partial and sometimes complete responses are seen in the majority of blastic phase patients, the responses tend to be short lived. Occasional patients however show dramatic responses and at least some durability in the short to medium term. Future studies plan to combine STI 571 with conventional chemotherapy.

Use of the signal transduction inhibitor, STI 571 (Glivec), in an expanded access program for treatment of chronic myeloid leukaemia (CML) in accelerated phase or second chronic phase. Results of the Australian experience

Chris Arthur¹, Tim Hughes⁴, Jeff Szer³, Kerry Taylor^{2*}, Richard Hermann⁵, Keith Fay¹, Naomi Mackinlay¹, Luke Coyle¹, Andrew Grigg³, Peter Browett⁶, Sue Wright², Kevin Lynch⁷, Kim Sheather¹, Rosie Hoyt³, Jenny Bourne², Sonja Downey⁵, Sonya Walsh⁴

¹ Royal North Shore Hospital, Sydney

² Mater Hospital, Brisbane

³ Royal Melbourne Hospital, Melbourne

⁴ Royal Adelaide Hospital, Adelaide

⁵ Royal Perth Hospital, Perth

⁶ Auckland Hospital, Auckland

⁷ Novartis Pharmaceuticals, Sydney

STI 571 (Glivec[®]) is a specific tyrosine kinase inhibitor of the Bcr-Abl protein present in CML. Inhibition of the Bcr-Abl protein blocks the overactive signal transduction pathways that cause the leukaemia. Results from a recent phase II study indicate that 90 percent of CML patients in accelerated phase will achieve partial or complete haematological remission with STI 571. An expanded access program/clinical trial was initiated for Australia and New Zealand in October 2000 for patients in accelerated phase of CML. This protocol also included Ph+ ALL but is not included in this report. At the end of June 2001, 84 patients have been entered. Patients received STI571 at 600 mg once daily. Follow-up data of at least 4 weeks is currently available on 73 patients. Treatment has been generally well tolerated and side-effects including cytopenia, fluid retention, nausea and rashes, reflected those seen in the previous phase II studies. Two patients withdrew due to adverse reactions, a rash in one patient and fatigue and nausea in another. At 3 months 34/39 (87%) patients showed improvement with resolution of leukocytosis ($WCC < 10 \times 10^9/L$). Cytogenetic analysis at 3 months shows complete cytogenetic remission in 13/45 (29%) and partial cytogenetic remission ($< 35\%$ Ph+ve) in another 8/45 (18%). By six months 9/21 (43%) evaluable patients had achieved complete cytogenetic remission. In four patients Bcr-Abl has become undetectable by PCR analysis. Progression has occurred in 5 patients between weeks 10 to 31 on study with death from disease in 1 patient. In conclusion, these results reflect the experience from other phase II studies and confirm that STI 571 is a major advance in the management of advanced CML. Although most patients have good early responses, by six months a small proportion have lost their response and longer follow-up is required to assess the durability of the remaining responses.

Response Profiles of Transformed Chronic Myeloid Leukaemia Cells to Arsenic Trioxide and STI 571

Dennis Carney* and Frank Firkin

Departments of Medicine & Clinical Haematology, St Vincent's Hospital, Fitzroy

Objective: To compare response profiles of K562 cells to arsenic trioxide (ATO) and STI 571 (STI), as a model for evaluation of mechanisms of toxicity, and potential for single and combined agent therapy in transformed Ph+ disease.

Methodology: Studies were performed on exponentially growing K562 cells. Maturation marker expression, cell cycle status, apoptosis, and loss of viability were assessed by flow cytometry.

Results: ATO produced concentration-dependent inhibition of growth of K562 cells, commencing at levels as low as 0.05 M. ATO also produced a sequence of concentration-dependent changes in cell cycle, commencing with accumulation in S phase at 0.5 M, followed by accumulation in G2/M phase at 2.5 M. Apoptosis became a major feature only at 5 M. Increased expression of surface glycoprotein A and CD 33 became prominent at the therapeutically attainable level of 1 M, and decreased at higher ATO concentrations. Evaluation of kinetics of response to ATO indicated S phase accumulation and increased maturation marker expression commenced within 24 hours, preceding significant effects on growth which were evident 48 hours later.

This response profile was compared to that of STI and thioguanine (TG), which were selected because of their activity in CML, known mechanism of action, and potential for use in combination with ATO in the clinical setting. TG also produced concentration-dependent growth inhibition, with a similar sequence of cell cycle changes, but no increased expression of maturation markers. The response to STI differed to the other two agents. Increased apoptosis was the major effect associated with increasing growth inhibition. Expression of CD 33 decreased, and glycoprotein A increased. The proposal that the mechanism of ATO action involves bcr-abl degradation is not supported by these findings, as the response profile differed from that of STI, which specifically inhibits bcr-abl.

Isobologram analysis of growth inhibitory responses to combinations of ATO and STI indicated at least additive activity, with the biological response parameters in cells exposed to both drugs predominantly resembling those induced by STI.

Conclusions: ATO produces a sequence of toxic effects in K562 cells which serve as a model of transformed CML. ATO has significant activity at therapeutically attainable levels, which does not appear to reflect impairment of bcr-abl function, and complements the growth inhibitory effect of STI. The findings suggest this drug combination has the potential to produce greater therapeutic activity in transformed, and possibly de novo Ph+ disease at sustainable concentrations than either drug alone.

BCR-ABL mRNA levels in peripheral blood may be predictive of response to STI571 therapy in chronic myeloid leukaemia patients when measured at three months

S Branford*, S Moore, Z Rudzki, TP Hughes

*Division of Molecular Pathology, Institute of Medical and Veterinary Science, Adelaide
Division of Haematology, Institute of Medical and Veterinary Science, Adelaide*

We have monitored treatment response by the measurement of BCR-ABL mRNA levels in blood in 21 chronic myeloid leukaemia (CML) patients in either chronic phase (n=16) or acute phase (n=5) receiving STI571 therapy for up to 11 months. Our objective was to determine if BCR-ABL levels at 3 months of therapy were predictive of treatment response and to measure minimal residual disease in patients who achieve a complete cytogenetic remission (CCR). Reverse transcription real-time quantitative PCR (QPCR) with TaqMan fluorescent hybridisation probes was used to determine BCR-ABL quantity. Normal BCR mRNA was quantitated to control for RNA degradation and for differences in the efficiency of the reverse transcription step. The results were reported as a percentage of BCR-ABL/BCR. The correlation between BCR-ABL mRNA levels in blood and the percentage of the Philadelphia chromosome in bone marrow in patients on STI571 ($r_s=0.93$, $n=44$) was similar to our previously published correlation with patients on interferon- α ($r_s=0.94$, $n=67$). After 3 months of therapy the QPCR values in 14/21 patients were <10%. These 14 patients achieved a CCR by 6 months of therapy at which time the QPCR values were <1.0%. The QPCR values continued to decrease in all but three of the 14 patients and two achieved PCR negativity. The QPCR values in 2/14 have reached a plateau at <1.0% while there was an increase in the third patient indicating relapse (acute phase patient). None of the 7 patients who had QPCR values above 10% at 3 months have achieved a CCR. Three have disease progression, one patient whose QPCR value increased at 3 months is off trial, one received an allograft and two are continuing with STI571 therapy. A subset of six patients was monitored by QPCR on a monthly basis. Four achieved a CCR by 6 months. The amount of decrease in the QPCR values in the first 2 months of therapy was not predictive of response. However, the amount of decrease from month 2 to month 3 was predictive of disease response. The four patients who achieved a CCR by 6 months had >3-fold reduction in QPCR values from month 2 to 3. In contrast, the remaining two patients had no reduction and one developed disease progression. We conclude that early monitoring by QPCR in blood may be predictive of response to STI571 therapy in CML.

STI571 is effective therapy for CML in relapse after allogeneic BMT

HLG Phan^{1*}, EA Gillett², GR Hill¹, S Wright¹, K Lynch³, K Taylor¹

1 Department of Haematology, Mater Adult Hospital, South Brisbane, Brisbane

2 Department of Haematology, Royal Brisbane Hospital, Herston, Brisbane

3 Novartis Pharmaceuticals

A 23-year-old male presented with low risk (Hasford score=58.4, Sokal index=0.51) Philadelphia positive, chronic phase (CP) CML. He was initially treated with hydroxyurea but evolved into blast crisis six months later and returned to a second CP following induction with MIDAC. He underwent unrelated BMT and developed steroid-responsive grade 2 acute GVHD post-transplant. Six months later, he developed cytogenetic relapse in the setting of an acute back pain syndrome. All immunosuppression was stopped. His back pain and cytopenias worsened and he remained transfusion-dependent (bone marrow was consistent with acute myelofibrosis and cytogenetics confirmed further clonal evolution). Given the setting of recent aGVHD in the unrelated BMT setting, DLI was felt to carry a high risk of inducing severe GVHD.

He was therefore enrolled on the STI571 expanded access trial for CML in accelerated phase and commenced therapy at 600 mg/day. Shortly thereafter, he developed liver function test (LFT) abnormalities in association with a new palmar rash consistent with GVHD. Therapy was ceased. Liver biopsy confirmed GVHD without evidence of drug-induced hepatitis and LFTs responded promptly to steroids. However, occasional aggregates of blasts were seen on liver biopsy and STI571 was restarted at 600 mg/day. His bone marrow after three months of STI571 confirmed a reduction in fibrosis and female karyotype in 100% of metaphases. A CT scan for investigation of deteriorating LFTs revealed hepatosplenic lesions. Chloromas were again documented on liver biopsy without evidence of significant GVHD. At this time he developed severe consumptive thrombocytopenia without evidence of hypersplenism, which failed to respond to Intragam. STI571 was restarted at an escalated dose of 800 mg/day. Within one month, his cytopenias improved and he has remained platelet independent. Repeat bone marrow after six months of therapy revealed marrow hypoplasia with minor fibrosis and no morphological evidence of leukaemia (karyotype remained 100% female). The hepatosplenic chloromas remain stable at the time of writing.

This case report illustrates that STI571 is effective therapy in CML patients who relapse following allogeneic BMT. GVHD does not appear to contraindicate the use of STI571 suggesting that it may be the treatment of choice for patients in whom active GVHD contraindicates DLI. In addition, there is clearly a dose-response curve for STI571 in the setting of advance disease and care must be taken to correctly diagnose the cause of cytopenias and disturbed liver function, so that STI571 dose may be appropriately adjusted.

A Polymorphic Mutation in the Cytolytic P2X₇ Receptor may predispose to Chronic Lymphocytic Leukaemia

James S Wiley*, Ben J Gu, Weiyi Zhang, Ronald Sluyter, Anne N Shemon, Simon Liang and Phuong Dao-Ung

University of Sydney Department of Medicine, Nepean Hospital, Penrith, NSW

Chronic lymphocytic leukaemia (CLL) is unique in showing a strong inherited predisposition to the disease. Since the first report of CLL in identical twin brothers by Dameshek in 1929, epidemiological studies have shown that the familial incidence of CLL is nearly three times higher than expected for the general population. The basis of this genetic predisposition to CLL is uncertain but probably involves the failure of apoptotic mechanisms which normally prevent an accumulation of B-lymphocytes in the body. Recently, the P2X₇ receptor has emerged as a death receptor expressed on normal and CLL-lymphocytes and whose activation by extracellular ATP leads to immediate uptake of ethidium and subsequent apoptotic death of the target cell. We have shown that P2X₇ has poor or absent function in some subjects and to study a possible genetic basis we sequenced DNA in the coding regions of the P2X₇ gene. In 11 of 54 normal subjects a heterozygous nucleotide substitution (1513A→C) was found, whereas 1 subject carried the homozygous substitution that codes for glutamic acid to alanine at amino acid position 496. Lymphocytes from the Glu496→Ala homozygote subject expressed non-functional receptor, whereas heterozygotes showed P2X₇ function that was half that of germline P2X₇ when receptor function was measured by the ATP induced uptake of ethidium at 37 °C. 14 of 34 B-CLL patients were heterozygous and one was homozygous for this polymorphic mutation (1513A→C) showing that B-CLL has a two fold greater prevalence of this polymorphic mutation (44% versus 22%; P<0.01 on Fishers exact test). Thus the Glu496→Ala polymorphic mutation represents a loss of function mutation in a lymphocyte death receptor. The results further suggest that familial B-CLL may have its genetic basis in a polymorphic mutation of the cytolytic P2X₇ receptor.

Nephrotic syndrome complicating advanced myeloma and high-dose aminobisphosphonate therapy

Mark Bentley*¹, Peter Hollett², Kerry Taylor¹, John Burke¹, Simon Fleming³, Susan Wright¹, Simon Durrant³, Ian Irving³

Mater Hospitals, South Brisbane, Australia

Princess Alexandra Hospital, Woolloongabba, Australia

Royal Brisbane Hospital, Herston, Australia

Aminobisphosphonates are almost universally incorporated into treatment regimens for myeloma during chemotherapeutic induction and maintenance in plateau phase or remission. Pamidronate has been demonstrated to significantly reduce skeletal complications of the disease and there is a trend towards improved overall survival within the first two years of therapy. Pamidronate has also been postulated to have a direct anti-myeloma effect which has led to attempts to salvage relapsing myeloma with higher doses or more frequent administration of the drug, with some early reports of benefit. We report three cases of relapsed plasma cell dyscrasias in whom high-dose pamidronate was used to regain disease control. All three patients developed acute renal failure characterised by non-selective proteinuria and histologic evidence of acute tubular necrosis and focal segmental glomerulosclerosis. The first patient, a 38yo male with relapsed myeloma 15 months following a second matched sibling allogeneic transplant, received pamidronate 180mg fortnightly and developed nephrotic syndrome after 5 months. The second case, a 56yo female with multiple plasmacytomata, relapsed 40 months following matched sibling allogeneic transplantation, received pamidronate 90mg fortnightly and developed nephrotic syndrome after 4 months. The third case, a 61yo male, relapsed 28 months following autologous transplantation and developed nephrotic syndrome after 4 months of pamidronate 180mg fortnightly. Renal biopsy was performed in each case and similar histologic features of acute tubular necrosis and focal segmental glomerulosclerosis were present in the tissues examined. All patients received concomitant thalidomide at variable dose and time interval to the development of renal failure. Two patients remain alive requiring dialysis and one patient succumbed to progressive renal failure after refusing further therapy. High-dose aminobisphosphonate therapy in advanced myeloma may be complicated by acute renal failure presenting with non-selective proteinuria and characterised by progressive irreversible glomerulosclerosis.

In vitro analysis of growth inhibition and induction of apoptosis by the bisphosphonate zoledronic acid, and possible synergy with other therapeutic agents in human myeloma cell lines

Cindy Baulch-Brown* and Andrew Spencer

Bone Marrow Transplant Programme, Alfred Hospital, Melbourne

Multiple myeloma (MM) is associated with a high incidence of osteolytic bone destruction caused by a marked increase in osteoclastic activity. Bisphosphonates (BPs) interfere with osteoclast recruitment, differentiation and action, and induce apoptotic cell death of these cells. Clinical studies have suggested that in addition to preventing osteoclast-mediated osteolytic bone disease, BPs may induce a reduction of the tumour burden and prolong the survival of MM patients.

We have investigated the effect of the BP zoledronic acid on human MM cell lines LP-1, OPM-2, U266, NCI-H929 and RPMI-8226 in vitro using flow cytometry and cell proliferation assays. After 4 days culture in the presence of 500 M zoledronic acid, the viability of MM cells was determined by staining with propidium iodide and AnnexinV FITC. Viability ranged from 42 to 85%, with OPM-2 being most sensitive and LP-1 least sensitive to the effect of zoledronic acid. The effect of culturing human MM cell lines with 500 M zoledronic acid (Z) in combination with 10 M dexamethasone (D) and/or 0.4 M tamoxifen (T) was assessed using a tetrazolium reduction assay to measure cell proliferation. Cells were also analysed by flow cytometry after staining with Apo2.7 PE or propidium iodide and AnnexinV FITC. Comparing results obtained by the three techniques, synergistic effects were more apparent when values for the MST assays were examined for the three cell lines studied.

Table 1: Percentage reduction in living cells compared to untreated cells, as determined by MST cell proliferation assay.

	Z	D	T	ZT	ZD	TD	ZTD
LP-1	16	30	9	36	37	18	36
U266	26	17	20	29	43	10	42
OPM-2	26	13	16	40	36	26	34

In summary, zoledronic acid effectively induces cell death in human MM cell lines. A synergistic effect is observed when zoledronic acid is combined with dexamethasone which is commonly used to treat MM. Furthermore, a synergistic effect is also observed when zoledronic acid and tamoxifen are used to treat cells. We are currently screening conventional therapeutic agents, and novel agents such as tumour necrosis factor related apoptosis inducing ligand (TRAIL), in combination with zoledronic acid for possible synergistic activity. Our data provides a rationale for the treatment of MM with zoledronic acid in combination other presently available therapeutic agents.

A Combination Treatment with Thalidomide(Thal) and Interferon- α -2b (IFN) for patients with multiple myeloma

LM Mileskin^{1*}, HM Prince¹, JJ Biagi¹, D Westerman¹, A Grigg², P Mitchell³, P Simmons¹

¹ Depts of Haematology and Medical Oncology; Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia

² Royal Melbourne Hospital, Melbourne, Victoria, Australia

³ Austin Repatriation Medical Centre, Melbourne, Victoria, Australia

Introduction: Both IFN and Thal are effective in the management of multiple myeloma (MM). The mechanisms of action of IFN in MM is unknown but may in part be through its anti-angiogenic effect. In Dec 1999, we initiated an ongoing prospective Phase II open-label, multi-centre (n=6), study attempting to combine Thal/IFN in patients(pts) with relapsed and/or refractory MM. The objectives were to determine response rate (RR), EFS and OS, as well as the toxicity profile of Thal/IFN combination.

Methods: Patients are initially commenced on Thal alone at a dose of 200 mg/d. After 14 days the dose is escalated by 200 mg/d every 14d to a maximum of 800 mg/d. At week 12, patients continue on their individual maximum tolerated dose of Thal(iMTD). IFN is commenced at 3 MU sc tiw provided that: ANC \geq 1.5x10⁹/l; platelets \geq 75x10⁹/l; bilirubin <2.0mg/dl and AST/ALT/ALP <3X ULN. IFN is reduced to 1.5 MU tiw if hemopoietic parameters fall below the above values or side effects (SE) develop. The combination continues while tolerated or until progressive disease (PD). All patients on study have 3 monthly bone marrow biopsies and blood specimens of platelet-poor plasma collected for correlative laboratory studies. Bone marrow trephine sections are stained with CD31, CD34 and vWF by immunohistochemistry in order to examine the prognostic significance of microvessel density in the sections. Plasma levels of IL-6, VEGF, bFGF and HGF are measured using an ELISA technique.

Results: The study has recently completed accrual. A planned interim analysis was performed in April 2001. At that time 38/62 pts (13 females, 25 males) were evaluable for Thal/IFN toxicity (minimum of 12 weeks prior Thal therapy) with a median age of 62 yrs (range: 42-83) and a median follow-up of 12 months (3-17). Median WHO performance status was 1 with a median number of prior chemotherapy regimens of 3 (range 1-7). At time of study entry, 8 pts had an elevated serum creatinine (range: 0.13-0.82 mmol/l), Chr. del 13 (n=5), elevated B2M (n=29), ANC <1.5 (n=10), platelets <75 (n=12), prior allograft (n=4), prior autograft (n=16). The iMTD of Thal has been 100mg (4pts), 200mg (6pts), 400mg (8pts), 600mg (6 pts) and 800mg (12pts). Utilizing SWOG response criteria RR were as follows: CR=1/38 (3%), PR=10/38 (26%), SD=22/38 (58%), PD=2/38 (5%), NE=3/38 (8%). The median time on study is 5mo with an estimated 47% still on study at 6 mo (95% CI: 24%-55%) The median event-free survival is 6 months. The median overall survival has not been reached. Of the 38 pts, 13 discontinued Thal before 12w (PD=6; neuropathy=3; constipation and/or somnolence=3; progressive renal failure=1). Of 15/39 pts who continued on Thal but did not start IFN, the reasons included: persistent neutropenia or thrombocytopenia (n=8), PD (n=3), fatigue (n=3) and ongoing GVHD (n=1). 7/38 commenced the combination with Thal (iMTD; 400-800mg/d) and IFN (1.5MU 3MU). Median time on IFN/Thal = 22w (2-55). SE from IFN were limited to neutropenia in 5 pts (2 pts grade 2, 2 pts grade 3, 1 pt grade 4), grade 1 nausea in 2 patients and fatigue in 2 pts. 2 pts discontinued IFN/Thal due to progressive disease. One pt discontinued interferon due to persistent grade

4 neutropenia. No pt had febrile neutropenia. Of note, 4/38 patients were entered on study following extramedullary relapse after allogeneic transplantation. All 4 pts responded to thal and 2 received additional IFN with no exacerbation or GVHD. Preliminary results of the correlative laboratory studies will shortly be available for presentation.

Conclusions: We conclude that the SE profile of the IFN/Thal combination is not additive, does not appear to differ from that of the individual drugs, and is generally well tolerated in this group of Thal responsive pts. The overall response rate of 30% correlates well with other recently presented phase II studies of thalidomide in multiple myeloma. Furthermore, 2 pts received thal/IFN post-allograft without worsening GVHD.

Efficacy Of Thalidomide Therapy For Extramedullary Relapse Of Myeloma Following Allogeneic Transplantation

James J Biagi¹, L Mileskin¹, AP Grigg², DW Westerman¹, HM Prince¹

1 Department of Haematology, Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia

2 Bone Marrow Transplant Unit, Royal Melbourne Hospital, Melbourne, Victoria, Australia

Background: Management options for relapse multiple myeloma (MM) following alloBMT are often limited by concomitant graft-versus-host disease (GVHD), haematologic cytopenias or poor performance status. Thalidomide has antineoplastic properties, through proposed mechanisms that include angiogenesis inhibition, immunomodulation, apoptotic mechanisms, and modulation of T-cell function. Recently published studies have demonstrated activity of thalidomide in relapsed or refractory MM.

Objectives: We have initiated a clinical trial to evaluate the efficacy of thalidomide, with interferon (IFN) maintenance treatment, in the management of relapsed or refractory MM. Four of the enrolled patients to date have relapsed after HLA-identical sibling alloBMT. Herein we report successful thalidomide treatment in these four patients, three of whom had predominantly extramedullary (EM) relapse.

Methodology: Patients were prospectively enrolled into an ongoing, Phase II study of thalidomide plus IFN for progressive or refractory MM requiring systemic therapy. Eligibility criteria included age ≥ 18 and an ECOG performance 0-2. Patients were not excluded for abnormal renal, hepatic or hematopoietic parameters. The primary endpoint was response rate, with secondary endpoints of disease-free and overall survival, toxicity, and tolerability of thalidomide combined with IFN. Thalidomide was initiated at 200 mg po nocte, with dose escalation every two weeks to 800 mg or the maximal individual tolerated dose below this. The treatment was discontinued if intolerable toxicities developed that did not resolve after appropriate dose reduction.

Results: Of the four patients in our study who had undergone alloBMT prior to study entry, three subsequently relapsed with EM disease, which responded in all three cases to thalidomide. It is also of interest that one of these patients, who relapsed while on thalidomide, did not have EM relapse. Patient characteristics are given in the table below.

Patient	Age at BMT	GVHD Severity	Years to Relapse post-BMT	EM Relapse after AlloBMT	Systemic Relapse	EM Disease Response**	Duration of Response to thalidomide
1 M	43	Moderate	4	Y	Y	CR	11 mo
2 M	50	Mild-mod	3	Y	N	CR	7+ mo
3 M	40	*	3	N	Y	N/A	5+ mo
4 F	46	None Mild	3	Y	Y	CR	4+ mo

* after DLI three years post-allotransplant; CR = complete response

GVHD is often considered a surrogate for a graft-versus-myeloma effect. However, we think it unlikely that induction of GVM was responsible for disease regression in our patients, as two patients had pre-existent chronic GVHD at relapse, and none had exacerbation of GVHD while on study.

Conclusions: Our preliminary experience suggests that thalidomide is an effective treatment option in patients with myeloma who relapse after and allogeneic transplant, and may be particularly effective if the relapse includes EM sites.

Serum Free Light Chain Immunoassays As An Aid In The Diagnosis And Monitoring Of Light Chain Monoclonal Gammopathies

GP Mead^{1,2*}, HD Carr-Smith¹, MT Drayson², PN Hawkins³ and AR Bradwell²

1 The Binding Site, PO Box 4073, Birmingham, UK

2 Dept Immunology, Birmingham University, UK

3 National Amyloidosis Centre, Royal Free Hospital, London, UK

Objective: The aim of this study was to assess automated immunoassays for serum free light chain (flc) measurement and evaluate the utility of the assays for the diagnosis and monitoring of Bence Jones myeloma (BJM), non-secretory myeloma (NSM) and light chain amyloidosis (AL).

Methods: The assays utilise sheep polyclonal antisera which are specific for free kappa and lambda light chains and do not react with light chain in whole immunoglobulin; the assays are designed for use on the Beckman Immage protein analyser and the Behring Nephelometer II. Normal ranges were established using 100 sera from blood donors. For the BJM study, archived sera from 224 patients (with confirmed BJM) in the UK MRC myeloma trials were assayed. From the same trials, sera from 28 patients, with a diagnosis of NSM, were assayed and these sera were also re-tested by immunofixation. Flc levels were also measured in sera from 48 patients with AL, selected for having very subtle underlying plasma cell dyscrasias.

Results: For the 100 normal sera, mean free kappa was 8.4mg/L (SD=2.66; 95% confidence interval 4.2-13.1mg/L) and mean free lambda 14.5mg/L (SD=4.4mg/L; 95% confidence interval 9.2-22.7mg/L) with a mean kappa/lambda ratio of 0.6 (95% confidence interval 0.36-1.01). Of the BJM sera, 104/104 lambda patients and 120/120 kappa patients could be diagnosed by comparison with the normal range data. Of the 28 NSM sera, 27 were abnormal when the kappa/lambda ratio as well as the flc concentrations, were considered and only 7 of the sera produced visible paraprotein bands when assayed by immunofixation electrophoresis. For the AL sera, clonal flc was identified in 48/48 of the serum samples obtained at diagnosis.

With all three classes of gammopathy, where serial serum samples were available, changes in the flc concentrations were in accordance with the clinical assessments.

Conclusion: The use of specific immunoassays for quantification of flc in the serum should prove useful in the diagnosis and monitoring of patients with all forms of light chain monoclonal gammopathies and may remove the need for urine testing.

Induction with Oral Chemotherapy (CID) and Early Autologous Stem Cell Transplantation (ASCT) for De Novo Multiple Myeloma (MM)

Andrew Spencer^{1*}, Michael Seldon², Sandra Deveridge², Paula Marlton³, Gavin Cull³, Arno Enno², Ralph Cobcroft³, Anthony Schwarer¹, Devinder Gill³

1 BMT Programme, Alfred Hospital, Melbourne

2 Hunter Haematology Unit, Mater Hospital, Newcastle

3 Department of Haematology, Princess Alexandra Hospital, Brisbane

In an effort to minimise the myelotoxicity of induction MM chemotherapy, avoid unnecessary hospitalisation and facilitate early ASCT we have prospectively evaluated an outpatient-based oral chemotherapeutic regimen (CID) comprising cyclophosphamide 100mg/m² days 1-4, idarubicin 10 mg/m² days 1-2 and dexamethasone 40mg daily days 1-4 (also days 8-11 and 15-18 in cycle 1) every 21 days for 4 cycles. Patients then underwent stem cell mobilisation (SCM) with cyclophosphamide (2000 mg/m²). Furthermore, 3 consecutive cohorts of 12 patients (C1, C2 and C3) exploring the use of escalating doses of filgrastim were planned. C1 filgrastim 5 g/kg daily from day +5 following IDC; C2 filgrastim 10 g/kg daily from day +5 following IDC, and C3 filgrastim 10 g/kg daily from day +5 following IDC plus filgrastim 5 g/kg daily from day +1 following ASCT.

Thirty-six newly diagnosed MM patients (aged 31 to 66 yrs, median 55.5 yrs) were enrolled between February 1997 and March 2000. Disease characteristics were Stage III disease 56%; β_2 M >4mg/L 28%; CRP >6mg/L 49% and median BM plasmacytosis 33% (range 2-90%). One patient withdrew from study after 1 cycle of CID for religious reasons and was not included in any further analyses. 136 cycles of CID were administered requiring 4 (3%) unplanned hospital admissions and producing 14 episodes (10.4%) of >grade 2 haematological toxicity. Thirty-two patients completed SCM. There was a non-significantly increased median CD34 cell yield in C2/C3 compared with C1 (6.04x10⁶/kg vs 4.42x10⁶/kg, respectively). Thirty patients underwent high-dose melphalan conditioned ASCT at a median of 5.5 months post-diagnosis. C2 demonstrated more rapid neutrophil engraftment and required shorter hospitalisation post-ASCT than C1 (12 vs 15 days, p=.01 and 16 vs 22 days, p=.03) but no additional advantage was seen with the addition of filgrastim post-ASCT. Response rates (PR+CR) based on an intention-to-treat basis were 66% (23 of 35, 9% CR) post-CID and 80% (28 of 35, 34% CR) post-ASCT. Five patients have died - 1 sepsis, 1 cardiac amyloidosis and 3 progressive MM. With a median follow-up of surviving patients of 36 months the median PFS is 33 months and predicted OS at 3 years post-diagnosis is 89%.

Our data demonstrate that CID in combination with early ASCT is an effective and well tolerated anti-myelomatous regimen and is a useful alternative to more toxic and invasive therapeutic approaches.

Targeting DCs for vaccination

Larry W. Kwak* and Arya Biragyn

Experimental Transplantation and Immunology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

Although many tumor antigens have been described as candidates for vaccine development, most are inherently non-immunogenic and must be first rendered immunogenic by chemical or genetic fusion to carriers which enable the induction of protective antitumor immunity in experimental tumor models. We tested a novel strategy of targeting receptors on DC in vivo with various ligands of innate immunity (defensins, chemokines, and viral chemokines) genetically fused to a lymphoma antigen and HIV antigens. Such fusion proteins induced chemotaxis of immature, but not mature, DC and bound specifically to chemokine receptors. Immunizations with DNA encoding these fusions also elicited humoral and protective and therapeutic anti-lymphoma immunity in syngeneic mice. No immunity was induced by DNA expressing free unlinked antigen with defensin or chemokine. The efficiency of defensins/chemokine-fusion as a strategy for general vaccine development was also apparent by its ability to induce antigen specific CTL responses in mice. We propose that chemokine fusion may represent a novel, general strategy for formulating existing or newly identified tumor and HIV antigens into vaccines for cancer and AIDS, respectively, which elicit CD8⁺ T-cell immunity.

Flt 3 ligand mobilisation of DCs

Eugen Maraskovsky

Ludwig Institute for Cancer Research

Abstract not available at time of printing

Dendritic cell mobilisation induced by ProGP with, and without, chemotherapy

David Ashley

Royal Childrens Hospital

Abstract not available at time of printing

QAP

Katherine Marsden

Abstract not available at time of printing

Haematology Morphology QAP

John Catalano and Surender Janeja

Abstract not available at time of printing

Comparison of three commercial fingerprinting systems for quantitative determination of donor engraftment post allogeneic stem cell transplantation

Ambrosoli, P.A.(1,2)*, Taylor, D.T.(1,2), Rodwell, R.R.(1,2), Baldry, D.(1,2), Henders, A.(3), Dumenil, T.(3), Martin, N.(3), Lachman, R.(4), Hill, G.(5) and Taylor, K.(1)

1 Haematology Division, Mater Hospitals, Brisbane

2 Lions Cord Blood and Leukaemia Research Unit, Mater Hospitals, Brisbane

3 Queensland Institute of Medical Research, Brisbane

4 Immunology Division, Mater Hospitals, Brisbane

5 Mater Medical Research Institute, Mater Hospitals, Brisbane

Objective: To determine optimal methodology for monitoring engraftment status post allogeneic transplant by polymerase chain reaction (PCR) of polymorphic markers. Three commercially available fingerprinting kits were assessed, and comparisons were made with respect to number of informative markers, capacity for analysis within the routine clinical laboratory setting and accuracy of quantitative results. The quantitative assessment of mixed chimerism provides a guide for implementation of treatment.

Method: Prior to allogeneic transplantation, sex-matched donor and recipient cells were harvested, DNA extracted and serial dilutions of the recipient and donor DNA performed. Post-transplantation, the recipient specimens used for evaluation were day 21 bone marrow (unsorted), and day 27 peripheral blood (sorted populations of CD3+/T cells and CD15+/myeloid). The three methodologies under evaluation were: (1) Biotech ApoB (single marker) kit with PCR product analysis by agarose gel electrophoresis (ethidium bromide) and spot densitometry; (2) Promega Geneprint STR system (9 markers) with PCR product analysis by acrylamide gel electrophoresis, silver staining and spot densitometry; and (3) Perkin Elmer Profiler system (10 markers) with subsequent PCR product analysis on the ABI 377/373 and analysis with Genotyper software. In the first instance, the serial dilutions from pre-transplant cells were assessed, followed by the recipients' post transplant specimens. Final validation of the methodology was obtained by participation with RCPA QAP samples.

Results: The single ApoB marker of the Biotech system was not informative for the donor/recipient pair. The Promega system was informative for two markers, in contrast to the Perkin Elmer kit, informative for four markers. The %recipient was calculated for the dilution series by each method and the R2 values assessed as 0.959 and 0.978 for the Promega and Perkin Elmer systems respectively. Comparable results were obtained for the post-transplant specimens using both Promega and Perkin Elmer kits. On analysis of the RCPA QAP DNA samples, the Biotech ApoB kit was informative and the R2 value determined as 0.999.

Conclusion: We found that the Biotech kit was least likely to be informative due to the single marker used for analysis, while the Perkin Elmer system with the most markers (ten), the most likely. The Biotech and Promega kits could be easily used in a routine clinical laboratory, with the Perkin Elmer kit requiring the services of a specialised external laboratory with an ABI system. The Biotech and Promega kits, although relying on fewer informative markers, provide a reliable result for laboratories, which do not have access to an ABI system. All tested methods allow accurate quantitative engraftment analysis for patients post-transplant and an informative guide to treatment.

Hodgkins Reed-Sternberg Cells Express a Novel Form of Cadherin-8

***Nicola Angel (1), Debra Norris (2), Peter Dash (2), Sonya Fitzpatrick (1), Dahlia Kahill (1), Slavica Vuckovic (1), Derek Hart (1), Masato Kato (1)**

1 Mater Medical Research Institute, South Brisbane, Qld

2 Department of Pathology, Mater Adult Hospital, South Brisbane, Qld

Cadherins are a family of homo/heterophilic adhesion molecules involved in cell-cell interaction, tissue development and cancer. Using suppressive subtractive hybridisation (SSH), we identified a cDNA encoding for a novel form of cadherin-8 (Cad-8S) specifically expressed in Hodgkin's Reed-Sternberg (H-RS) cell line, L428. Cad-8S lacks the transmembrane and highly conserved cytoplasmic domain of the transmembrane form of Cad-8 (Cad-8L). The expression of Cad-8S and Cad-8L mRNA was restricted to L428 amongst a range of haematopoietic cell lines assessed by RT-PCR. In freshly isolated blood leucocytes populations tested, including dendritic cells (DC), Cad-8S was expressed in CD14+ monocytes, CD3+ T and CD19+ B lymphocytes. Importantly, no Cad-8L was detected in any blood leucocyte populations. GM-CSF/IL-4 monocyte-derived DC expressed Cad-8S, but its expression diminished upon differentiation/activation with lipopolysaccharide (LPS). Using antibodies against the extracellular domain of Cad-8 (specific for Cad-8s and Cad-8L) or against the cytoplasmic domain (specific for Cad-8L), we found Cad-8 to be restricted to mononuclear histiocytes (dendritic cell/macrophage lineage) within the germinal centers and interstitial regions of the lymph nodes. In Hodgkin's Disease affected lymph nodes, Cad-8S but not Cad-8L was detected in Hodgkin's Reed-Sternberg (H-RS) cells. Cad-8 may have applications as a diagnostic marker for Hodgkin's Disease, and the expression and regulation of Cad-8 may participate in normal leucocyte physiology and pathological processes, particularly in Hodgkin's Disease.

RT-PCR-ELAHA for rapid detection of fusion gene transcripts associated with CML and ALL.

Armitage, MS*, Hyland, V., Williams, B., Rowell, J.

Haematology Division, Queensland Health Pathology Service, Royal Brisbane Hospital Campus, Herston, Queensland, Australia.

This study focussed on four well defined chromosome aberrations which frequently occur in CML or ALL: fusion gene transcripts t(9;22) with BCR-ABL, t(1;19) with E2A-PBX1, t(4;11) with MLL-AF4, and t(12;21) with TEL-AML-1.

Our aim was to develop a specific, sensitive and rapid method for fusion transcript detection in various subtypes of leukaemia to facilitate diagnosis.

Total RNA was extracted from 1-10 x 10⁶ leucocytes using TRIZOL reagent. cDNA was synthesised using MMLV reverse transcriptase and used as template for PCR amplification. One round of amplification was performed using primers on opposite sides of each breakpoint region. In a separate reaction ABL was amplified alone as an endogenous control for template integrity and reverse transcription efficiency. PCR was performed using DIG-11-dUTP to allow subsequent detection of products by enzyme-linked amplicon hybridisation assay (ELAHA). An equal volume of amplicon was mixed with specific biotin labelled oligo probes, and following denaturation then cooling, the amplicon-probe complex was incubated on streptavidin-coated microtiter wells. After incubation, washing and addition of HRPO-conjugated anti-DIG antibody, further incubation and washing, colour is developed from an enzyme-substrate reaction. The reactions were stopped by addition of HCl and absorbance recorded using a plate spectrophotometer. ELAHA results were compared with results obtained using our standard RT-PCR, PAGE and dot blot protocol for fusion transcript detection. Sixty five samples were analysed for the common CML associated t(9;22) fusion gene transcripts using our standard protocol and the RT-PCR-ELAHA. Of these, b2a2 transcripts were detected in 12 samples, b3a2 transcripts were detected in 11 samples and both b2a2 and b3a2 transcripts were detected in 1 sample, by both methods in the same samples. Using our standard protocol and the RT-PCR-ELAHA fifty four samples were analysed for ALL associated t(1;19), t(4;11), t(9;22), and t(12;21) fusion gene transcripts. t(9;22), e1a2 transcripts were detected in 4 samples, t(12;21) transcripts were detected in 4 samples, t(4;11) transcripts were detected in 4 samples and t(1;19) samples were not detected in any of the patient samples analysed. When compared, results from both methods showed 100% correlation. Cell lines containing the translocation/s of interest, as positive controls, and no-template negative controls were performed successfully in each run. The results indicate that the RT-PCR-ELAHA can be carried out in one working day while being as reliable as our standard RT-PCR protocol for fusion transcript detection. In addition, the RT-PCR-ELAHA has the potential become a semi-quantitative assay.

A prioritisation system for treatment of patients with chronic myeloid leukemia using STI 571 (Glivec®). Experience with the dilemmas of drugs in scarce supply, the Australasian solution with a comparison from overseas centers.

Dr Chris Arthur* (1), Dr. Kevin Lynch (6), Dr Kerry Taylor (2), Dr Jeff Szer (3), Dr Tim Hughes (4), Dr Richard Hermann (5)

(1) Royal North Shore Hospital, Sydney. (4) Royal Adelaide Hospital, Adelaide

(2) Mater Hospital, Brisbane (5) Royal Perth Hospital, Perth

(3) Royal Melbourne Hospital, Melbourne (6) Novartis Pharmaceuticals

The recent availability of STI 571 (Glivec®) is having a profound impact on the management of CML. Phase II studies show high haematological and cytogenetic remission rates. The efficacy and favourable side effect profile of Glivec has resulted in a heavy worldwide demand from doctors and patients wishing to access the drug via clinical trials or expanded access programs. An expanded access program was started in the Australia/NZ region in October 2000. Resource and drug limitations meant that in Aust/NZ only 10 patients/month could receive treatment. Patients in the accelerated phase or blastic phase were not subject to rationing. Australia and New Zealand were grouped as one region internationally and five satellite centres were initiated in major capital cities. It was estimated at least 100 patients in chronic phase would be eligible. Methods of prioritisation were discussed amongst the chief investigators and included factors such as spleen size, platelets, difficulty with controlling blood counts. Such factors however did not have sufficient discrimination or were difficult to objectively evaluate and compare. It was agreed that the highest priority was given to those predicted most likely to transform within the next 6-12 months. It is possible to make predictions based on established scoring systems using values obtained at diagnosis. The Sokal index and the Hasford score were calculated and used to prioritise. Published survival curves of these prognostic scores indicate the risk of transformation accelerates over the first three years and this fact was used to compare an individual's transformation risk at the time of study entry. Patients were therefore stratified according to the prognostic index and the time from diagnosis. Each of the five treatment centres was allowed to treat one patient/month according to their own criteria and the other five/month were allocated according to the prognostic index and time from diagnosis. Where possible patients were referred back to their originating physician to share the management so that experience gained with Glivec was as broad as possible. Overseas centres used a variety of prioritisation systems. These included direct negotiations via teleconferencing between doctors, random selection (Lotto), review of submitted case summaries and prioritisation by independent reviewers and finally in one country the whole process was handed over to the national haematology association. We believe the system chosen in Australia allowed patients most in need to receive treatment as soon as possible but also preserved the discretion of study doctors to select needy patients not identified by the prognostic scoring system.

Efficacy of low dose thalidomide therapy in relapsed/refractory multiple myeloma.

Sharon Avery*, Anthony Schwarzer, Partrick Elliott, Andrew Spencer.

Clinical Haematology and BMT Unit, Alfred Hospital, Melbourne.

Thalidomide in doses up to 800 mg daily has demonstrated activity in relapsed/refractory multiple myeloma producing an overall response rate of 30 - 40%. This, however, is at the expense of significant toxicity. Based on a lack of convincing evidence for a dose-response relationship we evaluated the toxicity and clinical efficacy of low-dose thalidomide in the management of refractory/relapsed myeloma patients.

Between November 1999 and June 2001 seventeen patients (9 females, 8 males, median age 65 years) with refractory (4 patients) or relapsed (13 patients) myeloma were treated with low-dose thalidomide (100 - 200 mg/day). Additional anti-myeloma therapy, principally alternate day corticosteroids, was given to 10 patients. Class IgG myeloma was diagnosed in 9 patients, IgA in 3 patients, IgD in 1 patient and in 4 cases light chain disease was recognised. The median time from myeloma diagnosis to initiation of thalidomide therapy was 31 months (range 4 - 112) and the median number of prior therapies was 3 (range 1 - 6). Four patients had previously undergone autologous stem cell transplantation. Response was based on a reduction in serum or urine paraprotein.

Three patients on treatment for less than four weeks are not evaluable for response. A single patient had early withdrawal of thalidomide therapy due to adverse effects leaving 13 patients presently evaluable. Two patients (15%) showed a paraprotein reduction of 75 - 100% (including 1 CR), 3 patients (23%) a response of 50 - 75% and one patient a response of 25 - 50% giving a total response rate of 46%. Two patients (15%) have stable disease while 5 patients (38%) demonstrated disease progression during the treatment period. Maximal monoclonal protein decrease was observed at a median of 56 days (range 28 to 218) after the start of therapy. Overall thalidomide was well tolerated with no adverse effects reported in 53% of treated patients. When reported, constipation (12%), paraesthesia (12%), fatigue (12%) and sedation (18%) were mild and did not necessitate treatment withdrawal. Haematologic side effects were infrequent. After a median follow-up of 212 days, all responding patients are alive and progression-free.

Our results demonstrate that the inclusion of low-dose thalidomide in the treatment of heavily pre-treated refractory or relapsed myeloma is well tolerated and effective. Furthermore, despite a median of 9 months of low-dose therapy, no patients have experienced progressive peripheral neuropathy necessitating cessation of therapy.

The Role of Exercise in Facilitating Patient Recovery Following Peripheral Blood Stem Cell Transplantation.

Hayes SC (1)*, Davies PSW (2), Parker TP (3), Bashford J (4), Green A (5)

1 Queensland University of Technology, Brisbane

2 The University of Queensland, Department of Paediatrics and Child Health, Brisbane

3 Queensland University of Technology, Brisbane

4 The Wesley Clinic, Brisbane

5 Queensland University of Technology, Brisbane

Fatigue and loss of functional capacity are common symptoms experienced by long-term bone marrow transplant (BMT) survivors. As cancer survival rates continue to rise, so too does the need to investigate rehabilitative procedures that maintain the potential to mitigate the disabling consequences of the disease and its treatments. Participation in a low- to moderate aerobic exercise program is one such rehabilitative strategy that has shown promising results in facilitating recovery post-cancer treatment. Unfortunately, research within this domain is predominantly limited to breast cancer patients participating in low intensity, aerobic exercise. The purpose of this investigation was to evaluate the impact of undertaking a peripheral blood stem cell transplant (PBST) on functional capacity, and to determine the role of a mixed type, moderate intensity exercise program in facilitating the recovery of patients following intensive cancer treatment. Aerobic capacity (VO₂ measures in L/min, ml/kg/min, ml/FFM/min) and muscular strength (upper body, lower body and hand grip strength) measures were assessed pre-transplant (PI), post-transplant (PII) and following a 12-week intervention period (PIII). Following PII, twelve patients were divided equally into a control group (CG) or exercise intervention group (EG). Those involved in the exercise intervention performed aerobic exercise of a moderate intensity, 20-40 minute duration, 3 times/week, and resistance training of moderate to high repetitions, moderate to heavy weight, twice per week. Mean values for peak ventilation, aerobic capacity and muscular strength were reduced following the transplant. However, differences between pre- and post-transplant data were only significant ($p < 0.05$) for upper body strength. The control group showed little change in peak ventilation, peak aerobic capacity and muscular strength between PI, PII and PIII measures. Participation in the exercise program led to significant improvements in peak ventilation ($p < 0.01$), peak aerobic capacity ($p < 0.05$) and upper and lower body strength ($p < 0.01$). Additionally, results recorded following the 12-week intervention period were significantly higher than pre-treatment levels, for peak aerobic capacity (L/min, $p < 0.05$ and ml/kg/min, $p < 0.01$) and lower body strength ($p < 0.01$). The magnitude of change between PII and PIII was significantly larger for the EG when compared with the controls, for peak VE ($p < 0.01$), peak VO₂ (L/min and ml/kg/min, $p < 0.05$) and upper ($p < 0.05$) and lower body ($p < 0.01$) strength. A mean negative change for peak VE, hand grip strength and lower body strength, was observed for the CG between PII and PIII. While the results of earlier work indicate that 40% of the BMT patients require 12 months to regain pre-treatment fitness levels, the results from this investigation demonstrate that it can take as little as three months. Moreover, this investigation highlights that through participation in an aerobic and strength training program, PBST patients can return to higher than pre-treatment function. Furthermore, failure to participate in an exercise program following treatment may exacerbate any physical functioning losses induced by the treatment process.

Peripheral Blood Stem Cell Mobilisation (PBSC) with ancestim (r-metHuSCF) plus filgrastim (r-metHuG-CSF) for patients failing mobilisation on filgrastim alone: down regulation of ckit and successful peripheral blood stem cell mobilisation are parallel events for ancestim successfully mobilised patients.

J Bashford (1)* S Durrant (2) A Schwarer (3) M Prince (4) J Gibson(5) B Cohen (6) J MacMillan (6) and B To(7).

1 Wesley Medical Centre, Brisbane

2 Royal Brisbane Hospital, Brisbane

3 Alfred Hospital, Melbourne

4 Peter MacCallum Cancer Institute, Melbourne

5 Royal Prince Alfred Hospital, Sydney

6 Amgen Australia Pty Ltd, Melbourne

7 Royal Adelaide Hospital, Adelaide

Prior chemotherapy and bone marrow involvement with malignancy are associated with the failure to mobilise adequate stem cells to allow autologous transplantation. Ancestim potentiates filgrastim-induced PBSC mobilisation alone or in combination with chemotherapy. 44 failed mobilisers with non-myeloid malignancies having $\geq 0.5 \times 10^6$ but $< 2.0 \times 10^6$ CD34+ cells/kg (13 filgrastim alone, 31 filgrastim plus chemotherapy) were studied with a program of adding ancestim to prior mobilisation regimen to assess the subsequent yield. Filgrastim only mobilisers received ancestim 20ug/kg for 3 days prior to filgrastim (10ug/kg/day) commencement. Injections continued daily through leukaphereses that began on day 5 of filgrastim administration. Filgrastim plus chemotherapy patients received ancestim 20ug/kg/day plus filgrastim 5-10ug/kg/day from day 2 post chemotherapy. Leukapheresis commenced when the WBC was $> 2 \times 10^9/L$ or peripheral CD34+ count was $> 5.0 \times 10^3$ cells/mL. Leukaphereses continued to a maximum of 4 procedures or until a target of 3.0×10^6 CD34+ cells/kg was reached. The median yield in patients treated with growth factors alone increased from 0.8 (0.6-1.1) to 2.4 (0.4-4.3) $\times 10^6$ CD34+ cells/kg. In patients treated with chemotherapy and growth factors, the yield increased from 1.0 (0.5-2.0) to 1.6 (0.0-5.5) $\times 10^6$ CD34+ cells/kg. 54% of patients treated with growth factors alone yielded $\geq 2.0 \times 10^6$ CD34+ cells/kg. and 45% of patients treated with chemotherapy plus growth factors achieved this threshold. Combining prior mobilisation yield with study mobilisation yield, 85% and 74% respectively yielded $\geq 2.0 \times 10^6$ CD34+ cells/kg. In patients successfully mobilising with ancestim, the CD34+ cells show a significant decrease in ckit expression compared with their prior unsuccessful mobilisation. For patients who failed mobilisation with ancestim, there was no change in ckit expression. There were no serious allergic reactions to ancestim. The combination of ancestim plus filgrastim allows patients mobilising poorly with or without chemotherapy to proceed to autologous transplantation.

Human Haemopoietic Progenitor Cell Engraftment in Murine and Human Hosts Correlates with Expression of the Chemokine Receptor CXCR4

Cindy Baulch-Brown (1)*, Jacob Jackson(1), Andrew Perkins (1,2), Andrew Spencer(1)

1 Bone Marrow Transplant Programme, Alfred Hospital, Melbourne

2 Department of Physiology, Monash University, Clayton

Expression of the chemokine receptor CXCR4 on haemopoietic stem cells (HSC) may play a crucial role in localizing HSC to the bone marrow compartment. To evaluate the importance of CXCR4 in vivo we transplanted varying doses of human HSC from normal donors and cord blood (CB) into sub-lethally irradiated NOD/SCID mice and assessed human haemopoietic cell engraftment at 4 weeks post-transplant by flow cytometric analysis.

We have previously reported that a significantly higher proportion of CB CD34+ cells express CXCR4 compared to adult CD34+ cells, and hypothesised that the increased engraftment potential observed for CB CD34+ cells is related to the higher level of CXCR4 expression. Preliminary data from our NOD-SCID engraftment studies is in line with this hypothesis. Greater numbers of adult CD34+ cells were required to engraft NOD-SCID mice compared to CB CD34+ cells (7.3×10^5 cf 2.6×10^5), however engraftment was achieved with similar numbers of adult or CB CD34+/CXCR4+ cells (1.65×10^5 cf 1.84×10^5).

The number of CD34 CXCR4 double-positive HSC infused into 16 adults undergoing allogeneic PBSCT was also enumerated. Overall the median number of CD34 cells expressing CXCR4 was 41% and the median number of double-positive HSC infused at the time of transplant was $2.5 \times 10^6/kg$ (range, $0.8-10.3 \times 10^6$). Recipients of $> 2.5 \times 10^6/kg$ double-positive cells demonstrated a significant shortening of time to platelet engraftment compared to recipients of lower cell doses (10 days vs 14.5 days, respectively, $p = .02$) with all but one of the high cell dose recipients achieving platelet engraftment by day 11. Other transplant characteristics within this patient group including donor type (related vs unrelated) and matching (matched vs mismatched), GvHD prophylaxis (methotrexate vs no methotrexate) and CD34 dose ($>$ or \leq median) did not significantly influence the rate of platelet engraftment.

These observations indicate that human progenitor cell engraftment in murine and human hosts may correlate with the expression of CXCR4 and that CD34 CXCR4 double-positive cell dose may be a more relevant biological predictor of post-transplant engraftment than total CD34 cell dose.

Fludarabine/Cyclophosphamide/Rituxan is an effective regimen for non-myeloablative allogeneic stem cell transplantation (SCT) for lymphoid malignancies.

Leanne C. Berkahn, David R. Simpson, Anastasios Raptis, Hans G. Klingemann

Rush Bone Marrow Transplant and Cell Therapy Program, Chicago, USA.

Aim: To assess the safety and efficacy of a non-myeloablative preparative regimen for allogeneic stem cell transplantation for advanced lymphoid malignancies.

Methods: Eleven patients (pts) underwent a non-myeloablative allogeneic SCT from a 6/6 HLA-matched sibling donor between November 1998 and December 2000. Pts with CLL (6), mantle cell lymphoma (2), or follicular mixed B-cell lymphoma (1) received Flu/Cy/Rituxan while 2 patients (CLL/PLL with WBC >400, peripheral T-cell lymphoma) received Flu/Cy alone without Rituxan. The preparative regimen doses were Rituxan 375 mg/m² day -6, Fludarabine 30mg/m² x 4, Day -5 to -2, and Cyclophosphamide 1g/m² x 2, Day -3, -2. Donor stem cells were mobilized using G-CSF (10 mg/kg/day) for 4 days. GVHD prophylaxis was cyclosporine/methotrexate. The median age at SCT was 59 years (range 51-65 years). Patients had received a median of 2 (range 1-5) prior regimens.

Results: Pts received a median of 4.0 x 10⁶ CD34+ cells/kg. The median time to neutrophil engraftment was 12.5 days (range 9-24 days). Most patients (5/9) did not require blood product support and two pts required only a single blood or platelet transfusion. The median duration of hospital stay was 20 days (range 12-33 days). There have been 2 deaths. One patient with refractory CLL died of sepsis 63 days post transplant with evidence of engraftment and disease response. One patient with CLL/PLL refractory to CHOP and Fludarabine had an excellent initial response with a fall in pre-SCT WBC from >400 x 10⁹/l to 8 x 10⁹/l by day 10 post SCT. This pt developed progressive disease and underwent a second transplant using TBI/Cy preparative regimen and died of fungal infection in remission. Steroid-responsive grade II-III acute graft versus host disease was seen in 5/8 evaluable pts. Chimerism analysis revealed at least 30% donor cells by day+30, 60% by day+60 and 90% by day+100 in 6/7 evaluable pts. With a median follow-up of 12.5 months (range 2-27 months) the Kaplan-Meier estimate of overall survival is 78 ± 20% at 27 months. Progression free survival is 73 ± 15% at 27 months. All 6 evaluable patients are in complete remission including negativity by consensus PCR for the immunoglobulin heavy chain rearrangement.

Conclusions: Non-myeloablative SCT using Flu/Cy/Rituxan is very well tolerated in older patients and has potent anti-tumor effects even in advanced CLL and aggressive lymphomas. Patients with symptomatic indolent lymphomas or CLL should be considered for non-myeloablative transplantation after failure of first-line therapy or in first remission for poor-risk aggressive lymphomas.

Modified DICE Chemotherapy in Patients With Lymphoma: Results of Phase II ALLG Study

James J Biagi*, H M Prince, C Smith, E Abdi, M Leahy, C Folkson, M Wolf, H Januszewicz, J Seymour, J Stone, B Dale

Dept of Haematology, Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia, on behalf of the ALLG

Background: Salvage treatment for patients with relapsed Hodgkin's or non-Hodgkin's lymphoma is aimed at achieving a high clinical response with acceptable morbidity. Patients who respond to salvage regimens may proceed to high dose chemotherapy with autologous stem cell transplantation (autoSCT).

Rationale and Objective: DICE combination chemotherapy is reported to produce an ORR of 67% with 23% CR rate in relapsed or refractory lymphoma. The objective of our study was to determine the effectiveness of an alternative DICE regimen, modified to: 1) reduce the inpatient requirement for intravenous (IV) infusion from four days to one, and 2) to provide a lower dose regimen for older patients (>55 yrs). The endpoints were response rate, toxicity and survival.

Methodology: Treatment schedule - every 4 weeks for 6 cycles, or until PD or withdrawal -

	Patients ≥ 55 years:	Patients < 55 years:	
Dexamethasone	10mg QID orally	10mg QID orally	D1 - D4
Ifosfamide (ifos)	2.5g/m ² IV	4g/m ² IV	D1
Cisplatin	50mg/m ² IV	100mg/m ² IV	D1
Etoposide	50mg IV	100mg IV	D1
Etoposide	100mg orally	200mg orally	D2 - D4
MESNA	1g/m ² pre/post ifos	1.6g/m ² pre/post ifos	D1x3

Results:

Patient Characteristics	Number of Patients at diagnosis: (unless stated in brackets)
Patients Accrued	40 enrolled / 39 evaluable (July 1997–Apr. 2000)
Male : Female	22 : 18
Median Age	(55 yrs, range 25-79)
Relapsed/Resistant	32 relapsed / 8 resistant
Disease	I = 5 / II = 5 / III = 11 / IV = 19
Stage at Registration Cycles Completed	I = 5 / II = 5 / III = 11 / IV = 19
Dose Schedule	higher dose = 12 / lower dose = 28
Gr 3-4 Toxicity, non-haem:	Nausea = 12 Thrombosis = 1 Stomatitis = 1
Gr 3-4 Toxicity, haem:	Neutropenia = 34 Anaemia = 6 Th'penia = 18 Febrile neutropenia = 5
Outcomes: CR/PR/SD/PD	3 (8%) / 15 (38%) / 12 (30%) / 8 (24%)
Survival at 2 Years	28% Progression-Free Survival / 52% Overall Survival
Off Protocol Due To:	Completion of Chemo = 8 Relapse or Progression = 14 Proceed to autoSCT = 6 Radiation = 2 Other Reason = 10
Deaths	20 disease related

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; Gr = grade; ORR = overall response rate

Conclusion: DICE is an effective regimen in relapsed/refractory lymphoma, with promising response rates in this poor prognosis group of patients, and is suitable as a salvage regimen prior to autoSCT.

Multi-centre Clinical Trials of Denileukin Diffitox (Ontak®) in Primary Cutaneous T-Cell Lymphoma (CTCL)

James J Biagi* (1), Jill Davison (1), C McCormack (2), G Ryan (1), C Baker (2), JF Seymour (1), HM Prince (1).

Divisions of 1) Haematology and Medical Oncology and 2) Radiation Oncology, Peter MacCallum Cancer Institute and 3) Department of Dermatology, St. Vincent's Hospital, Melbourne, Victoria, Australia

Background: Primary CTCL usually is confined to the skin but may involve blood, lymph nodes, bone marrow and visceral organs with survival strongly related to disease stage; median survival ranging from 12+ years for early stage disease to <2 years for advanced stage disease. Patients (pts) with extensive skin disease or systemic involvement usually respond to various topical, radiotherapy and systemic therapies but the disease course is characterized by frequent relapses and infectious complications. Novel treatment approaches are required.

Rationale and Objective: The human interleukin-2 receptor (IL2-R) consists of 3 interacting isoforms of variable binding affinity, that influence development and progression of CTCL. An immunohistochemical (IHC) antibody to the cell surface antigen CD25 is used in detecting one of the IL2-R isoforms. Denileukin diffitox (Ontak®) is a protein derived from a recombinant DNA fusion gene that consists of the active A and B chains of the diphtheria toxin linked to the human IL2-R. A recent randomized Phase II study using Ontak monotherapy demonstrated a 30% response rate in pts with CD25 (+) CTCL, with acceptable toxicity. Two subsequent multinational trials are currently underway and we report our experience to date.

Methodology: Patients (pts) were eligible if they had biopsy-proven Stage IA-III CTCL, no greater than three prior therapies, ECOG performance status 0-1 and serum albumin \geq 30 g/L (to avoid vascular leak syndrome [VLS]). Patients with CTCL expressing CD25 by IHC entered a three-armed Phase III randomised study (blinded placebo vs 9 μ g/kg/d vs 18 μ g/kg/d), (Study 11). Patients with CD25 (-) CTCL, or those that progressed on the placebo arm of Study 11, were entered onto a Phase II study (18 μ g/kg/d) (Study 14). Ontak was made available for compassionate use in a patient not eligible for either study. Treatment was administered as a 30 min infusion daily for 5 days, q3w for 8 cycles. Endpoints included 1) frequency and duration of clinical response, 2) change in functional status and 3) safety and toxicity.

Results: 7 pts (4M / 3F), median age 64 years (range 44-75) were enrolled from Aug. 2000. Three patients were entered to Study 11, two of whom proceeded to Study 14, and three enrolled directly to Study 14. One patient, who had more than 3 prior treatments, received Ontak on a compassionate basis. At enrollment 2 pts were stage IIA or less and 5 pts IIB-III. The mean number of treatment cycles to best response was 3.6 (range 2-6). Of 5 pts evaluable for response, there have been 3 partial responses (PR) and 2 complete responses (CR), one of these on Study 14 after progressive disease (PD) on Study 11. Durability of response has been maintained for a median of 6 mo (range: 2 – 9+ mo, follow-up ongoing). Symptoms were improved in all 5 evaluable patients. Common infusion-related side effects were fever and hypotension. Two responding patients discontinued treatment because of toxicity: one with lethargy and renal impairment and the other because of vasculitis. Two patients developed anemia requiring transfusion. VLS was observed in one patient.

Conclusion: Ontak is effective and well tolerated, and activity in CD25 (-) pts has been demonstrated.

Molecular Pathogenesis and Geographic Variation in the Development of Follicular Lymphoma

James J Biagi*, JF Seymour

Dept of Haematology, Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia

Background: It has been repeatedly observed that the incidence rates of follicular non-Hodgkin's lymphoma (FL) between geographic regions are markedly different. Extremely low rates are reported among most Asian countries versus comparatively high rates in North America and Western Europe. Although widely accepted, this observation remains unexplained. The t(14;18)(q32;q21) translocation, detectable with high sensitivity by polymerase chain reaction (PCR), is the hallmark of FL and is characterized by one of two common breakpoints known as major breakpoint region (MBR) and minor cluster region (mcr).

Objective: The aim of this study was to compare the rates of bcl-2 translocation in FL across geographic regions. We suggest that a difference in such rates would support a hypothesis that the molecular pathogenesis is different between geographic regions, that nonetheless results in a morphologically similar phenotype of FL.

Materials and Methods: A literature search of previously published data was performed to compare the incidence of t(14;18) across geographic regions among patients with FL. All published studies, where PCR was used to detect both MBR and mcr loci in FL tissue specimens, were included. Sensitivity of PCR in the studies was at least 1×10^{-5} . A 95% confidence interval (CI) was applied to the resulting patient subtotals.

Results: The number of studies available from Asian populations was limited to two, with a total of only 46 evaluable patients. Conversely, there were seven studies with 239 patients and five studies with 323 patients from Europe and USA, respectively. The frequencies of bcl-2 positivity with corresponding CI demonstrate significant heterogeneity across geographic regions ($P < 0.0001$), with the highest frequency of bcl-2 gene rearrangements in the USA population. This pattern is also apparent amongst both the MBR ($P=0.001$) and mcr subgroups ($P=0.003$).

Region Evaluable	No. Patients (%) Patients	MBR +ve (%) Patients	mcr +ve (%) Patients	Total bcl-2 +ve	
Asia	46	43 (29-59)	4 (0.5-15)	48 (33-63)	(95% CI in brackets)
Europe	239	49 (43-56)	5 (3-9)	55 (48-61)	
USA	323	65 (60-70)	13 (10-17)	79 (74-83)	

Conclusion: Although based on small numbers of patients, and studies that do not compare results directly between geographic regions, we conclude that a significant gradient exists in the frequency of bcl-2 gene rearrangement between populations. This observation supports our hypothesis that the molecular pathogenesis varies across geographic regions and, further, may explain the differences in the incidence rates commonly reported. If these findings were confirmed in prospective studies, it would imply that different etiologic or genetic factors may influence the development of FL across separate regions.

Primary Cutaneous B-cell Lymphoma: A Clinicopathological Study of Thirteen Cases

R Blum¹, L M Yap², JF Seymour¹, HM Prince¹

Division of Haematology and Medical Oncology; Peter MacCallum Cancer Institute, Melbourne¹, and Department of Dermatology; St. Vincents's Hospital², Melbourne.

Tel: 61-3-9656 1700, Fax: 61-3-96561408, Email: mprince@petermac.unimelb.edu.au

Background: Primary cutaneous B-cell lymphoma (PCBCL) is a very rare condition with few reported series in the literature. The classification and treatment remains controversial.

Aim: A retrospective study of the pathologic features, clinical course and treatment outcomes of patients (pts) with PCBCL treated at Peter MacCallum Cancer Institute was performed.

Methods: The computerized patient record database was searched for all pts with a diagnosis of cutaneous involvement with lymphoma between 1986 and June 2001. Individual case records were then reviewed and all pts with PCBCL identified. Original skin biopsy specimens were obtained where possible, reviewed and classified according to the proposed WHO classification (JCO 1997; 17:3835-49).

Results: 13 cases of PCBCL were identified (6 female, 7 males), aged 32-85 yrs (median=59). 9 pts had primary cutaneous follicle centre lymphoma (FCL), 2 pts had diffuse large B cell lymphoma (DLBCL) of the leg, and 2 had marginal zone lymphoma (MZL). 11 pts (85%) had localised lesions (Stage 1A) and 2 pts (15%) had skin involvement of at least two nonadjacent sites (Stage 2A and 3A). Localised skin lesions involved the head (n=4), trunk (n=5), lower leg (n=2), and multifocal skin lesions involved the head and trunk (n=1) and both arms (n=1). 11 pts (85%) received involved field irradiation (30-40Gy). 4 pts received chemotherapy (CHOP, n=3: 2 pts with FCL and 1 pt with DLBCL of the lower leg) or VP16 (n=1: DLBCL of the lower leg; age 79). One patient had excision of the lesion alone. The median follow-up was 45 mo (range= 4-161). 12 pts (92%) achieved CR. 1 pt with DLBCL of the leg progressed on CHOP and subsequently died. 1 pt with FCL developed local recurrence after radiotherapy and responded to chemotherapy. The 5yr actuarial disease-free survival and overall survival for pts with FCL/MZL were 88% and 100%, respectively. Both patients with DLBCL of the lower leg progressed and died within 13 months.

Conclusion: Our study findings support the view that most PCBCL present with localised disease (predominantly head and trunk), have an indolent course, with a good response to local radiotherapy. Patients with localized FCL and MZL have an excellent outcome with RT alone and DLBCL of the lower leg has an unfavorable prognosis despite aggressive combined modality treatment.

Non-myeloablative allogeneic blood stem cell transplantation for low grade non-Hodgkins lymphoma

KF Bradstock, DJ Gottlieb, M. McGurgan, P. Stavros, J.Charity, M. Hertzberg

Blood and Marrow Transplant Service, Westmead Hospital, Westmead, NSW, Australia

Background

Relapsed follicular and mantle cell lymphoma following combination chemotherapy has a poor prognosis. The curative impact of autologous stem cell transplantation remains uncertain in this setting. The anti-lymphoma effects of non-myeloablative allogeneic stem cell transplantation have not been systematically explored to date for this indication.

Patients.

Eight patients with relapsed follicular or mantle cell lymphoma underwent non-myeloablative allogeneic stem cell transplantation at Westmead Hospital between July 1999 and June 2001. Patients were aged 34 to 56 (median 48) years. Four were females and 4 males. They had received between 2 and 6 different modalities of therapy prior to transplant, including high dose therapy and autografting in 1 patient. One patient was in second remission at BMT, while the remainder had evidence of residual disease.

Methods

All patients received conditioning therapy with Fludarabine 25 mg/m² for 5 days and Cyclophosphamide 60 mg/kg for 2 days, with a G-CSF-mobilized blood stem cell transplant containing 0.85 to 18.9 (median 5.0) x 10⁶ CD34⁺ cells /kg being infused on day 0. Cyclosporin was given as GVHD prophylaxis.

Results

All patients demonstrated complete donor T cell and myeloid chimerism by microsatellite PCR analysis post transplant. Neutrophil engraftment to 0.5x 10⁹/L occurred on median day 10 (range 8 to 13), while platelet recovery to 20x 10⁹/L was seen between day 10 and 30 (median 15). One patient had a platelet nadir >20, while one patient did not achieve platelet transfusion independence at the time of death from GVHD. Acute graft versus host disease was seen in 5 of 7 evaluable patients, with grade 3 or 4 disease in 3 cases. Chronic GVHD occurred in 4 of 5 surviving evaluable patients. Two patients died of GVHD and its complications on days 51 and 73. No other deaths occurred. One patient is too early post-transplant to evaluate for response. The remaining 5 surviving patients all achieved a complete remission, and remain alive between 464 and 714 days post-transplant.

Conclusions.

Non-myeloablative stem cell transplantation has a high risk of acute and chronic GVHD in this older high risk patient population, but is associated with a high rate of sustained complete disease response.

Unrelated Cord Blood Transplantation in Adults: Experience at Westmead Hospital and The Children's Hospital at Westmead

Bradstock KF(1) *, Hertzberg MS(1), Antonenas V(1), Bleakley M(2) and Shaw PJ(2)

(1) The Blood and Marrow Transplant Unit, Westmead Hospital, Westmead, Sydney

(2) The Oncology Unit, The Children's Hospital, Westmead, Sydney

Background:

Unrelated cord blood transplantation is being increasingly considered for children requiring allogeneic stem cell transplantation but lacking a matched related or unrelated marrow donor. Since 1997, we have performed 9 unrelated cord blood transplants in children (median weight of 23.8kg) with engraftment in 6 patients. Recent studies have shown that nucleated cell dose in the cord unit is important for engraftment, thus limiting applicability in adults. We report here our experience of unrelated cord blood transplants in 3 adults with high-risk acute leukaemia since 1999.

Results:

The 3 patients all had high risk acute leukaemia: primary induction failure of AML, T-ALL in CR2, and ALL in CR3. One patient had relapsed after a previous autograft. The median age of recipients was 21y (range 17-24), with median weight 53kg (range 45-63). The conditioning therapy consisted of TBI/ATG, with Cyclophosphamide in 2 patients and Melphalan in one. GvHD prophylaxis consisted of Methylprednisolone and Cyclosporin. All 3 cords were mismatched for 2/6 HLA loci. The median infused cell dose was 1.84x10⁷ TNC/kg (range 1.68-2.0) and median CD34⁺ cell count 0.91x10⁵/kg (range 0.55-1.6). One patient with refractory AML died of an intracerebral event on day +13, prior to engraftment. The 2 other patients achieved an ANC > 0.5x10⁹/L on days 16 and 27 and platelets > 20x10⁹/L on days 84 and 90 post transplant. Neither patient developed acute GvHD, while one had limited chronic oral GvHD. Both remain in complete remission at 20 and 25 months.

Conclusion:

Unrelated cord blood is an alternative source of stem cells in adults with leukaemia lacking a matched unrelated donor, and can successfully engraft adult patients despite a high level of HLA disparity and low cell dose.

Mobilisation of Peripheral Blood Stem Cells following IVAC chemotherapy for Non-Hodgkin Lymphoma.

C. Burton*, A. Enno, M. Seldon, S. Deveridge, I. Kerridge.

Hunter Haematology Unit, Mater Hospital, Newcastle, NSW.

IVAC (Etoposide 60mg/m² Days 1-5, Ifosfamide 1500mg/m² Days 1-5, Cytarabine 2g/m² Days 1-2) is frequently used as salvage chemotherapy in patients with relapsed or refractory intermediate and high grade NHL. IVAC is often used to obtain disease control prior to high dose chemotherapy and autologous stem cell transplant. Mobilisation of PBSC is often difficult in these patients due to heavy pretreatment. Since March 1997 our unit has evaluated the efficacy of PBSC mobilisation following IVAC. Comparison was made with other mobilisation regimes including G-CSF, GIVE, Ifosfamide/Etoposide and ICE.

Twenty two patients have received IVAC chemotherapy. Of these, nine proceeded to PBSC harvesting. Eight patients achieved adequate CD34+ cells for transplantation (CD34+ cells > 2 x 10⁶/kg), with a median count of 10.68 x 10⁶/kg (range 2.62 – 36.1 x 10⁶/kg). One patient failed to mobilise, achieving only 0.9 x 10⁶/kg CD34+ cells. This patient was successfully harvested with Cyclophosphamide/Ancestim/G-CSF. Median days to recovery was 14 (range 7 – 27).

Patient	1	2	3	4	5	6	7	8	9
Diagnosis	FCC	DLC	DLC	DLC	Tcell NHL	DLC	Tcell NHL	TcellNHL	FCC
Stage	4A	3B	2B	2A	1A	4B	1B	1A	4A
Status	PR	PR	RD	PR	R	PR	RD	PR	PR
Days/recovery	15	7	14	15	13	10	19	27	14
Aphereses	3	3	2	1	1	2	4	1	2
CD34+ x10 ⁶ /kg	19.8	4.72	8.26	30	36.1	0.9	2.62	19.1	13.1

IVAC appears to be an effective regime for PBSC mobilisation in poor prognosis patients with Non-Hodgkin Lymphoma. The interval between chemotherapy and PBSC harvesting, number of aphereses and CD34+ yield were comparable to other mobilisation regimes.

Sibling Allogeneic Bone Marrow Transplantation (BMT) at Royal Perth Hospital (RPH) : 1974 – 2001

Cannell P K, Herrmann R P, Buffery S I and Trent M

Royal Perth Hospital, Wellington St, Perth.

Objectives: To report the results of sibling allo BMT in our institution since the first allograft in 1974.

Methodology: The RPH transplant database was analysed with respect to routine transplant outcome measures including overall survival, 100 day transplant related mortality and relapse rate. Patients were grouped into standard disease defined risk groups and outcome measures were compared with published national and international results.

Results

	n	Overall Survival	100 day treatment related mortality	Relapse	Average Time from diagnosis to transplant – months	Median Age - years
Aplasia 1974-79	4	25%	25%	-	2	17
Aplasia 1980 - 2001	1 4	93%	7%	-	2	22
CML CP/AP < 12/12	2 5	68%	16%	0%	6	38
CML AP/CP > 12/12	19	37%	26%	4%	27	38
CML BC	9	11%	0%	78%	13	36
AML CR1*	2 6	58%	19%	12%	5	33
AML >CR1	1 0	20%	30%	10%	11	34
ALL CR1	6	50%	0%	17%	5	34
ALL >CR1	1 5	7%	20%	60%	9	35
MDS	2 1	43%	14%	19%	6	41

*Survival since 1990 is 74%

Prior to 1980 only 4 transplants were performed, all for aplastic anaemia. A cohort of patients transplanted between 1992 and 1997 was compared with ABMTRR data – no significant difference in overall survival or TRM was found. (OS RPH vs ABMTRR 59%vs 54% p= 0.9, TRM RPH vs ABMTRR 24.8% vs 22.1%)

Conclusions

This 27 year data set shows expected survival outcomes and treatment related mortality for a data set spanning more than 2 decades in which time there have been substantial changes in transplant practice. In acute leukemia transplant in CR1 is associated with improved outcomes. Transplantation of patients with relapsed ALL rarely results in long term survival. The results show that BMT recipients experience similar outcomes to patients transplanted elsewhere in Australia with disease and patient related risk factors having a significant impact on the prospect of cure.

Autologous Bone Marrow Transplantation (BMT) at Royal Perth Hospital (RPH) : 1974 – 2001

Cannell P K, Herrmann R P, Buffery S I and Trent M

Royal Perth Hospital, Wellington St, Perth.

Objectives: To report the results of autologous bone marrow and peripheral blood stem cell transplantation in our institution since the first procedure in 1981.

Methodology: The RPH transplant database was analysed with respect to routine transplant outcome measures including overall survival, 100 day transplant related mortality and relapse rate. Patients were grouped into standard disease defined risk groups and outcome measures were compared with published national and international results.

Results

	n	Overall Survival	100 day treatment related mortality	Relapse	Average Time from diagnosis to transplant – months	Median Age - years
ALL	4	0%	0%	100%	11	29
AML	51	47%	4%	49%	7	40
Hodgkins Disease	43	63%	0%	37%	23	33
Myeloma	73	77%	3%	10	51	
NHL	10	47%	4%	51%	16	42
	1					
Breast Cancer	7	43%	0%	57%	5	42
Germ Cell Tumour	6	50%	0%	67%	19	30
Rheumatoid Arthritis	9	100%	0%	-	-	46

A cohort of patients transplanted between 1992 and 1997 was compared with ABMTRR data – no significant difference in overall survival or TRM was found. (OS RPH vs ABMTRR 75%vs 73.4% p= 0.8, TRM RPH vs ABMTRR 2.3% vs 5.3%). Of the 12 patients transplanted prior to 1990, one with AML transplanted in CR2, remains alive and disease free. Multiple transplants were performed in 63 patients (GCT 4, HD 11, Myeloma 27, NHL 21) with a TRM of < 2%.

Conclusions

This 20 year data set shows expected survival outcomes and treatment related mortality. Multiple autografts can be safely undertaken with no increase in TRM. The greatest barrier to long term survival in patients undergoing autologous BMT remains disease relapse.

The RCPA CD34+ Cell QAP: Report of the first survey.

Annabella Chang (1)* & David DF Ma (2)

(1) Dept. Haematology, PaLMS. Royal North Shore Hospital. Sydney.

(2) Dept. Haematology & Stem Cell Transplantation. St. Vincent's Hospital. Sydney.

A CD34+ cell QAP has been established to ensure accurate and reliable CD34+ stem cell results for clinical decisions in the management of haemopoietic stem cell transplant patients, and for the accreditation of transplant laboratories. The first survey conducted in 2001 consisted of a mobilised peripheral blood (PB) and a peripheral blood stem cell harvest (PBSC). Data on instruments, reagents, methodology and results were collected from 36 centres.

The ISHAGE protocol was used by 29 centres, (23 dual, 6 single platform) with 7 centres using other methods (5 dual, 2 single platform) The distribution of the values obtained were summarised by boxplots (see table). The stem cell dose was calculated for a 76 kg patient.

Parameter	No. of results	Median	50% of results within	Smallest observed value not outlyer	Largest observed value not outlyer	No. with outlying values* values #	No. with extreme
PB wcc x 10 ⁶ /ml	34	8.5	7.8 – 9.2	6.2	10.4	2	1
PB % CD34	36	0.17	0.15 – 0.21	0.07	0.25	4	0
PB CD34/ul	35	14	12.4 – 19.4	3.3	28	3	2
PBSC wcc x 10 ⁶ /ml	34	33.8	32.2 – 35.7	7.3	39.9	1	1
PBSC %CD34	36	0.82	0.72 – 0.92	0.61	1.1	2	1
PBSC CD34/ul	35	286	253 - 320	216	393	2	5
PBSC CD34 x 10 ⁶ /kg	35	8.3	7.4 – 9.3	6.3	11.4	2	5

The submitted results showed clerical errors, miscalculations, or errors in the use of units. After correction of these errors, there were no longer any extreme values. In the clinical setting, these results could give rise to different decisions as to whether or not this patient is sufficiently mobilised to initiate leukapheresis, and the number of transplants available from this PBSC.

Although centres may have established their own internal ranges and correlations with their clinical data, CD34+ cell results need to be comparable between institutions in order to compare data, or for collaboration in multi-centre studies. While the boxplot shows the distribution of data, acceptable limits over different ranges needs to be defined for the CD34+ QAP to be clinically relevant. These acceptable limits will be established in consultation with transplant centres.

* Outliers are values that are between 1.5 and 3 box-lengths from the upper and lower edge of the box.

Extremes are values that are more than 3 box-lengths from the upper and lower edge of the box.

Novel cytogenetic abnormalities in two patients with myeloid malignancy.

Chen VMY* (1), Francis CH (1), Arthur CK(1).

(1) Department of Haematology, Royal North Shore Hospital, Sydney.

Cytogenetic abnormality is recognised as the most important variable in classification and prognosis in myeloid malignancy. We report two myeloid malignancies with novel chromosome translocations. The first patient presented with fatigue and splenomegaly. Morphological analysis of peripheral blood and bone marrow revealed classical features of chronic myeloid leukaemia. Cytogenetic analysis showed no evidence of the Philadelphia chromosome. Instead two translocations were present that have not previously been reported in association with myeloid malignancy: t(1;21)(q32;q22) and t(5;11)(q13;q21). Molecular gene rearrangements studies by nested PCR confirmed the presence of dual bcr/abl fusion products: b2a3 and E1a2. FISH analysis confirmed the presence of a masked Philadelphia chromosome, but with loss of the abl/bcr from chromosome 9 and a large deletion of sequences flanking the translocation - a finding previously correlated with poor prognosis. The patient was treated with ST1571 according to the accelerated phase protocol. At three months, he was in morphological remission and the two novel cytogenetic translocations were no longer detectable.

The second case is of a 49 year old man with acute myeloid leukaemia with multilineage dysplasia on morphological analysis, but no prior history of myelodysplasia. Cytogenetic analysis revealed an pericentric inversion of chromosome 3; inv3(p21;q23) which had not been previously reported in the literature. The inversion 3 persisted in 100% of metaphases after induction therapy at which time the marrow elements were replaced by an infiltrate of abnormal histiocytes. Analysis of peripheral lymphocytes by G banding revealed normal cytogenetics, confirming that the inversion 3 was related to the malignant clone and not a constitutive abnormality.

EPIC salvage chemotherapy for relapsed or refractory Non-Hodgkin lymphoma.

P. J. Crispin(1)*, L.A. Coupland(1), I.W. Prosser(1)

Clinical Haematology Unit, The Canberra Hospital, Canberra, ACT.

Objectives: A retrospective review of the efficacy and toxicity of EPIC salvage chemotherapy was performed in patients attending a single institution for treatment of relapsed or refractory NHL. EPIC consists of Etoposide 100mg/m² D1-4, Prednisolone 100mg PO D1-5, Ifosfamide 1g/m² D1-5 with MESNA, Carboplatin 240mg/m² D12, repeated q28 days.

Method: Patient characteristics including histological classification, disease staging and prior treatment were determined. Response and toxicity were analysed according to WHO criteria. G-CSF, fluconazole and cotrimoxazole were given prophylactically.

Results: 22 patients were treated. 12 patients have complete data sets and have been analysed. Mean age was 49 years (range 34 – 70) with median remission prior to EPIC of 9.4 months (0 – 36). 8 patients had diffuse large B-cell histology, with 3 follicular and one T cell. Pre-treatment LDH was elevated in 8 patients. Performance status was ECOG 1 in 8 patients (67%), 2 in 2 patients (25%) and 3 in 1 patient (8%).

Dose reduction for myelosuppression was required for etoposide (7 cycles), Ifosfamide (8 cycles), and carboplatin (2 cycles). Ifosfamide was omitted due to neurotoxicity (1 cycle), and carboplatin due to myelosuppression (7 cycles). Non-prophylactic antibiotics were required in 7 cycles.

6 patients (50%) achieved CR, 1 (8%) PR, and 5 (42%) had NR or PD. Overall response (CR + PR) was 58%. Mean duration of remission in responders was 227 days (61 – 290). 6 patients proceeded to autologous transplant (5 CR + 1 PR following EPIC) and 1 to allogeneic transplant (PD following EPIC). 1 patient who achieved CR was not transplanted. 2 patients (17%) remain in CR at 2 and 6 months following autologous transplant.

9 patients have died. Mean survival following CR/PR was 399 days (287 – 627), and in non-responding patients 255 days (158 – 544).

42 cycles of EPIC were given to 12 patients (mean 3.8, range 2-6). Haematological toxicity: Grade 4 neutropenia occurred in 11 cycles (26% - 6 patients), Grade 4 thrombocytopenia in 5 cycles (12% - 2 patients). Grade 3 anaemia occurred in 4 cycles (10% - 2 patients).

Conclusions: EPIC chemotherapy is generally well tolerated and can be administered in an outpatient setting. Haematological toxicity was dose limiting but extramedullary toxicity was minimal. Overall response was favourable (CR + PR = 58%) in comparison to other salvage regimens for aggressive NHL. Stem cell mobilisation was achieved in all patients for whom high-dose chemotherapy was considered appropriate. However, long-term survival is poor in this refractory group of patients despite good disease response.

Towards In Vivo Selection For Liver Directed Gene Therapy

JA Curtin*, PB Rowe, IE Alexander

Gene Therapy Research Unit, The Children's Hospital at Westmead and Children's Medical Research Institute, Locked Bag 4001, WESTMEAD NSW 2145.

Background: It remains a challenge to achieve stable, high level expression of a therapeutic transgene in a significant proportion of hepatocytes in vivo. Clonal expansion of gene-modified cells would provide a means to overcome inefficiencies of current delivery systems. This could be achieved by co-delivery of a therapeutic gene with a gene that provides a growth advantage when an exogenous selection pressure is applied. Increasing the ability of cells to produce glutathione (GSH), a natural cellular protectant, could be utilised in an in vivo selection strategy for gene therapy.

Aim: To show that cells with an increased capacity for GSH production can be selected using paracetamol.

Methods: 293 cells (human embryonic kidney cells) were serially transfected with plasmids encoding the heavy and light subunits of g-glutamylcysteine synthetase, (the rate limiting step in GSH synthesis) generating a polyclonal cell line 293HNClip. This cell line was characterised and the resistance to NAPQI, (the toxic metabolite of paracetamol) was determined by LDH release, clonogenic growth assay and mixing studies.

Results: The polyclonal cell line 293HNClip exhibited an enhanced capacity to synthesise GSH, with a doubling of their steady state GSH levels, a 1.5 fold increase in GCS activity, and resistance to GSH depletion by diethylmaleate. The 293HNClip cells demonstrated less cytotoxicity, (measured by LDH release) and increased survival (measured by clonogenic growth assay) on exposure to NAPQI. Mixing studies with marked, transiently transfected 293 cells and unmarked control cells, confirmed that it was possible to select the marked cells overexpressing GCS by exposure to NAPQI. Under optimal conditions it was possible to achieve a high degree of selection (to 97%) with a single dose of NAPQI.

Conclusion: Transfection of cells with the heavy and light subunits of GCS provides them with an increased capacity to produce GSH and a survival advantage when exposed to the toxic metabolite of paracetamol. These results establish "proof of concept" for our selection strategy and provide a solid foundation upon which in vivo studies will be built.

Equivalent Peripheral Blood Progenitor Cell Collections With Delayed G-CSF Introduction In A Cyclophosphamide Containing Mobilisation Schedule

Doocey R.T.*, Browett P.J., Norcott J., Petrasich M., Hawkins T.E.

Haematology Department, Auckland Hospital, Auckland

Department of Molecular Medicine, University of Auckland, Auckland

The role of granulocyte-colony stimulating factor (G-CSF) in combination with chemotherapy is well established in the mobilisation of peripheral blood progenitor cells (PBPCs) for use in autologous haematopoietic stem cell transplantation. G-CSF is usually given early in the mobilisation schedule to allow adequate predictability of the optimal time for leucapheresis and to increase PBPC yield whilst protecting against prolonged chemotherapy-induced neutropenia. There have been limited studies investigating the optimal time of commencing G-CSF in PBPC mobilisation. A later introduction may allow cost savings from decreased G-CSF dosing without compromising predictability or adequacy of PBPC collection.

A prospective analysis was performed on leucapheresis data for 16 patients (Group A) who were mobilised with Cyclophosphamide 2g/m² followed by G-CSF (Filgrastim) 5µg/kg/day administered from Day 6 until completion of adequate CD34⁺ cell collection. The data was compared with a cohort of 61 patients (Group B) who were mobilised on a protocol with Cyclophosphamide 2g/m² on Day 1 followed by G-CSF given on Day 2. Patient characteristics were equivalent for both groups including diagnosis, status of disease and previous cycles of therapy.

The days of G-CSF administration were significantly reduced in Group A as compared to Group B (mean 6.7 days vs 10.7 days, $p < 0.01$). There was no difference between the two groups in median number of days to first leucopheresis, median number of leucopheresis required to obtain a minimum of 2×10^6 CD34⁺ cells/kg and median CD34⁺ cell harvest yield. There were no failures of CD34⁺ cell harvesting or mobilisation related sepsis seen in Group A.

Equivalent PBPC yields can be obtained from the later introduction of G-CSF on Day 6 of a mobilisation schedule that commences with Cyclophosphamide 2g/m² on Day 1. This was achieved in our series without any failures of mobilisation or infection-related sequelae. The decreased growth factor consumption has beneficial resource implications with a potential cost saving per patient mobilised.

Multilineage dysplasia is an important prognostic factor in refractory anaemia and refractory anaemia with ring sideroblasts

S. Dunkley*(1), Y.L.Kwan (2,3) and A. Manoharan (3)

(1) Department of Haematology, Prince of Wales Hospital, Sydney.

(2) South Eastern Area Laboratory Services, St George Hospital, University of New South Wales, Sydney.

(3) Department of Clinical Haematology, St George Hospital, Sydney.

Introduction: The international prognostic scoring system (IPSS) represents the most useful prognostic index in myelodysplastic syndrome (MDS). MDS of the FAB classification refractory anaemia (RA) or refractory anaemia with ring sideroblasts (RARS) are most often classified as "low risk" by the IPSS as there is less than 5% blasts, by definition, and frequently normal cytogenetics. Further prognostic indices are therefore important in these subgroups. Morphological evidence of multilineage dysplasia is an important prognostic factor in MDS including cases of RA and RARS. These findings have led to the proposed WHO classification of MDS.

Aim: Patients with RA and RARS at St George hospital were studied to confirm the prognostic significance of multilineage dysplasia in these FAB subsets. The prognostic significance was examined along side the IPSS to evaluate whether multilineage dysplasia added further prognostic information.

Methods: We studied 64 consecutive patients with myelodysplastic syndrome, 44 with refractory anaemia and 20 with refractory anaemia with ring sideroblasts, and established their median survival in relationship to morphological evidence of dysplasia. Bone marrow aspirates were examined to see if they fulfilled previously defined criteria for lineage dysplasia. In addition, the IPSS was calculated.

Results: There was a significant difference in survival between refractory anaemia patients with single lineage dysplasia (32.8 months) and those with multilineage dysplasia (14.2 months) ($p < 0.05$). A significant difference was also observed in the combined group of refractory anaemia and refractory anaemia with ring sideroblasts patients with single lineage dysplasia and multilineage dysplasia, 36.3 months and 14.9 months respectively ($p < 0.01$).

Combining the IPSS and multilineage dysplasia in a prognostic scoring system enabled greater differentiation in survival groups than the IPSS alone (IPSS "low" risk: 42.5 months with single lineage dysplasia and 11.1 months with multilineage dysplasia ($p < 0.05$); IPSS "INT-1" risk: 29.6 months without multilineage dysplasia and 10.2 months with multilineage dysplasia ($p < 0.05$)).

Conclusion: Morphological evidence of multilineage dysplasia is an important risk factor for RA and RARS. When combined with the IPSS it adds further prognostic definition. These findings support the proposed WHO classification of myelodysplastic syndromes.

Development of a Haematology Advanced Practice Nurse Role in the Acute Care Setting.

S.Eerhard*, Clark, L, Spencer, D, Gale, J & Prince, M

Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia, 8006.

The development of a Nurse Practitioner role reflects the continuing evolution and strengthening of professional nursing in Australia. It substantially extends the career pathway for clinical nurses and recognises expert nursing practice. This role enables the nurse to conduct comprehensive health assessments, demonstrate a high level of autonomy and possess expert skills in the diagnosis and treatment of complex responses of individuals and their families to actual or potential health problems and experiences. The Nurse Practitioner formulates clinical decisions to manage acute and chronic illness and promote wellness. Nurses in advanced practice integrate clinical expertise with ethical decision-making, collaboration, consultation, education, research, and clinical and professional leadership. These skills applied in patient focused care can facilitate improved patient access and continuity of care, and promote safe and best practice. The Peter MacCallum Cancer Institute (PMCI) Haematology Nurse Practitioner Project was one of eight Nurse Practitioner Models of Practice funded by the Victorian Government Department of Human Services from May 1999 to June 2000 to both explore, and demonstrate sustainable advanced practice roles in nursing which enhance health service delivery. This presentation will demonstrate how the PMCI model aims to further develop and adjust the boundaries of advanced haematology nursing practice in the acute care setting, within a collaborative multidisciplinary health care team.

Establishment of cDNA microarray technology for analysis of gene expression profiles in haematological diseases

T.H. Loi * (1) D.D.F. Ma (1) presented by Thomas Exner

(1) Department of Haematology and Stem Cell Transplantation, St Vincent's Hospital, Sydney.

Microarray analysis is a new and powerful technique which can be used to analyse expression levels of multiple genes simultaneously. This technique is particularly useful in distinguishing between normal/control and diseased/test tissue. The aim of our study is to establish and optimise the cDNA microarray technique and to utilise it in the investigation of differential gene expression in (1) apoptotic cells induced by bcl-2 inhibition; (2) selected cells from patients undergoing bone marrow transplant and patients treated for lymphoma using MabThera and CHOP. The Microarray technique is divided into 3 main steps: 1) Fabrication of human cDNA microarrays on glass slides, 2) RNA extraction and fluorescent probe production followed by DNA hybridization and detection, and 3) Data analysis of the relative gene expression in all genes represented on the array. The arrays used in the experiments are made by the Microarray Consortium based at the University of New South Wales. The fluorescent probes are produced by reverse transcribing RNA, in the presence of either cyanine 3 or 5 -dUTP nucleotides to be incorporated into the cDNA. Our preliminary experiments using 96 human cDNA microarray revealed distinct gene expression patterns between normal peripheral blood and Chronic Lymphocytic Leukemia samples, and between CLL and a B cell Non-Hodgkins Lymphoma cell line. Furthermore, it was found that 10-20µg of RNA is required to permit reproducibility. Due to the small number of cells obtained from some specimens, we have investigated an RNA amplification strategy to compensate for the limited RNA extracted. In the near future, we plan to use 4000-8000 gene microarrays to identify distinct gene expression profiles and to determine the molecular pathways involved in the pathogenesis of disease.

A retrospective review of toxicity and efficacy of hyperfractionated total body irradiation (TBI) with lung shielding at a tertiary referral centre.

Michael Fay^{1*}, John Seymour², Michael MacManus¹, Andrew Wirth¹, Gail Ryan¹, Miles Prince² and Andrew Grigg³.

1 Dept. of Radiation Oncology, Peter MacCallum Cancer Institute. Melbourne.

2 Dept. of Haematology and Medical Oncology, Peter MacCallum Cancer Institute, Melbourne.

3 Dept. of Haematology, Royal Melbourne Hospital, Melbourne.

Hyperfractionated TBI with lung shielding is a relatively new technique to Australia and has been used as part of the conditioning regimen prior to allogeneic and high risk autologous bone marrow/stem cell transplantation (BMT) at Peter MacCallum Cancer Institute. Unlike other TBI techniques, partial transmission blocks are placed over the lungs to reduce the central lung dose by 50%. We have retrospectively reviewed the outcome for all patients treated with this technique between May 1998 and June 2001. Clinical and treatment details were obtained from medical records. Toxicity was scored using "common toxicity criteria" version two.

A total of 32 patients received hyperfractionated TBI as part of their conditioning regimen for BMT. Median age=33 (range 16-57) years. There were 12 females. Underlying diseases were chronic myeloid leukaemia (n=1), chronic lymphocytic leukaemia (n=1), acute myeloid leukaemia (n=3), acute lymphocytic leukaemia (n=13), Hodgkin's disease (HD)(n=6) and non-Hodgkin's lymphoma (NHL)(n=8).

In the cohort treated for HD+NHL there was one allogeneic BMT. The most serious toxicity was interstitial pneumonitis (2 of 14 patients, 14%). One patient died with pneumonitis; the other was subclinical. Both patients were being treated for HD and had previously received bleomycin containing chemotherapy. One patient developed acute pericarditis. Nausea and vomiting were the most common acute toxicities; occurring in more than 90% of patients (grade 2-3). No veno-occlusive disease was seen. The 3yr actuarial survival rate was 54%(+/-14%).

We conclude that this regimen is well tolerated and has a low incidence of late toxicity with only one late toxicity greater than grade one.

Reduced CD5 Fluorescence in the identification of T large granular lymphocytosis.

Susan Francis*, Harry Kronenberg, Douglas Joshua.

Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW.

Large granular lymphocyte (LGL) disorders can arise from a CD3+ T-cell lineage or from a CD3- NK-cell lineage. CD3+ T-LGL lymphocytosis/leukaemia is the most frequent form of LGL leukaemia and usually affects older people. This T cell population is not always evident but with CD5 surface marking phenotyping this can be identified. The main clinical manifestations are neutropenia (with resulting recurrent infections), anaemia and thrombocytopenia and there is a strong association with rheumatoid arthritis. Bone marrow, liver and spleen involvement is common in T-LGL leukaemia. T-LGL lymphocyte clonal expansion has been shown to inhibit erythropoiesis. Adult-onset-cyclic neutropenia is also associated with T-LGL leukaemia. The clinical presentation therefore can be varied. The most common phenotype of the T-cell population is CD3+, CD8+, CD57+, TCR-alpha/beta+. On presentation not all patients have a lymphocytosis and when present it is usually only moderate. Cases with normal lymphocyte counts therefore may be difficult to diagnose. CD3+CD8+ phenotyping histograms and/or normal to slightly low CD3+CD4+/CD3+CD8+ T cell ratios may show no indication of an abnormal T-LGL cell population. In this study we evaluated 16 patients (age 21-80 years, average 55 years) all of whom have been phenotyped as having the T-LGL CD3+CD8+CD57+ phenotype. Their WCC range 2.6-14.6 $\times 10^9/L$, neutrophils 0.4-4.8 $\times 10^9/L$ and lymphocyte counts 1.0-10.3 $\times 10^9/L$. A characteristic common to all the patients was a decrease in CD5 fluorescence on the CD3+CD8+ T cell population and this can even lead to the appearance of two distinct CD5 populations. This decrease in CD5 fluorescence varied considerably between patients but was invariably present in all cases and thus proved to be a constant marker for the T-LGL phenotype. This characteristic therefore is a very easy and useful marker for the identification of T-LGL lymphocyte populations.

Use of CD80 (B7.1) to enhance the allo-immune response to melanoma and myeloid leukaemia

A Bartlett (1), M Marangolo (1), J Li (1), M. Millington (2), KF Bradstock (1), LJ Bendall (1), DJ Gottlieb (1)*

1 Leukaemia Research Laboratory, Westmead Institute of Cancer Research, University of Sydney at Westmead Hospital, Sydney

2 Johnson and Johnson Research Laboratories, Strawberry Hills, Sydney

The co-stimulatory molecule CD80 (B7.1) is important in the generation of a primary immune response. Many tumours do not express CD80 and may therefore be relatively protected from the effects of anti-tumour immune responses. Tumour expression of CD80 could be applied to the generation of donor-derived anti-tumour cells for use in adoptive therapy after allogeneic stem cell transplantation.

Objective of study: to assess the effect of tumour CD80 (B7.1) expression on generation of anti-tumour responses from HLA identical siblings of patients with melanoma and myeloid leukaemia

Methodology: A CD80 negative tumour cell line (WMMH) was derived from the biopsy of a metastatic melanoma lesion. A retroviral construct coding for CD80 was used to transduce the cell line. A mixed lymphocyte tumour culture (MLTC) was established using peripheral blood mononuclear cells from the patient's two HLA identical siblings. A similar approach was taken with malignant cells from patients with myeloid leukaemia. Proliferation and cytotoxicity of MLTC cells to the parent tumour cells was studied.

Results: Proliferation and cell number in culture were increased when HLA identical donor lymphocytes were incubated with WMMH/B7.1 compared with WMMH (3H uptake 3280+169 cpm v. 1933+121 cpm and cell count 1.8 v. 0.5 $\times 10^6$ respectively). Cells from one of the two HLA identical donors stimulated with WMMH/B7.1 killed the stimulating cells as well as WMMH transduced with the empty vector pLXSN and parent untransduced cells in chromium release assays (mean cytotoxicity 23+3% at E:T ratio of 25:1 against untransfected parent cells). Untransduced myeloid leukaemia cells (3 AML, 1 CML) induced 10 fold proliferation of lymphocytes from HLA identical donors in mixed cultures in 2 of 4 cases but cytotoxicity was generated only in one of these (AML). An attempt was made to transduce leukaemic cells from patients with AML (n=5) and CML (n=1) with a retroviral vector encoding CD80. Only one AML case and the case of CML demonstrated increased CD80 expression following 48 hours incubation on a CD80 producing packaging cell line. In two AML cases, lentiviral infection with a construct encoding green fluorescent protein demonstrated significantly higher expression than seen using a retrovirus.

Conclusion: Expression of CD80 in tumour cells may help in expansion of anti-tumour lymphocytes derived from HLA identical donors. However lack of tumour cell proliferation results in poor retroviral infection of AML cells. Alternative methods of enhancing CD80 expression including lentiviral infection need to be explored.

CD83 Expression in Dendritic Cell / T-lymphocyte Interactions

D. J. Munster, M. Kato, D. J. N. Hart*, K. P. A. MacDonald.

Mater Medical Research Institute, Brisbane.

CD83 is a member of the immunoglobulin superfamily and is expressed predominantly on the surface of activated dendritic cells (DC). Gaps remain in the characterization of the expression of this molecule and data regarding a potential ligand is conflicting. Its role in DC interactions is unknown although preliminary data has been published describing anti-CD83 antibody mediated blockade of lymphocyte function.

We employed flow cytometry to characterize the expression of CD83 in co-cultures of DC and T-cells, and this was correlated with anti-CD83 mediated blockade of the allogeneic mixed lymphocyte reaction (MLR).

We confirmed that, although cell surface expression of CD83 is minimal on immature monocyte derived DC (MoDC), it is present in the cytoplasm and it is released as a soluble form (sCD83). LPS activation of MoDC consistently induced surface CD83 expression on MoDC and enhanced secretion of sCD83. In contrast, surface CD83 was not consistently induced on MoDC when co-cultured with allogeneic T-cells even though the T-cells were induced to proliferate. The previously observed and presumed functional blockade of the allogeneic MLR with rabbit polyclonal anti-CD83 (RA83) was confirmed using immature MoDC as stimulators, despite the sometimes minimal expression of CD83 on the latter cells. However, we found that the blockade was due to antibody-dependent cellular cytotoxicity (ADCC) by co-purifying NK-cells. Inhibition was not observed when highly purified T-cells were used instead of erythrocyte-roseette purified T-cells in the allogeneic MLR, but blockade could be restored by adding NK-cells.

Prior fixation of the MoDC did not abrogate blockade, and blockade also occurred when purified T-cells were stimulated with immobilized CD3 and CD28 in the presence of RA83 and NK-cells. We concluded that other cells important in the MLR expressed surface CD83 and this was confirmed by flow cytometry. After 96 hr of co-culture of T-cells with allogeneic MoDC we found that CD4⁺ CD25⁺ T-cell blasts expressed CD83.

In conclusion, anti CD83-mediated NK-cell ADCC can be used to deplete allo-reactive T-cells and this may be useful in allogeneic bone marrow transplantation. The function of CD83 remains to be determined but its identification on a subset of responding T-cells generates a new direction to the investigation.

Microangiopathic haemolysis (MAHA) induced by Sirolimus (Rapamune)

Chi-hung HUI

Haematology unit, Institute of Medical & Veterinary Science, Frome Road, Adelaide, Australia

Introduction: Sirolimus is a recently available immunosuppressant structurally similar to tacrolimus, with reported successes in the prophylaxis and treatment of acute renal allograft rejections as well as rejections associated with other solid organs and stem cell transplantation. Its toxicity profile, distinct from cyclosporin (CyA), includes dyslipidaemia and thrombocytopenia. Thrombotic thrombocytopenic purpura (TTP), however, has never been reported in the medical literature except a single report in an abstract. We report a case of TTP with an obvious temporal relationship to Sirolimus and highlight the peculiar features.

Case report: A 41-year-old woman with cystic fibrosis had bilateral lung transplantation in 1994. She had been on triple immunosuppressants since transplant but was complicated by bronchiolitis obliterans. She developed progressive renal failure 6 years later when a renal biopsy revealed severe glomerulosclerosis, scarring and arteriolar hyalinosis but no intra-glomerular thrombi, which was believed to be due to a combination of CyA toxicity, hypertensive and diabetic nephropathy. The cyclosporin was substituted by sirolimus at 0.6 ml/day with prednisolone, azathioprine, without any change in her other medications. One month after sirolimus, she developed anaemic symptoms when blood smear revealed florid red cell fragmentation not dissimilar to those in TTP-HUS. Investigations showed direct Coombs negativity, reduced haptoglobin, mildly raised LDH (peak 820), but no evidence of coagulopathy or haematinics deficiency (iron, folate, vitamins E, B1 & B12). Echocardiogram showed no abnormality. No occult malignancy could be detected. When sirolimus was ceased in December, the MAHA disappeared in 2 weeks and she became transfusion-independent.

Discussion: The temporal relationship with sirolimus strongly suggested a drug-induced red cell fragmentation. Unlike the first reported case, the onset was insidious and the full-blown picture of TTP was absent in our patient. Other than the haemolysis, there was no neurological deficit or thrombocytopenia, and plasma exchange was not required. Nevertheless, sirolimus should be considered as a differential diagnosis in drug-related MAHA and not-so-typical presentation of TTP.

Use of an IgG monoclonal antibody against tumour necrosis factor alpha (TNF alpha) in two patients with haemophagocytic syndrome.

Jackson D.S.W. (1)*, Arthur C. (1), Isbister J. (1)

(1) Haematology Department, Royal North Shore Hospital, Sydney.

Haemophagocytic syndrome can occur in association with infection or malignancy, or rarely be congenital. Reactive forms have been described in association with elevated TNF α levels. We describe two patients, both with malignant lymphoproliferative disorders, who presented with clinical features of the haemophagocytic syndrome and biopsy confirmed haemophagocytosis. Each were treated initially with multiagent chemotherapy and then followed by weekly intravenous infusions of infliximab, a monoclonal IgG antibody that binds to human tissue necrosis factor alpha. Both patients presented with pancytopenia, elevated liver function tests, coagulopathy, fever, sweats, hepatosplenomegaly, gastrointestinal symptoms and weight loss. Bone marrow biopsies confirmed the presence of haemophagocytosis with proliferating histiocytes and marked haemophagocytosis. Initially therapy with multiagent chemotherapy was given with vincristine, cyclophosphamide and methylprednisolone however there was no initial clinical or laboratory evidence of improvement. Infliximab was given intravenously and in one patient there was a progressive improvement in liver function, full blood count parameters, resolution of fevers and gastrointestinal symptoms. In the second only a temporary improvement in blood parameters occurred. Unfortunately the first patient died of overwhelming sepsis and the second of progressive haemophagocytosis. Notably infliximab has been associated with severe sepsis in patients concurrently on immunosuppressive therapy. These cases highlight the potential use of TNF α blockade in patients with haemophagocytic syndrome.

A Phase II Study of Liposomal Doxorubicin (Caelyx) in Combination with Vincristine and Dexamethasone in Multiple Myeloma

H Januszewicz, J F Seymour, H M Prince, M Wolf, D Westerman, C O'Kane,

Department of Haematology, Peter MacCallum Cancer Institute, East Melbourne, VIC.

Doxorubicin is an effective drug in the treatment of multiple myeloma. Liposomal doxorubicin has several advantages over doxorubicin (1) longer half life of 40 hours, approximately 100-fold over doxorubicin, therefore enabling chemotherapy to be given in one day rather than a four day infusion (2) liposomal encapsulation may overcome multi-drug resistance and (3) concentration of the drug within tumours.

We treated 15 patients from Feb 2000 to June 2001. Treatment consisted of Caelyx 40 mg/m² IV over 2 hours, vincristine 1.4 mg/m² stat. and dexamethasone 40 mg/d po for four days. Cycles were repeated every 28 days for four to six cycles. Pyridoxine 150 mg/d po was given to prevent muco-cutaneous toxicity (palmar plantar erythrodysesthesia or PPE). Eight were newly diagnosed and seven were relapse patients. Age ranged from 34-78 with a median of 63 years. One patient had previous VAD and PBSC autograft. Of the other relapse patients, three had anthracycline combinations and three had melphalan and/or cyclophosphamide.

Nine patients have had three or more courses and are evaluable for response. Five of the eight patients achieved a partial response and four stable disease. One patient continued to progress.

Serious adverse events were three anaphylactic reactions, two in the first cycle and one in the second. Two of the patients continued to receive further courses with pre-medication. Five patients developed PPE, all grade 1 to 2. One patient had grade 3 oral mucositis. Two patients were admitted with febrile neutropenia and no patient required platelet transfusion.

Caelyx VAD is a tolerable and effective regime in multiple myeloma.

Multilectin Receptor DEC-205 is Widely Expressed but Differently Regulated by Blood Leucocytes Including Dendritic Cells.

Kelli MacDonald, David Munster, Derek Hart and *Masato Kato

Mater Medical Research Institute, South Brisbane, Queensland.

DEC-205 belongs to the macrophage mannose receptor family of multilectins, and is a putative antigen-uptake/processing receptor for dendritic cells (DC). We generated the MMRI-7 hybridoma from mice immunized with a panel of DEC-205-Ig fusion proteins. The MMRI-7 mAb recognized the DEC-205-Ig containing carbohydrate recognition domain 1 and 2 by ELISA, and a single 200 kDa band in KM-H2 cell lysate by Western blot analysis. MMRI-7 and another DEC-205 mAb M335 (recognizing the cysteine-rich domain), were used to examine DEC-205 expression by peripheral blood mononuclear cells and in lymphoid tissues. DEC-205 was expressed at moderate to high levels on freshly purified blood CD11c⁺ myeloid (+) and CD11c⁻ plasmacytoid DC (+), CD14⁺ monocytes (++) and CD19⁺ B (++) cells but at low levels on CD3⁺ T (+) and CD16⁺/56⁺ NK cells by flow cytometry. Following activation by overnight culture in RPMI 1640/FCS, both blood DC subsets increased cell surface DEC-205 markedly. After LPS activation, monocyte DEC-205 expression reduced markedly, however, B cell levels increased slightly. DEC-205 expression on T and NK cells was unaffected by PMA/calcium ionophore activation. In tonsil, both DC subsets expressed high levels of cell surface DEC-205, whereas other leucocytes expressed lesser levels. Thus, DEC-205 is widely expressed, not only on DC, but also on most leucocytes. DC increase cell surface DEC-205 upon activation, however, lineage⁺ cells appear to regulate their expression differently, depending on the cell type. DEC-205, which is also expressed on thymic epithelia, may play multiple roles depending on the cell type.

Neutrophil dysplasia characterized by a pseudo Pelger-Huet anomaly occurring with the use of mycophenolate mofetil and ganciclovir post renal transplantation: a report of 5 cases

Glen A Kennedy (1)*, Troy D Kay (2), David W Johnson (2), Carmel M Hawley (2), Scott B Campbell (2), Nikky M Isbel (2), Paula Marlton (1), Ralph Cobcroft (1), Devinder Gill (1) and Gavin Cull (1)

1 Department of Haematology, Princess Alexandra Hospital, Brisbane

2 Department of Nephrology, Princess Alexandra Hospital, Brisbane

Aim. The pseudo Pelger-Huet (PH) anomaly is characterized by reduced nuclear segmentation and excessive chromatin clumping in neutrophils, and has been associated with a variety of primary haematological disorders, infections and drugs. Recently, the development of dysgranulopoiesis characterized by a pseudo PH anomaly has been reported in 2 patients with the use of mycophenolate mofetil (MMF) in the setting of heart +/- lung transplantation.

Methods. We present a further 5 cases of MMF-related dysgranulopoiesis characterized by a pseudo PH anomaly occurring post renal transplantation. In our series, however, the observed dysplastic granulopoiesis appeared related to the combination of MMF and ganciclovir.

Results. The 5 cases were identified over a 12 month period, during which time 105 renal transplants were performed at our institution. Median age was 48 years (range 35 to 62 years). All patients received standard immunosuppression protocols consisting of MMF, prednisolone and cyclosporin or tacrolimus. Oral ganciclovir was used for CMV prophylaxis in each case. All cases had normal neutrophil morphology and white blood cell differentials on review of blood films pre / peri transplantation. The development of dysgranulopoiesis occurred a median of 96 days (range 66 to 196 days) after transplantation. There was no association between the development of dysgranulopoiesis and underlying renal disease, transplant type (cadaveric vs live unrelated) or other complications. Moderate or severe neutropenia ($<1.0 \times 10^9/L$) developed in 3 cases, and appeared to be directly correlated with the percentage of circulating dysplastic neutrophils. Cytogenetic analyses performed on bone marrow aspirate samples in 3 cases were normal. In all cases the dysgranulopoietic changes were reversible with dose reduction and / or cessation of both MMF and ganciclovir, within a median of 61 days (range 26 to 175 days) after initial development.

Conclusion. We have identified 5 cases of the pseudo PH anomaly occurring with the combination of MMF and ganciclovir post renal transplantation. This was associated with significant neutropenia in 3 cases. Given the increasing use of both MMF and CMV prophylaxis in transplantation, this pattern of drug-related bone marrow toxicity could potentially be increasingly recognized. We recommend that all patients receiving these drugs have regular peripheral blood films reviewed; if dysplastic granulopoiesis develops, dose reduction and / or cessation of both MMF and ganciclovir should be considered until the dysgranulopoietic changes resolve.

Modulation of hydroxylase levels in bone marrow stroma: a potential mechanism for regulation of apoptosis in haemopoietic progenitors

J McLeod(1), R. Sparrow(2), K Patton(2), MA Kirkland(1)*

(1) Douglas Hocking Research Institute, Barwon Health, Geelong, Victoria

(2) Research Unit, Australian Red Cross Blood Service, Southbank, Victoria

Background: Oxysterols (OS) are a group of hydroxylated derivatives of cholesterol with diverse biological functions. They have been implicated in regulation of cholesterol synthesis, cell cycle regulation and cytotoxicity, through the induction of apoptosis, in a number of cell types.

We have previously reported that one of the family of OS binding proteins (ORP-3) is upregulated in haemopoietic progenitors. Further, we have demonstrated that a number of OS can induce apoptosis in CD34+ cells from bone marrow and cord blood, and inhibit the growth of CFU-GM and haemopoietic cell lines.

Aim of Study: To assess the potential physiological relevance of these findings we have examined the regulation in bone marrow stroma of hydroxylases responsible for the enzymatic production of OS.

Methods: Primary bone marrow stroma cultures were exposed to a range of cytokines for 24 hours before lysis and extraction of RNA. Expression of 25 hydroxylase and 7 α hydroxylase was assessed by Real Time PCR.

Results: These studies have demonstrated upregulation of both hydroxylases by IL3, GM-CSF, and maximally by IFN α and TNF (>10 fold). Results with M-CSF were variable. TGF β upregulated 25 hydroxylase but not 7 α hydroxylase.

Conclusions: These results suggest that hydroxylase enzyme levels, and therefore potentially OS production, may be regulated in bone marrow stroma by cytokines and inhibitors. Combined with our earlier findings this raises the possibility that modulated production of OS by stroma may be a mechanism for controlled induction of apoptosis in haemopoietic progenitors.

Hereditary Hyperferritinaemia-Cataract Syndrome - The Australasian Complement.

Janet McLeod^A, Jamie Craig^B, Sarah Gumley^A, Sarah Roberts^A, Mark Kirkland^{*A}

A Douglas Hocking Research Institute, Barwon Health, Geelong, Vic 3220

B Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Vic 3000

Hereditary hyperferritinaemia-cataract syndrome (HHCS) is a rare autosomal dominant condition which presents clinically as high serum ferritin levels (600 to >3000µg/L) in the presence of normal iron saturation, and a distinctive cataract morphology due to accumulation of ferritin deposits in the lens. These features are the result of point mutations or deletions in the iron responsive element (IRE) at the 5' end of the ferritin light chain gene. Under normal circumstances, iron regulatory protein (IRP) binds to the IRE, suppressing L-ferritin translation. Mutations in the IRE prevent IRP binding and result in excessive unregulated ferritin production independent of iron levels. To date, ten different mutations and three deletions in the L-ferritin IRE have been identified.

Using PCR amplification of genomic DNA, restriction digest and sequencing analysis, we have identified a pedigree containing ten members who share a previously described mutation at position ±40 of the L-ferritin IRE (A-G, Paris-1). In addition, the characteristic pattern of high serum ferritin levels, in the absence of other causes of hyperferritinaemia, and cataract formation at an early age, has allowed clinical identification of a further three pedigrees.

DNA analysis of the first of these three pedigrees revealed a novel mutation (C-A) at position ±39 in the L-ferritin IRE in affected family members in two generations.

The second pedigree presented as an isolated case of bilateral cataracts in an eight year old child; which we have identified as a sporadic mutation at position ±32 (G-U), a mutation that has previously been reported in HHCS. In this pedigree both parents do not carry the mutation, have normal ferritin levels and are free of cataract.

The third pedigree is a small New Zealand kindred where two family members have presented with unexplained high serum ferritin (1200-1300µg/L). However, cataracts have not been detected in either affected individual. Molecular analysis of the L-ferritin gene in this kindred has indicated no mutations are present in the IRE. We are currently investigating the ferritin heavy chain gene IRE in light of a recent report identifying high ferritin and iron levels associated with an H-ferritin IRE mutation at position A49U in a Japanese kindred.

HHCS is considered a rare cause of elevated serum ferritin levels and cataract, though our findings suggest that it may be more common than previously thought. It is important that this condition be distinguished from haemochromatosis as it is not associated with iron overload and venesection is inappropriate.

A Highly Effective Salvage Regime for Relapsed/Refractory Multiple Myeloma

Stephen Larsen (1)*, Monica Callachor (1), John Gibson (1), Mark Hertzberg (2), Harry Iland (1), Graham Young (1), Doug Joshua (1).

(1) Institute of Haematology, Royal Prince Alfred Hospital, Sydney.

(2) Department of Haematology, Westmead Hospital, Sydney.

Introduction

Most patients with multiple myeloma who initially respond to standard chemotherapy eventually relapse. The treatment of relapsed disease is problematic with only limited therapeutic options available. There has been marked interest in the anti-angiogenic agent thalidomide and recent studies suggest that when dexamethasone is given in combination with thalidomide efficacy is greater. We report on a regime that combines thalidomide and dexamethasone with combination chemotherapy.

Objective

To determine the efficacy and toxicity of DT-PACE in patients with refractory or relapsed multiple myeloma.

Method

Eight patients with refractory or relapsed multiple myeloma were treated using dexamethasone 40mg daily for 4 days; thalidomide 400mg daily continuously; cisplatin 10mg/m²/day for 4 days as a continuous infusion; doxorubicin 10mg/m²/day for 4 days as a continuous infusion; cyclophosphamide 400mg/m²/day for 4 days as a continuous infusion and etoposide 40mg/m²/day for 4 days as a continuous infusion. One patient received reduced doses due to renal failure. The interval between cycles was 4 weeks. The average age was 47 years (range 29 to 62). All patients had previously been treated with combination chemotherapy and all but 2 had received high dose chemotherapy with autologous stem cell support. The remaining 2 patients had been treated with 6 courses of PCAB (prednisone, cyclophosphamide, doxorubicin and carmustine).

Results

Reassessment data is available after 2 courses of DT-PACE in 6 patients; 5 patients had ≥ 75% regression (CR) and 1 patient had ≥25% <50% regression (<PR). In 1 patient reassessment after 1 course of DT-PACE showed ≥75% regression. The remaining patient died from complications of disease a few days after receiving the first course. In 4 of the patients who achieved CR, serum paraproteinaemia was reduced to undetectable levels. The regimen was very well tolerated. The average day of neutrophil recovery to > 1 x 10⁹/L was 15 (range 13 to 23). Five patients developed febrile neutropenia requiring admission to hospital however there were no life threatening infections.

Conclusion

Our results demonstrate that DT-PACE was very effective with minimal toxicity in patients with relapsed/refractory multiple myeloma.

Once Weekly Erythropoietin (Epo) is Effective in Improving Hb levels in Haematological Disease.

Minakshi Taparia and Michael F Leahy*,

Department of Haematology, Fremantle Hospital, Western Australia

Anaemia is a major problem in the management of haematological diseases and is associated with fatigue, dizziness, headache and decreased quality of life. Blood transfusion is the mainstay of treatment in patients with chronic anaemia associated with haematological malignancy and chemotherapy. However, blood is becoming an increasingly scarce resource especially with the reduction in donor pool because of vCJD. Erythropoietin in addition to improving the Hb is known to have beneficial effects on quality of life (QoL) separate from that associated solely with the rise in Hb. Typically Epo has been administered thrice weekly but this is often disliked by patients especially those unable to self administer. Once weekly Epo has been shown to be as effective at increasing Hb and improving QoL. This mode of administration has not been routinely used in Australia.

The Aim of this study was to assess the value of Epo in the management of anaemia associated with haematological disease in the standard dose of 10,000 units thrice weekly or 40,000 units once weekly. Patients were either transfusion dependent or symptomatic of anaemia without previous transfusions. Seven patients received Epo 10,000 units SC thrice weekly and seven patients 40,000 units SC once weekly.

Of those receiving the thrice weekly dose, four had myeloma, one CLL and two MDS. The myeloma patients all responded with a rise in Hb (mean 89 g/l to 119 g/l). One MDS patient responded the Hb rising from 92g/l to 111g/l. The CLL patient, who was to receive radiotherapy for a metastatic squamous carcinoma responded well, the Hb rising from 103 g/l to 126 g/l. Of those receiving 40,000 units once weekly two had myeloma; both responding with rises in Hb (mean 9.3 to 11.3). Of three with MDS one responded: the Hb rising from 96g/l to 124g/l. A patient with red cell aplasia unresponsive to immunosuppressives responded, the Hb rising from 96 g/l to 156 g/l. A patient with HCL with pancytopenia and severe anaemia related fatigue who refused a blood transfusion also responded well, the Hb rising from 110g/l to 124 g/l. Those on thrice weekly injections expressed a dislike of the frequency of injections, considering once weekly preferable treatment.

This small study indicates that Epo may be as effective when administered once weekly as when given thrice weekly. This may lead to improved compliance and reduced overall costs relating to the use of this expensive drug. Greater use of Epo in selected patients will reduce transfusion requirements and release blood for more acute clinical situations.

Patient Number	Diagnosis	Epo	Dose	Predose	Hb	Max	Response	QoL
1	MDS	40000						

Expression studies of angiogenic-inducing agents in acute myeloid leukaemia

Lay-Cheng Lim*, Gee-Fung How.

Dept of Hematology, Singapore General Hospital, Singapore

Recent studies suggest an important role for angiogenesis in the pathogenesis of acute myeloid leukemia (AML). The 2 most potent and specific angiogenic regulators are vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (bFGF). To study their possible roles in AML, reverse transcription-polymerase chain reaction (RT-PCR) was performed to determine mRNA expression of VEGF and bFGF in bone marrow aspirate samples from 112 newly diagnosed AML patients. Bone marrow samples from 16 healthy marrow donors were also studied.

Number of samples with or without VEGF/bFGF expressions (%)					
FAB subtype	VEGF-/bFGF-	VEGF+/bFGF-	VEGF+/bFGF+	VEGF-/bFGF+	Total
AML-M0	0 (0)	1 (50)	1 (50)	0 (0)	2
AML-M1	7 (18)	12 (32)	17 (45)	2 (5)	38
AML-M2	6 (14)	10 (23)	25 (58)	2 (5)	43
AML-M3	4 (40)	4 (40)	2 (20)	0 (0)	10
AML-M4	0 (0)	0 (0)	2 (100)	0 (0)	2
AML-M5	2 (29)	1 (14)	4 (57)	0 (0)	7
AML-M6	0 (0)	2 (25)	4 (50)	2 (25)	8
AML-M7	1 (50)	0 (0)	1 (50)	0 (0)	2
Total	20 (18)	30 (27)	56 (50)	6 (5)	112
Normal	7 (44)	1 (6)	6 (37.5)	2 (12.5)	16

77% of AML patients expressed VEGF transcripts while 55% expressed bFGF transcripts. In contrast, 44% and 50% of normal controls expressed VEGF and bFGF respectively. The results suggest a possible role of VEGF in mediating angiogenesis in AML. Further studies to establish if leukemic cells secrete VEGF would be important.

The mechanism of apoptosis induction by STI571 in the bcr/abl expressing cell line K562.

RM Domaschenz, TP Hughes and *AB Lyons

Melissa White Laboratory, Hanson Centre for Cancer Research, Division of Haematology, IMVS, Frome Rd., Adelaide SA.

Chronic Myeloid Leukaemia (CML) is characterised by expression of the bcr-abl fusion protein, which results in deregulation of cell growth due to constitutive tyrosine kinase activity. Targeting of the bcr-abl tyrosine kinase activity is a potentially valuable form of therapy. A newly developed tyrosine kinase inhibitor STI571 (Glivec, Novartis) blocks bcr-abl kinase activity, inducing apoptosis in CML cells and has shown remarkable efficacy in obtaining haematological and cytogenetic responses in CML patients. Our study aimed to examine the kinetics of apoptosis induction and to determine the pathway of apoptosis induced by STI571.

The bcr-abl expressing human cell line K562 was used as our model system. After culture with STI571, the kinetics of apoptosis induction was examined using Annexin V in combination with the nuclear stain 7-AAD and analysis by flow cytometry. In addition, the flow cytometric data was used to determine appropriate times during the initiation of apoptosis to measure the expression of several apoptosis-associated proteins, using western blotting and quantification using a fluorimager.

The flow cytometric examination of the kinetics of apoptosis induction showed that STI571 recruited K562 cells into apoptosis sequentially over the culture period. It is likely that STI571 induces apoptosis in an indirect way, starting with inhibition of tyrosine kinase activity, followed by later effects as a number of signal transducing molecules are perturbed. STI571 sensitivity may also be influenced by cell cycle position.

Western blotting demonstrated that the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL exhibited a rapid decline to six and three fold decrease respectively after 48 hours culture in the presence of STI571. This pattern may represent a subtle distinction in STI571's action, as a number of other drugs inducing apoptosis result in an inverse expression of Bcl-2 and Bcl-xL. By 48 hours, the expression of the pro-apoptotic protein Bax showed a 20% increase, suggesting its expression may require prior down-regulation of Bcl-2. The pro-apoptotic protein Bak showed a two fold decrease, which was initially surprising, but similar results have been reported elsewhere for other cell lines undergoing apoptosis, suggesting a variable role for Bak. The expression of the pro-apoptotic protein Bad and the effector molecule pro-caspase 3 showed a complex pattern of expression over the 48 hour culture period, but exhibited an inverse relationship to each other. These results suggest that the apoptosis induced by STI571 may be initiated predominantly by a decrease in Bcl-2 expression.

A better understanding of the kinetics and pathways involved in the induction of apoptosis by STI571 may enable development of combination approaches to treatment to lessen the likelihood of resistance development.

CD38 Expression in B-cell Chronic Lymphocytic Leukaemia (B-CLL).

MacCallum SJ, Thomas ME, Drivas N, Mulligan SP

Department of Haematology, Mayne Health Pathology, North Ryde. Sydney. 2113

Aims: CD38 expression in B-CLL has been associated with adverse prognosis. We reviewed the incidence and clinical correlations of CD38 expression in B-CLL in our laboratory.

Methods: The laboratory services a broad referral base in NSW and southern Queensland. Lymphocyte immunophenotyping was reviewed together with morphology and basic haematological parameters for 220 consecutive cases. Two and three colour analysis was performed on a Becton Dickinson (BD) FACS Calibur flow cytometer using FITC and PE conjugated antibodies. The antibodies were all BD monoclonals, except for CD10 and FMC-7. CD38 analysis was restricted to B-cell expression and positivity defined as >30% B-cells

Results: Presentation for immunophenotyping was incidental / asymptomatic in 60%, clinically suspected new diagnosis in 29% and known B-CLL in 11%. The overall incidence of CD38 expression was 21%, with no difference between mode of presentation. CD38 expression was associated with moderate-strong surface immunoglobulin (slg) expression (21% vs 4%), anaemia (39% vs 10%), thrombocytopenia (28% vs 10%), atypical morphology (35% vs 7%), and clinical stage. CD38 expression was not found in Stage A patients, but was present in 67% of Stage C.

Conclusion: CD38 is expressed on the leukaemic B-cells of 21% of patients with B-CLL. In this study, CD38 expression was associated with anaemia, thrombocytopenia, atypical morphology high intensity slg, and advanced clinical stage.

Maintenance therapy with weekly chlorambucil (Chl) in intermediate-risk chronic lymphocytic leukaemia (CLL)

A. Manoharan.*

St. George Hospital, University of NSW, Sydney, Australia.

Chl has a proven and well-accepted track record in the treatment of patients with advanced or progressive CLL, albeit only as palliative therapy. However, there is little information on its role as maintenance therapy in patients showing optimal response to initial treatment. This report describes 18 patients with intermediate risk disease (modified Rai stage II or Binet stage B) who were treated with Chl for one or more indications according to the National Cancer Institute guidelines (Blood 1996;87:4990) and were kept on longterm maintenance therapy.

Patients – male 12; female 6; age 50-84 yrs (mean 72.5 yrs). Indications for treatment [A] bulky disease; B) progressive disease; C) constitutional symptoms; D) falling haemoglobin/platelets (non-immune); E) falling haemoglobin/platelets (immune)] A=3, B=5, C=1, A+B=2, A+C=2, A+D=3, B+E=2.

Initial treatment comprised Chl 0.06mg-0.08mg/kg daily for two to four months. Following documentation of complete remission (resolution of clinical symptoms/signs, normal blood counts), the frequency of therapy was reduced to two consecutive days each week for 4-6 months and then to once weekly.

Results – Thirteen patients have remained in complete remission (all alive) without lymphocytosis in the peripheral blood for 6+ to 147+ months (median >45 mo). Three patients have remained in remission, with slight/stable lymphocytosis ($6-12 \times 10^9/l$) for 18 to 102 months; one is alive at 63 months, whilst the other two have died due to unrelated causes (road accident, cardiac failure). Two patients developed progressive disease at 9 mo and 3 yrs, respectively, and are on combination chemotherapy. There have been no adverse side-effects attributable to Chl therapy.

Conclusion – Weekly maintenance therapy with Chl appears to sustain remission status in the majority of patients with Chl-sensitive, intermediate-risk CLL.

Clinical, radiologic, physiologic and histologic correlates of cytosine arabinoside (Ara-C) induced pulmonary toxicity.

C McNamara*, D Ritchie, A Grigg.

1 Department of Diagnostic Haematology

2 The Royal Melbourne Hospital, Parkville, Victoria.

Most reported cases of Ara-C pulmonary toxicity describe the clinical feature of non-cardiogenic pulmonary oedema rather than the underlying histological change antemortem. We describe a 65 year old man diagnosed with acute myeloid leukaemia (AML) who received treatment with Ara-C (3 g/m^2 over 2 hours then 1.5 g/m^2 d1-4) and idarubicin (9 g/m^2 d1-3). Induction therapy was complicated by culture-negative fever (40°C) on d5-8. Complete remission was achieved. Consolidation with the same dose and schedule of Ara-C and one day of idarubicin was again complicated by culture-negative fever, a maculopapular rash and paralytic ileus. Fevers of variable severity persisted despite broad spectrum antimicrobials including antifungal treatment. High resolution CT scan on day 15 showed diffuse, predominantly upper lobe, ground glass opacification. Count recovery occurred at d17. He was discharged but readmitted on d36 with fever and arterial hypoxaemia. Repeat CT chest showed widespread ground glass opacification. High dose cotrimoxazole was commenced. BAL was microbiologically unrewarding. Open lung biopsy on d43 showed diffuse alveolar damage with no evidence of pneumocystis, CMV or fungi. High dose methylprednisolone was commenced with prompt resolution of fever and dyspnoea, radiologic abnormalities and physiologic changes of impaired gas exchange (DLCO). Steroids were given for 7 weeks; the DLCO and CT at d73 showed continued resolution of previous abnormalities. Ara-C should be considered as a cause of hypoxaemia with fever in patients undergoing therapy with this agent.

Morphologic and Cytogenetic Effects of STI 571 on the Bone Marrow and Blood of Patients with Philadelphia Chromosome (Ph) Positive Chronic Myeloid Leukaemia (CML).

C McNamara*, A Grigg, J Szer, A Roberts, R Hoyt, K Lynch, S Juneja

Departments of Diagnostic Haematology & Clinical Haematology and Medical Oncology, Royal Melbourne Hospital, Melbourne and Novartis Pty Ltd, North Ryde.

The effectiveness of the tyrosine kinase inhibitor STI571 in CML has been recognised recently. We have examined morphologic changes in the bone marrow and blood in 20 evaluable patients with CML 3 months after commencement of STI571. Fourteen patients had CML in chronic phase (CP) of whom 12 were intolerant of or unresponsive to interferon (IFN). Six patients had accelerated (AP) or blastic phase (BP) disease. The median patient age was 50 years (range 19-71) and median interval from diagnosis to commencement of STI571 was 47 months (1-132). At three months, 18 of 20 patients achieved complete hematologic response (CHR) as defined by WBC count $<10 \times 10^9/l$ and platelet count $<450 \times 10^9/l$. In 19/20 patients the marrow cellularity was reduced by a median of 50% (5-95%). There was a reduction in granulocytic cells and an increase of erythroid cells with a consequent median 2.7 fold decrease in the myeloid to erythroid ratio. The number of megakaryocytes was reduced in 13/19 patients. Importantly this was accompanied by an increase in the proportion of morphologically normal appearing megakaryocytes. The proportion of Ph+ metaphases was reduced by a median of 47% in CP and 46% in AP and BP patients (range 0-98%). Nine of 14 CP and 3 of 6 AP and BP patients had a reduction in Ph+ cells. There was no statistical correlation between the degree of reduction of cellularity and cytogenetic response ($r^2=0.04$). In summary, in CML patients treated with 3 months of STI571, there was a significant clearance of leukaemic cells as evidenced by CHR in the blood together with reduced marrow cellularity, restoration of morphologically normal haemopoiesis and cytogenetic response.

Portal or Hepatic Vein Thrombosis as the First Presentation Of Myeloproliferative Disorders with a Normal Platelet Count.

Christopher McNamara*, Surender Juneja, Max Wolf, Andrew Grigg.

Royal Melbourne Hospital & Peter MacCallum Cancer Institute, Melbourne, Australia

Myeloproliferative disorders are associated with an increased risk of thrombotic complications. We describe three cases which presented with portal or hepatic vein thrombosis and on investigations were found to have an underlying myeloproliferative disorder. At diagnosis, although the platelet count was within normal range, essential thrombocythemia (ET) was considered to be the most likely subtype of myeloproliferative disorder on the basis of megakaryocyte morphology and in vitro bone marrow culture studies (so called 'latent' or early ET). There is an ongoing debate in the recent literature as to relative importance of various criteria including the platelet count for the diagnosis of essential thrombocythemia. Patients presenting with unexplained portal or hepatic vein thrombosis should be systematically investigated for the presence of myeloproliferative disorders, including latent ET, which may not be apparent using conventional diagnostic criteria.

High-Dose Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation for patients aged ³60 years.

LM Mileskin*, HM. Prince, JF Seymour, M Wolf, P. Gates, T. Joyce

Depts of Haematology and Medical Oncology; Peter MacCallum Cancer Institute, Melbourne, Australia

Introduction: Age \geq 60 yrs has been a relative contraindication to high dose therapy (HDT) with autologous stem cell transplantation for lymphoma (NHL) and multiple myeloma (MM). We present the PMCI experience of all patients (pts) aged \geq 60 yrs treated with HDT compared with a control group aged $<$ 60 yrs.

Methods: Pts \geq 60 yrs at HDT were identified from the transplant database. Medical records were retrospectively analysed to assess efficacy and safety of HDT. Event-free and overall survival rates were compared with a matched control group of pts, matched by disease type, chemotherapy sensitivity and conditioning regimen. NHL pts were also matched by International Prognostic Index (IPI) score.

Results: 26 patients aged \geq 60 yrs were identified. Median age was 64 yrs (range: 60 - 76) with 13 MM and 13 NHL. Median number prior chemotherapy regimens was 2 (1-4) and performance status ECOG 1 (0 -2). Median IPI for NHL pts was 2 (1-5), 8/12 (67%) having aggressive histology. 50% of pts had \geq 1 comorbidities. Indications for transplant in MM pts were relapsed disease in 3 pts (23%) and high-risk disease (CR1 or PR1) in 10 pts (77%).

Conditioning regimen for MM pts was high-dose melphalan (200mg/m²) \pm amifostine. 10 pts with NHL received BEAM and 2 pts received Busulphan/Melphalan. Mobilisation of CD34+ cells was achieved in MM patients with high-dose cyclophosphamide (4g/m²) and G-CSF (10 μ g/kg/day). Median CD34+ cells collected was 7.18 X 10⁶/kg (range 2.33 - 21.75) and similar in controls (p=0.8)

Response rates (RR) following HDT for MM were as follows: 3CR (23%), 9 PR (69%), and 1 not evaluable (NE) (8%) giving an overall response rate (ORR) of 92%. For NHL the RR were 3 CR (23%), 6PR (47%), 2 PD (15%) and 2NE (15%), giving ORR of 70%. RRs in the control arms were equivalent (p=0.23). Transplant-related mortality (TRM) was 11% compared to the control group of 8%. Median time to neutrophils $>$ 0.5 X 10⁹/L = 11d, $>$ 1.0 x 10⁹/L = 12d, platelets $>$ 20 X 10⁹/L = 16d, $>$ 50 X 10⁹/L = 26d. Median hospital stay 21 days (range 9-73) was similar in controls (p=0.20). Important toxicities:

	$>$ 60 yrs	$<$ 60 yrs	P value
Median days IVantibiotics	14(5-61)	12(5-73)	0.35
Pts given IVamphotericin	6/23(23%)	5/26(19%)	1.0
Pts given TPN	12/26(46%)	14/26(54%)	0.78
Median days TPN	10(3-57)	14(1-51)	0.97
ICU admissions	5/26(19%)	3/26(12%)	0.70
NCI grade 3/4:			
-mucositis	22/26(85%)	21/26(81%)	1.0
-renal	2/26(8%)	1/26(4%)	1.0
-cardiac	10/26(38%) mainly AF	2/26(8%)	0.019
-pulmonary	7/26(27%)	4/26(15%)	0.49

At median follow-up of 15 mo, there is no difference in EFS (26mo vs 19 mo: p=0.18) or OS (69% vs 58%: p=0.94)

Conclusions: HDT is a feasible and effective treatment in selected patients \geq 60 years with MM and lymphoma. With the exception of atrial fibrillation, toxicities are similar.

The treatment effect of 5-HU (syrea) in patients with major B-thalassemia (intermediate) of Amirkola Thalassaemia Center

A Tammadoni MD, D Javaheri MD, S M Miri MD, P Karimi MD

Objectives: According to the studies on 5-Hydroxyurea, there was a positive effect on HbF and total Hb increases in the patients with intermediate thalassaemia.

The target of this studies identifying the effectiveness of 5-HU on total Hb blood transfusion intervals and drug complications.

Materials & Methods: The 46 samples were selected from the patients of Amirkola Thalassaemia Center during 6 months. The patients were divided to two groups of with transfusion history and without it. 5-HU was administered as a capsule (500 mg) 3 times a week for who were under 12 years-old and 4 times a week for whom were older.

Results: From 46 patient, whom were studied, 3 patients were eliminated in order not to return. In group 1, the mean Hb difference before drug administration and after 3 months drug administration was significant ($p < 0.0001$) and also it was significant ($p < 0.0001$) in group 2. 16 patients had transfused the blood during 6 months of using the drug.

All the patients who underwent blood transfusion were among those who were under regular blood transfusion (group 1).

Conclusion: According to our results, 5-HU increases total Hb and blood transfusion intervals and there was any complication during the treatment.

Key words: intermediate thalassaemia, 5-HU, transfusion, hemoglobin(Hb).

Incidence and Predictors of Infusion related Reactions to Liposomal Anthracyclines in Patients with Haematologic Malignancies

Moloney M* (1), Prince HM (2), Seymour JF (2), Januszewicz H (2), Wolf M (2), Dooley M (1), Martinac G (1), Eaton K (1)

(1) Department of Pharmacy, Peter MacCallum Cancer Institute, East Melbourne

(2) Department of Haematology, Peter MacCallum Cancer Institute, East Melbourne

Background: The liposomal anthracyclines (LP-Doxorubicin – Caelyx and LP- Daunorubicin – DaunoXome) are registered for use in solid tumors, but are under investigation in the haematologic malignancies. Infusion related events have been observed in 5-10% of patients (pts) with solid tumors receiving LP-Dox and 14% with LP-Daun. These adverse infusion related events are characterised by flushing, shortness of breath, facial oedema, headache, chills, back pain, tightness of the chest and throat, and/or hypotension. There is no data on the frequency and predictors of such reactions in pts with haematologic malignancies

Method: We retrospectively reviewed the cases of all pts who have received LP-Dox or LP-Daun at Peter MacCallum Cancer Institute (PMCI).

Results: 29 received liposomal anthracyclines from 3/99 - 4/01. LP-Dox was administered over 60-80mins in 250ml glucose 5% (dose < 90 mg) and in 500ml glucose 5% (dose ≥ 90 mg) and LP-Daun was administered over 2 hours in 250-500ml glucose 5%. Premedication was administered in 2 pts receiving LP-Dox. All pts had haematological malignancies (myeloma = 15, cutaneous lymphoma = 4, Kaposi's sarcoma = 2, NHL= 6, ALL = 1, AML = 1). The median age was 67 yrs (range 31 to 79). 20/29 received LP-Dox (total 84 cycles, median cycles 4 [range 1-11] Dose range 20-90mg). 6/20 (30%) experienced adverse infusion-related events. Four of these occurred during cycle 1, two of whom received no other doses due to the severity of the reaction (anaphylaxis). The remaining two (\downarrow blood pressure, from 114/61 to 103/40 and 107/44 to 89/39 respectively) proceeded with further cycles without incident, with no change in infusion rate or premedication. Two other patients reacted on cycle 2 (chest & throat tightness) and 4 (\downarrow blood pressure) respectively but were able to continue with subsequent cycles.

9/29 received LP-Daun (31 cycles, median = 4 [range 1-6] Dose range 145mg-210mg). No standard premedication was given. None of these patients experienced adverse infusion related reactions ($P = 0.07$ vs. LP-Dox)

Within the whole group of 29 pts, comparing the features of "reactors" and "non-reactors", there were no differences in median age (56 yrs, range 49-71) vs. (68 years, range 31-79), median infusion time (60minutes, range 60-80) vs. (75 minutes, range 60-120) (each $P \geq 0.16$) There was no difference in reaction rates for central (17%) vs. peripheral lines (25%) $P = 0.62$.

Conclusion: Patients receiving LP-Dox developed the expected 10% severe infusion-related reactions that required discontinuation of therapy. No infusion related events were observed in the LP-Daun group.

A performance study of the T Cell-DLI Kit in conjunction with the Eligix™ Cell Separation System in a simulated clinical setting for processing CD8 depleted DLI

H Houde, H Zhu, R Schmittling, D Cook and R Monroy, Eligix, Inc. Medford, MA, USA

Donor leukocyte infusions (DLI) depleted of CD8+ T cells have been shown mediate a graft-versus-leukemia effect with a reduced incidence of graft-versus-host disease relative to unmanipulated DLI (Aylea et al, Blood 91: 3671). We have previously demonstrated a prototype method to deplete CD8+ T cells from apheresis collections using CD8-HDM, which consist of an anti-CD8 monoclonal antibody conjugated to high density microparticles. The CD8-HDM method was developed to replace cumbersome methods of CD8+ T cell depletion using antibody and complement. Recently, the Eligix™ Cell Separation System using CD8-HDM in the TCell-DLI kit has been developed incorporating a disposables kit and two accessory bench-top devices for clinical implementation of the HDM depletion procedure. To assess the performance of this System, apheresis products were obtained from 22 normal volunteers. By splitting products with high cell number, 34 CD8+ depletion processes were performed in a simulated clinical setting. The initial number of processed nucleated cells ranged from 0.2 to 2.7 x 10¹⁰ cells, and all processes were performed aseptically in a biological safety cabinet. The median depletion of CD8+ T cells from these products as measured by flow cytometry was 2.2 logs (range: 1.7 logs to 3.4 logs) and depletions frequently were below the limit of detection of the assay. The number of depleted CD8+ target cells ranged from 0.4 to 5.8 x 10⁹ CD8+ T cells. The CD4+ T cell recovery was highly reproducible with a mean of 88% (SD ± 6%) and a range of 76% to 99.8%. The percent recovery of CD4+ cells was consistent over the entire range of cells entered into the process. The median number of CD4+ T cells recovered was 3.2 x 10⁹ with a range of 0.4 to 8.5 x 10⁹ cells. Percent hematocrits of 2% to 8% and platelet counts ranging from 400 to 3000/microliter did not affect processing. Processing times were less than 2 hours. The Eligix™ Cell Separation System using the TCell-DLI Kit represents an improved method both in processing time and purging efficacy and is appropriate for use in clinical trials for patients requiring DLI therapy.

A simulated use controlled study of 22 normal volunteers using the Bcell-SC Kit in conjunction with the Eligix™ Cell Separation System demonstrates highly efficient depletion of CD20+ B Cells and high recovery of CD34+ and CD3+ Cells

H. Houde, H. Zhu, R. Schmittling, M. Graney, D. DeLucia, D.Cook and R. Monroy. Eligix, Inc. Medford, MA, USA

Clinical devices are available that select for CD34+ cells from mobilized peripheral blood stem cell (PBSC) collections as a means of isolating stem cells for subsequent infusion after high dose therapy. As a consequence of the selection process, contaminating tumor cells are reduced in the autograft. However, such a selection process often results in a significant loss of stem cells, depletion of T cells, and may leave cells coated with antibody. Additionally, several studies have documented a pronounced immunodeficiency in some patients who were infused with autologous CD34-selected cells. A functionally closed Eligix™ Cell Separation System using the BCell-SC Kit has been developed to effectively purge CD19+ and CD20+ tumor cells from autologous PBSC of patients with non-Hodgkins lymphoma (NHL) with minimal stem cell loss. A normal volunteer study was conducted in which twenty-two G-CSF mobilized apheresis products were processed in a simulated clinical setting. The depletion of CD20+ B cells and recovery of CD34+ and CD3+ cells were documented. A central processing lab was used to measure cellular phenotypes by flow cytometry and to detect CD20+ cells by immunocytochemistry using an assay validated to detect as few as 4 B cells per 107 total nucleated cells. Median percent recoveries for CD34+ and CD3+ cells and median log depletion are listed in following table. A median of 3.2 x 10¹⁰ nucleated cells (range: 1.7-6.2 x 10¹⁰ cells) were processed.

	% Recovery CD34+	% Recovery CD3+	Log Depletion CD20+ B cells
Median	103	94	4.7
Range	68 – 228	65 - 125	2.8 - >6.0

Contaminating RBCs and platelets did not interfere with cell processing. The final cell product was sterile and free of murine antibody. This study reproducibly demonstrated a significant depletion of CD20+ B cells and highly efficient recoveries of CD34+ and CD3+ cells, while retaining high cell viability (>98%) and CFU-GM (median 84%). The system will be implemented in a pivotal trial to purge B cells from PBSC of NHL patients.

Haemopoietic cell transplantation in Australia and New Zealand: a review from the ABMTRR.

I Nivison-Smith*.

Australasian Bone Marrow Transplant Recipient Registry, St Vincent's Hospital, Darlinghurst NSW.

The Australasian Bone Marrow Transplant Recipient Registry (ABMTRR) has recorded bone marrow and blood stem cell transplants in Australia since 1992, and in New Zealand since 1998.

In the 2000 calendar year, 997 transplants were recorded in Australia and New Zealand (Australia 932, New Zealand 65). This is a slight decrease on the number carried out in 1999 (Australia 958, New Zealand 91, total 1049). Of the transplants carried out in 2000, 288 were allogeneic transplants with a related donor (Australia 263, New Zealand 25) including 6 with identical twin donors (Australia 5, New Zealand 1) and 110 were allogeneic transplants with matched unrelated donors (Australia 106, New Zealand 4). A further 535 were autologous transplants involving a single infusion (Australia 501, New Zealand 34) and 64 were staged autologous transplants involving more than one infusion (Australia 62, New Zealand 2). Numbers for each of these transplant types are similar to 1999, with the exception of staged autologous procedures, which have decreased in number from 122 in the previous year.

The median age for transplant in Australia and New Zealand in 2000 was 49 years for autologous procedures (range 0-76), 44 years for allogeneic related (range 0-68) and 25 for allogeneic unrelated (range 0-56).

In 2000, peripheral blood or blood plus marrow was used as the stem cell source in 96% of autologous transplants. Peripheral blood or blood plus marrow was used in 57% of allogeneic procedures, compared to 49% in 1999.

The commonest indications for single autologous transplant in Australia and New Zealand in 2000 were non-Hodgkin's lymphoma (NHL - 210), myeloma (181) and acute myeloid leukaemia (AML - 36).

The commonest indications for staged autologous transplant were breast cancer (14) and medulloblastoma (7). The commonest indications for allogeneic related transplant were AML (62), chronic myeloid leukaemia (CML - 54) and acute lymphoblastic leukaemia (ALL - 36). The commonest indications for allogeneic unrelated transplant were ALL (25), AML (21) and CML (19).

Current patterns of haemopoietic cell transplantation show a consolidation of these procedures as standard treatment for a wide range of indications. The ABMTRR is a valuable trans-Tasman resource providing timely and detailed information on transplant activity and outcome.

Fibrillary glomerulopathy in a patient with diffuse large B-cell lymphoma

Huong LG Phan(1)*, Keith Horwood(2), Zoltan H Endre(3), Rick Abraham(2), David Wyld(2)

(1) Department of Haematology, Mater Adult Hospital, Brisbane

(2) Department of Medical Oncology, Royal Brisbane Hospital, Brisbane

(3) Department of Renal Medicine, Royal Brisbane Hospital, Brisbane

We report a case of the fibrillary glomerulopathy (FGN) in association with a localised small bowel diffuse large B-cell lymphoma (DLCL). FGN is only rarely associated with B-cell lymphoproliferative disorders, treatment of which has been reported to result in improvement of renal function and proteinuria. However, our patient progressed to end-stage renal failure despite successful treatment of the lymphoma.

A 43-year-old Caucasian female with a past history of endoscopically proven eosinophagitis and type II diabetes mellitus presented acutely in August 1999 with severe epigastric pain. At laparotomy she was found to have a dilated small bowel due to an obstructive mass (55 mm in diameter) extending through the bowel wall. Histological examination of the mass revealed a DLCL with clear resection margins. Gallium scan suggested retroperitoneal nodal involvement but there was no evidence of other extra-intestinal involvement.

She was commenced on standard CHOP chemotherapy. Two weeks later, a sudden increase in the serum creatinine (0.19 mmol/L, normal range 0.04 - 0.11), and new-onset hypertension was noted. Although this was initially considered to be secondary to chemotherapy and/or aminoglycoside toxicity, subsequent investigations revealed nephrotic range proteinuria (14 g/24 hours, normal range < 0.15) and normal sized kidneys on ultrasound. Renal biopsy revealed a non-amyloid FGN. No evidence of cryoglobulinaemia, serum or urinary paraproteins was detected. Further CHOP was recommended, but was declined by the patient who subsequently did not attend outpatient follow-up for six months.

On representation in February 2000, repeat staging investigations showed no evidence of residual lymphoma. However, evidence of chronic renal failure (serum creatinine 0.49 mmol/L, serum urea 23.2 mmol/L (normal range 3.0 - 7.5), serum albumin 23 g/L (normal range 30 - 42), measured creatinine clearance 6.0 mL/min (normal range 91 - 130) was present and hospital-based haemodialysis was therefore commenced. Twenty-two months after diagnosis, she remains in remission from her lymphoma but remains on haemodialysis three times a week.

FGN is a distinct, rare entity characterized by extracellular deposition of immunoglobulin and complement fibrils in the mesangium and glomerular basement membrane. It is associated with significant proteinuria, often in nephrotic range, as well as microscopic haematuria and hypertension, and progression to end-stage renal failure in about 50% of cases. Whilst a link between lymphoid proliferation and the development of FGN has been suggested, the pathophysiology of the fibrillary deposits in our patient is difficult to establish in the absence of paraproteins or cryoglobulins.

Epoetin Alfa Therapy Increases Hemoglobin Levels and Improves Quality of Life in patients with Cancer-Related Anemia who are Receiving Chemotherapy and in patients with Cancer-Related Anemia who are not Receiving Chemotherapy.

I. Quirt (1)*, C. Robeson (2), C.Y. Lau (2), M. Kovacs (3), S. Burdette-Radoux (4), S. Dolan (5) S.C. Tang (6), M. McKenzie (7), F. Couture (8), and the Canadian EPREX* Oncology Study Group. (Supported by Ortho Biotech Inc.)

1 Princess Margaret Hospital, Toronto, Ontario

2 Ortho Biotech Inc., Toronto, Ontario

3 London Regional Cancer Centre, London, Ontario

4 Royal Victoria Hospital, Montréal, Québec

5 Saint John Regional Hospital Facility, Saint John, New Brunswick

6 Dr. H. Bliss Murphy Cancer Centre, St. John's, Newfoundland

7 British Columbia Cancer Agency, Vancouver, British Columbia

8 Hôtel-Dieu de Lévis, Lévis, Québec and Hôtel-Dieu de Québec, Québec City, Québec

Objective: To determine the impact of treatment with epoetin alfa on hemoglobin (Hb) levels, transfusion requirements and quality of life (QOL) in patients with cancer-related anemia receiving chemotherapy and not receiving chemotherapy.

Methodology: 401 cancer patients with anemia from 34 centres across Canada were enrolled into this prospective, open-label, 16 week study. The trial had two cohorts: patients who were not receiving chemotherapy and patients who were receiving chemotherapy. All patients initially received epoetin alfa 150 IU/kg subcutaneously three times per week. The dose was doubled after 4 weeks if the patient's Hb did not increase by at least 10 g/L.

Results: In the 183 patients in the nonchemotherapy cohort, statistically significant and clinically relevant improvements in QOL were observed using both the FACT-An and linear analog scale assessment. Hemoglobin levels increased a mean of 25 g/L from baseline to the end of study ($p < .001$). This increase correlated with improved QOL and ECOG score. 48% of patients experienced an increase in Hb of 20 g/L or more without transfusion. Transfusion rates decreased from 29% at baseline to 19% by the end of study ($p < .02$).

The 218 patients in the chemotherapy cohort experienced similar improvements in QOL, transfusion rates and Hb levels. Epoetin alfa was well tolerated in both cohorts.

Conclusions: Epoetin alfa therapy resulted in significantly improved quality of life, increased hemoglobin levels and decreased transfusion use. Although these benefits have previously been reported in studies of patients receiving chemotherapy, this study demonstrates the same degree of benefit in patients with cancer-related anemia who were not receiving chemotherapy.

G-CSF mobilisation of peripheral blood stem cells in a patient with early chronic myeloid leukaemia and mild neutrophilia.

S. Ramanathan (1) *, C. Arthur (1) , J. Isbister (1)

(1) Department of Haematology, Royal North Shore Hospital, Sydney.

The optimal management of the newly diagnosed, treatment naïve patient with chronic myeloid leukaemia is in a state of flux. Allogeneic transplantation, interferon and hydroxyurea are well established but there is renewed interest in the use of autologous stem cell transplantation. The role of ST1571 remains to be defined. It is considered good practice to cryopreserve stem cells early in the course of CML while there is still residual normal haematopoiesis. Most strategies for stem cell collection involve apheresis of patients presenting with high white cell counts or in the recovery phase following chemotherapy. We report a case of a CML patient, presenting with an absolute neutrophil count of $14 \times 10^9/l$, mobilised with G-CSF alone. A baseline CD 34+ cell count was performed and found to be 12 cells/ microlitre; a count normally considered insufficient to attempt peripheral stem cell harvest. 300 micrograms of G-CSF was administered daily for two days. The absolute neutrophil count rose to $80 \times 10^9/l$ and the CD34+ count to 97 cells/ microlitre on day 3. Apheresis was performed and a product containing $259 \times 10^9/l$ white cells and 7.37×10^6 /kg of CD34 cells. The patient experienced no significant side effects. Our experience demonstrates the safety and efficacy of low dose G-CSF in mobilising peripheral blood stem cells in a patient with chronic phase chronic myeloid leukaemia. The greater than expected rise in CD34 count may indicate an increased responsiveness to G-CSF in chronic myeloid leukaemia.

Consistent use of a reliable antiseptics technique for collection of cord blood ensures its sterility for use in transplantation.

Rodwell RL* (1,2), Arnold J (2), Ruthenberg S (2), Eastment R (1,2), Maksoud A (1,2), Taylor DL (1,3) Tilse M (4), Mollee T (4) (Bentley M (2), Wright S (2), Taylor K (2).

1 Lions Cord Blood and Leukaemia Research Unit at the Mater Brisbane.

2 Queensland Cord Blood Bank at the Mater, Brisbane.

3 Haematology Division Mater Misericordiae Hospitals, Brisbane.

4 Microbiology Division, Mater Misericordiae Hospitals, Brisbane.

There is a worldwide demand for umbilical cord blood (UCB) for use in transplantation. The major impetus has come from the global network of cord blood banks that are developing cryopreserved repositories of HLA typed UCB stem cells as a readily available substitute for bone marrow in transplantation. Sterility during collection and processing is vital to ensure the safety and quality of the final product.

Objective. The aim of this study was to develop and validate an antiseptics technique for collection of UCB and then to assess its usefulness in the initial phase of a cord blood-banking programme.

Method. The study populations for the validation and banking phases comprised mothers with healthy term pregnancies who had consented to either (a) participate in a research study to develop methods for the cord blood bank (n=150) or (b) to be a cord blood donor (n=500). Following the third stage of labour the placenta was transported to an adjacent site and suspended in a supporting structure. Antiseptics included a 30-second 70-percent isopropyl alcohol wash, followed by a 30-second scrub with a povidine-iodine (10%) swabstick, with 30-second intervals for drying. Blood was collected into triple bag blood collection bags (OPTIPAC Baxter Healthcare Ltd CA, USA) with the CPD volume adjusted prior to collection. Volume reduction of cord blood units was performed by buffy coat extraction on the Fenwal Optipress II (Baxter Healthcare Ltd). A closed system was maintained throughout by the use of "connector sets," a sterile tubing welder and the attachment of "syringe ports" (Terumo, NJ, USA). Subsequent manipulations prior to cryopreservation were performed in a laminar flow cabinet. Sterility at collection was assessed by drawing 3mL samples from the RBC fraction into anaerobic and aerobic adult blood culture bottles (Bactec) and at completion of processing by drawing 0.5 mL of the final buffy coat/cryopreservation mix into anaerobic and aerobic pediatric blood culture bottles. These were cultured for 7 days on the Organo Bactec system.

Results. None of the 150 cord blood units tested in the validation phase were contaminated and only two of 500 were contaminated during laboratory processing in the early banking phase. This gives an overall contamination rate of 0.3%.

Conclusion. The consistent use of a reliable antiseptics method, strict adherence to aseptic technique and use of a closed system can minimize bacterial contamination during the collection and processing of UCB and ensures its sterility for use in transplantation.

A real-time interactive computer system designed to meet regulatory codes for the Queensland Cord Blood Bank at the Mater.

Rodwell RL* (1,2), Carey J (3), Whelan B (3), Schreiweis L (3), Taylor D (1,2), Eastment R (1,2), Cooper M (1,2), Bentley M (2), Wright S (2), Taylor K (2).

1. Lions Cord Blood and Leukaemia Research Unit at the Mater Brisbane.

2. Haematology, Mater Misericordiae Hospitals, Brisbane.

3. Information Management, Mater Misericordiae Hospitals Brisbane.

Objective. To develop a real-time interactive computer system for the Queensland Cord Blood Bank (QCBB) At The Mater to manage its operations, to facilitate compliance with good manufacturing (GMP) principles and regulatory codes, and to facilitate the attainment of a Therapeutic Goods Administration (TGA) licence.

Method. Preliminary investigations included a feasibility study to several banks, a period of research and planning, flow-charting of the cord blood banking (CBB) process, and a pilot study using a Microsoft Office Excel spreadsheet based programme. Ultimately, two existing hospital networked computer systems – the obstetric database (ODB) and the Kestral Pathology Laboratory system (PLS) Haematology were utilized, modified and refined.

Results. The ODB was modified to manage and record donor recruitment and consent, cord blood unit (CBU) collection, donor assessment and follow-up. Medical history forms, successful or unsuccessful letters, which thank mothers for their participation in the programme and reminder letters when mothers fail to return for follow-up are generated. The PLS was modified to manage CBB operations with the CBU being regarded as the donor. Test requests are ordered as "profiles," which automatically request the appropriate battery of tests and ensure uniformity of testing. Bar code labels are generated as required at different stages of the process. "Worklists" are used to divide the CBB process into manageable steps, to identify at any time-point samples to be processed, to transfer data automatically to the processing worksheet and to maintain audit trails. All calculations for testing and quality control are performed automatically. Cell counters are incorporated for performance of white blood cell differentials and counting of CFUs. Computerized freezer inventories are maintained, which detail the type and location of stored CBUs and corresponding cord blood and maternal aliquots. Reagent and consumable receipt into the laboratory is incorporated and the data is transferred into the CBU processing log. Test results are transferred electronically from the clinical laboratories and hard copy reports generated automatically. A "validation" code provides a summary of results and allows sequential scientific and medical validation and indicates the CBU status eg quarantine or available. Numerous data extraction codes simplify data analysis and quality control.

Conclusions. This state of the art computer system, which is integrated with the Obstetric Service and MLS Services streamlines and manages the CBB operations. It maintains audit trails, processing logs and freezer inventories and eliminates the potential for calculation and data transcription errors. It provides real-time quality control and simplifies compliance with GMP principles and regulatory codes.

A quality system to AS/NZS ISO-9002 for the Stem Cell Processing Laboratory and Queensland Cord Blood at the Mater.

Rodwell RL*(1,2), Taylor D (1, 2), Ambrosoli P (1, 2), Eastment R (1,2), Baldry D (1, 2), Kerwick AM (2), Maksoud A (1, 2), Coulston J (1, 2), Cooper M (1, 2), Hinds J (3), Hutchings R (4), Schreiweis L (3), Taylor KT (2).

1. Lions Cord Blood and Leukaemia Research Unit, Mater Hospitals, South Brisbane.

2. Haematology Division, Mater Hospitals, South Brisbane.

3. Mater Laboratory Services, South Brisbane.

4. Quality Business Documents, Brisbane.

The regulatory codes of the Therapeutic Goods Administration and the Federation for the Accreditation of Haemopoietic Cell Therapy require a quality system for stem cell processing laboratories (SCPL) in order to comply with the principles of Good Manufacturing Practice (GMP).

Objective. To develop a quality system to the AS/NZ ISO-9002 standard and to regulatory codes for the SCPL and the Queensland Cord Blood Bank (QCBB) at the Mater.

Method. A quality officer (QO) was appointed to the Mater Laboratory Services (MLS) quality team, which was led by an external quality management consultant. The role of the QO was to assist with the development and implementation of uniform integrated policies for the clinical laboratories of MLS, to devolve information and to ensure regulatory compliance.

Results. The major components of the quality system are described in the MLS, SCPL and the QCBB Operations Manuals and include systems for compliance with the elements of ISO-9002 and regulatory codes, the management structure, finance, information technology, workplace health and safety and the audit programme. Commercial computer programmes are used for the management of equipment (MAINPAC), electronic monitoring of equipment (MICROSCAN), calibration of pipettes (SARTIOUS PICASSO), procurement and purchasing (ONLINE 2000), staff rostering and salary generation (ROSTA 2000), staff training records

(orientation, specific training and competency checks) (EXCEL), routine operations of the SCPL (EXCEL) and the QCBB (Kestral Pathology Laboratory system [PLS]) and system reports for complaints, non-conformance and improvements (Access). Manuals are colour-coded for easy identification. All controlled documents (policies, standard operating procedures, information brochures, forms, records, labels and letters) are managed via a computerised document masterlist and amendment distribution list. Receipt of goods into the QCBB is via the PLS, which generates barcodes and automatically provides details when the item is used. Expenditure at a laboratory level is provided monthly by an institution wide cost-centre programme and at an individual test level (SCPL) by the national benchmarking projects. The MAINPAC equipment programme provides bar-code identification, an equipment inventory, a calibration master list and schedule of maintenance and generates reminders, which are actioned and documented by an equipment officer. The MICROSCAN system with audible alarms, automatic dial-out, remote modem access and an "events log" to record alarms and corrective action continuously monitors equipment,

Conclusions. Our system although labour intensive to develop simplifies quality and regulatory compliance, provides early detection of non-conformance, management of risk and incorporation of continuous improvement strategies. The SCPL achieved AS/ANZ ISO-9002 in 2000 and this will be sought for the QCBB.

A Rapid RT-PCR Screening Assay Incorporating Multiplexed Validated Control Genes for CBF Rearrangements at Diagnosis in AML

Russell Saal*(1), Paula Marlton (1), Georgina Timson (1), Marcus Waugh (1), Francisca Springall (2), Karen Grimmett(1), Devinder Gill(1), and Harry Iland (2).

(1) Queensland Health Pathology & Scientific Service, Department of Haematology, Princess Alexandra Hospital, Brisbane.

(2) Kanematsu Laboratories, Royal Prince Alfred Hospital, Sydney.

Introduction. The cytogenetic abnormalities *inv(16)*, *t(16;16)* and *t(8;21)* are associated with good prognosis AML and require specific therapeutic considerations. Both are associated with CBF rearrangements detectable by RT-PCR assays. Cytogenetic screening of AML cells at diagnosis does not detect all patients with a CBF rearrangement found by RT-PCR. Routine RT-PCR screening of AML patients at diagnosis has therefore been advocated. In order to standardise such screening the Biomed-1 Concerted Action Group has proposed primer sets for universal use.

Aim. In this study our aim was to advanced their work by developing a robust single step assay for universal use in screening de-novo AML. Our objective was to establish a multiplexed assay using the Biomed-1-proposed primers to detect AML-1-ETO [*t(8;21)*] transcripts and 9 different CBF-MYH11 [*inv(16)* and *t(16;16)*] transcripts simultaneously. In addition we aimed to incorporate in the multiplexed assay carefully chosen control genes.

Methods. After reviewing many candidate control genes BCR and ABL were chosen. A series of experiments were carried out to confirm that these genes fulfilled the following criteria: (1) Expression levels and product sizes differ to provide a spectrum for comparison with the target product and thus RNA quality. (2) False positive amplification due to contaminating genomic DNA does not occur. (3) The presence of target transcript does not influence control gene expression. The final multiplex RT-PCR assay was tested for sensitivity using dilutions of cell lines in normal cells. It was then applied to samples from 50 AML patients and results were compared with those obtained by cytogenetics.

Results. Control gene testing confirmed the designated criteria were fulfilled. Sensitivity of the assay was found to be 1 in 10⁶ for AML1-ETO transcripts and 1 in 10³ for CBF-MYH11. Of the 50 patient samples tested, 4 RT-PCR results did not correlate with the cytogenetic result. In 3 cases RT-PCR detected cryptic CBF rearrangements in cytogenetically negative cases (1 AML-1-ETO and 2 CBF-MYH11). One negative RT-PCR result was found in a patient with *inv(16)*. This result was associated with detection of only the more highly expressed of the 2 control genes thus casting doubt on the quality of the RNA.

Conclusions. We have described a robust single step multiplex RT-PCR for the detection of CBF rearrangements at diagnosis in AML incorporating experimentally validated control genes that have assisted in allowing accurate interpretation of results. Application of this methodology to a large series of uniformly treated patients will enable us to determine if cryptic CBF rearrangements share the same prognostic significance as their cytogenetic counterparts.

Telomere Length in CD34+ Cells Is Equivalent to that of Cells from BM, PB and Apheresis Collections

Fiona E Scorgie (1)*, Lisa Lincz (1), Jennette A Sakoff (2), Esther De Waal (2), Madhu Garg (2), James Denham (3), Stephen P Ackland (2), Arno Enno (1,3)

1 Hunter Haematology Research Group, Mater Misericordiae Hospital, Newcastle, NSW

2 Medical Oncology Department, Mater Misericordiae Hospital, Newcastle, NSW

3 Department of Radiation Oncology, Mater Misericordiae Hospital, Newcastle, NSW

4 Hunter Haematology Unit, Hunter Area Pathology Service, Newcastle, NSW

Telomeres are tandemly repeated hexanucleotide sequences located at the end of linear chromosomes. Telomere length can be used to determine the replicative capacity of haemopoietic progenitor cells and may be important for predicting the onset of cellular immune dysfunction. However, routine measurement of telomere length in these cells is hampered by the requirement for invasive bone marrow biopsies and laborious stem cell isolations. In order to overcome these obstacles we sought to determine the relationship between telomere length of progenitor cells and their progeny. Southern blotting was used to calculate the mean telomere length from PB cells, isolated MNC and BM aspirates from each of 22 patients ranging in age from 45-81 years (median 66 years). Correlation analysis confirmed that mean telomere length (+ 1 SD) of BM aspirates (9200 + 2290 bp) was equivalent to that of PB (8716 + 2019 bp) ($r = 0.94$, $P < 0.001$) or circulating MNC (9431 + 2296 bp) ($r = 0.87$, $P < 0.001$). Once this was established, the analysis was taken one step further to determine if telomere length in these more mature cells reflected that of their original CD34+ progenitor cells. In order to obtain sufficient numbers of progenitor cells for DNA analysis, CD34+ cells were isolated from samples of apheresis collections obtained from 16 patients preparing for autologous peripheral blood stem cell (PBSC) transplant after myeloablative therapy for NHL ($n=11$), MM ($n=1$), CML ($n=1$), Hodgkin's disease ($n=1$), or breast cancer ($n=2$). Telomere length of isolated CD34+ cells (8656 + 1701 bp) was slightly greater than that of the whole apheresis collection (8816 + 2061 bp). However, there was a significant positive correlation between these two variables ($r=0.61$, $p<0.01$). Analysis of variance of telomere length in MNC, PB, BM, PBSC and CD34+ cells showed no significant difference between all sample populations ($P = 0.7$). Thus, telomere length in haemopoietic stem cells can be determined from that of whole or fractionated PB in future studies of haematological disorders.

The New Zealand experience of treating Gaucher's patients with enzyme replacement: 1997-2001.

RL Spearing* (1), H Blacklock (2), L Teague (3) on behalf of New Zealand Cerezyme Treatment Panel.

(1) *Department of Haematology, Christchurch Hospital, Christchurch*

(2) *Department of Haematology, Middlemore Hospital, Auckland*

(3) *Department of Haematology/Oncology, Starship Children's Hospital, Auckland.*

Gaucher's Disease, an autosomal recessive lysosomal disorder due to the deficiency of glucocerebrosidase, leads to the accumulation of glucocerebroside within tissue macrophages. Enzyme replacement (Ceredase and now Cerezyme) has been available since the early 1990s. However it is extremely costly at approximately \$NZ800 dose if given in the amount recommended by many of the US experts. In 1996 a paper published by Hollack's group in the Netherlands, supported the use of the drug in approximately one eighth (15u/kg/m) of the doses recommended. A decision was taken by Pharmac (the NZ drug funding agency) to fund treatment of type 1 Gaucher's patients, who fulfilled specific criteria, at this low dose with a requirement that the patients underwent regular monitoring.

The 13 patients accepted for treatment, had a high incidence of bony complications having had bone crises and/or avascular necrosis. One had spent most of the preceding year in hospital requiring narcotic analgesia, 2 others were in wheel chairs and 2 required crutches. Five had undergone prior splenectomy. One patient has left NZ and another (a teenager) is taking the treatment intermittently. Of the remaining 11 patients, 8 have been treated for 4 years and the other three, 2-3 years. Seven are also receiving bisphosphonates, mainly pamidronate (Aredia).

Of the 11 patients with ongoing data, all have improved in all symptoms. One patient who discontinued treatment for 6 weeks last year had 2 mild bone crises. Another patient had 2 crises in the first 2 years but none since. One, previously in a wheel chair had a further crisis during the first year associated with further avascular necrosis, but has been free of further events for more than three years. Having successfully undergone bilateral hip replacements, he is back at full time work. The other, also out of her wheel chair, is now walking with crutches and has had a successful pregnancy. None of the others has experienced further bone crises and only one requires ongoing mild analgesia.

Splenomegaly (up to 20cm below the costal margin) was a feature in 7 patients. Only one has persistent splenomegaly, reduced now from 14 to 5cm. Other surrogate markers have improved albeit in most cases not completely as yet.

In conclusion, low dose therapy has produced a satisfactory response in most of this patient group. The role of the bisphosphonate therapy is unclear but may be contributing. However, ongoing careful monitoring will be important.

Immunomagnetic CD138 Isolation Of Myeloma Plasma Cells Increases The Detection Of 13q Deletion, An Adverse Prognostic Marker In Multiple Myeloma

MJ Sturm*, J O'Reilly, RP Herrmann, S Ward, J Cooney, M Trent, PK Cannell

Department of Haematology, Royal Perth Hospital, Perth, Australia

Multiple myeloma is a B cell malignancy characterised by terminally differentiated plasma cells. Of the cytogenetic abnormalities found in myeloma, deletion of 13q (del 13q) is an adverse prognostic marker and has been detected by conventional cytogenetics in about 15% of cases. In this study, CD138 immunomagnetic beads were used to isolate myeloma plasma cells and increase the sensitivity of detection of del 13q by fluorescence in situ hybridization (FISH).

Bone marrow aspirates from 60 patients (32 at diagnosis, 28 at relapse/ restaging) were analysed for del 13q. Plasma cells were quantitated using flow cytometry by high CD38 and low CD45 expression, and examined for co-expression of CD138. For samples with plasma cell loads <50%, CD138 magnetic microbeads (MACS) were used to isolate plasma cells. FISH was performed on the bone marrow or purified plasma cells using a probe to 13q14 at locus D13S25 (Vysis). Interphase cells were scored for the presence of fluorescent signals. The partial or complete deletion of chromosome 13q was indicated by the presence of only one signal in at least 5% of scored cells.

Plasma cell purity after CD138 selection was >80% and resulted in del 13q being detected in patients with plasma cell loads as low as 2%. Del 13q was detected in 57% (34/60) of patients and these patients had a significantly worse overall survival with only 63.6% of del 13q surviving at 5 years as compared to 92.6% of patients without the deletion ($p=0.027$) and irrespective of treatment.

CD 138 purification of myeloma plasma cells increases the detection of 13q deletion, which is associated with a worse prognosis.

Where it all stems from: An educational tool for Stem Cell Transplant Patients.

J Suffolk, B White

Princess Alexandra Hospital, Brisbane, Qld.

To aid in the education of our Autologous Stem Cell Transplant patient's and their significant others, we have produced a therapy flowchart. The poster is used as a visual aid, in conjunction with existing methods of education, to illustrate the continuum of care provided within our Oncology/Haematology Unit.

With increasing numbers of Autologous Stem Cell Transplants being performed, a need was seen for a flowchart that summarised a patient's continuum of care during the Transplant program. The poster shows where a patient will expect to be at any time during the peri-transplant process. Patients and their significant others are easily able to visualize their continuum of care, increasing their awareness of what to expect in the treatment process. Incorporating photos, quotes and input from past patients in the poster, has been found useful by patients currently undertaking treatment.

The poster demonstrates a typical continuum of care, which commences when the patient presents from the community to our Outpatients Department (OPD), for their first consultation. It follows through diagnostic testing to treatment for their disease in Daycare and our Inpatient facilities. Then on to planned Stem Cell Apheresis and takes them through the Autologous Transplantation process. Finally following on to recovery and further monitoring via OPD.

We feel that this poster effectively illustrates the flow of care provided for our stem cell transplant patients by our multi-disciplinary team within this unit.

Reappearance of Polycythaemia Vera following treatment of Myelofibrosis with Thalidomide.

Ram Tampi (1 2)* Paul Cannell (1 2) Richard Herrmann (1)

(1) Haematology Department, Royal Perth Hospital, Perth, WA

(2) Clinipath Pathology, West Perth, WA

A 44 year old female presented with classical symptoms of Polycythaemia vera (PV) and splenomegaly in 1995: Hb 189g/l, wc 10.3/nl, plts 510/nl. Following a diagnostic marrow, cytogenetic (normal) and red cell mass studies she underwent periodic venesections followed by maintenance with Hydroxyurea until 2000 when a progressive decrease in her Hb, leucoerythroblastic blood film and moderate splenomegaly heralded myelofibrosis which was confirmed on bone marrow biopsy. She then became transfusion dependent having failed a trial of erythropoietin. After informed consent Thalidomide was commenced in November 2000, maintenance dose 300mg oral daily. This was well tolerated with only a skin rash as a side effect. Her Hb corrected within 3 months, splenomegaly regressed and she was no longer transfusion dependent. In June 2001 there was a return of her original PV symptoms her Hb rose to 159g/l HCT 0.48, RCC 6.1/pl. A bone marrow demonstrated substantial regression of the myelofibrosis with profound erythroid hyperplasia in keeping with a return of her PV. Cytogenetics revealed a del 20q abnormality. Thalidomide was then decreased and finally ceased. Hydroxyurea and periodic venesections were then reinstated. Thalidomide therapy in secondary myelofibrosis can lead to haematological improvement, but it is unusual for the original disease to re-present and may point to the unpredictable action of this drug when used in this situation.

Pneumonitis Occurring After Fludarabine Therapy - The Value Of Gallium Scan In Diagnosis.

Minakshi Taparia*, F T Cordingley, Michael F Leahy.

Department of Haematology, Fremantle Hospital, Alma Street, Fremantle.

Fludarabine is an effective treatment for CLL. Major side effects of Fludarabine are myelosuppression, immunosuppression, neuropathy, and hepatocellular toxicity and, less commonly, pulmonary toxicity has been described. We present 3 cases, in which the diagnosis of post Fludarabine pneumonitis was made only after Gallium scans showed abnormal pulmonary accumulation.

Case 1: A 72-year-old man, with CLL for 6 years, previously heavily treated, presented with progressive disease. He was a heavy smoker. He received 2 cycles of Fludarabine. One month later he developed abdominal pain and was found to have a leaking aortic aneurysm requiring urgent repair. Postoperatively he developed fever, which was unresponsive to antibiotics. No focus was found on detailed investigations, which included CXR, Thoracic CT and bronchoscopy. Gallium scans showed marked pulmonary accumulation. He subsequently developed ARDS. Despite steroid therapy and antibiotics he deteriorated and died due to respiratory failure.

Case 2: A 66-year-old woman with CLL for 5 years previously treated with Chlorambucil had progressive disease. Treatment was given with 2 cycles of Fludarabine. Following the first cycle, she presented with febrile neutropenia, which appeared to resolve with antibiotics. Following the second cycle she again developed pyrexia unresponsive to antibiotics. Apart from mild shortness of breath she had no respiratory symptoms. Pulmonary function studies showed mildly reduced gas transfer and mildly reduced peak flow. CXR and high resolution CT scan of the lungs were normal. Serology for atypical infections was normal and blood cultures were negative. Gallium scans showed increased pulmonary activity indicating low-grade diffuse pneumonitis. Treatment was commenced with oral Prednisolone and lead to rapid resolution of symptoms, which did not recur.

Case 3: A 66-year-old male with CLL for 6 years previously heavily treated developed progressive refractory disease. He was a heavy smoker. He received two cycles of Fludarabine. Following the second cycle of Fludarabine, he developed shortness of breath but had no other respiratory symptoms. Pulmonary function studies showed moderate impairment of gas transfer. CXR was normal. Gallium scans showed a diffuse pneumonitis. Treatment with, oral prednisolone lead to rapid resolution of symptoms, which did not recur.

We conclude that Fludarabine pneumonitis should be considered whenever patients have respiratory symptoms or fever of unknown cause following treatment. Patients may not have prominent respiratory symptoms but this reaction may be severe, leading to death and may not be responsive to steroid therapy. Gallium scanning appears to be the most sensitive test for diagnosis. Smoking may be a risk factor for developing pneumonitis

Fetal isoimmunization in WA: clinical and haematological outcomes

S.Taparia*¹, J.Travadi¹, R.Hagan^{1,2}, R.Kohan¹, L.Marshall³

1 Department of Neonatal Paediatrics, 2 University Dept of Obstetrics and Gynaecology, & 3Department of Haematology; King Edward Memorial Hospital for Women, Perth, Western Australia

Objective: To review, the clinical and haematological outcomes of fetal isoimmunization, since the introduction of intrauterine intravascular transfusion.

Setting: Geographical population of WA. Fetal intrauterine intravascular transfusion was first performed in WA in 1987.

Methods: Cases (positive maternal antibodies) from 1987 to 2000 were identified in WA from i) blood bank records, ii) ultrasound department records, iii) neonatal databases. Clinical and laboratory data were extracted from maternal and infant charts using pre-set proforma, data checked, coded and entered onto computer database.

Results: There were 180 cases identified in these years. Abstract is based on analysis of data in first 31 cases. Four groups were identified. Group-1: no intrauterine transfusion (IUT) or exchange transfusion (ET); Group-2: no IUT but ET after birth; Group-3: IUT only and, group-4: both IUT and ET required. There were no differences in gestational age or birth weight between the groups (Table- median and iqr shown). There were significant differences in the cord Hb and bilirubin levels and the peak bilirubin level reflecting the more severe disease in groups 3 and 4. Overall, 41 IUTs, 28 ETs and 27 top-up transfusions were performed. Sixteen (51%) babies required blood transfusion for late anaemia. Babies in group 3 and 4 (IUT groups), more commonly required transfusion.

	Group-1 (n=7)	Group-2 (n=13)	Group-3 (n=6)	Group-4 (n=5)
gest age (w)	37(36-38)	37(34-37)	36(36-37)	36(36-36)
Birth wt (g)	2900(2700-3090)	2325(2185-3260)	3107(3040-3140)	2675(2600-3105)
cord Hb (g)	146(127-150)	127(102-157)	116(106-129)	110(89-139)
cord bilirubin+ (mM/L)	42(34-55)	75(44-90)	68(61-81)	112(94-134)
max bilirubin+	169(125-304)	319(212-349)	174(126-226)	309(274-332)
phototherapy duration (hrs)	133(33-197)	116(113-127)	109(90-165)	115(110-120)
discharge Hb	146(121-174)	132(121-140)	128(125-135)	133(127-140)

+ p<0.05

Conclusion:

Fetal isoimmunization is still a major problem in neonatal nurseries with a continuing requirement for fetal and neonatal transfusions.

Autologous Platelet Transfusion - Revitalising an Old Methodology

Taylor D^{1*}, Rodwell R¹, Ambrosoli P¹, Coultson J¹, Baldry D¹, Bentley M¹, Williams K¹, Kelly C¹, Fitzsimmon A¹, Wright S¹, Taylor K¹, Jardim-Surman K², Lanzalone S², Morganti A².

1. Mater Hospitals, South Brisbane, Australia.

2. Pharmacia, Sydney Australia, Milan Italy.

AIM: A clinical trial using recombinant human thrombopoietin-derived (rhTPO, Pharmacia) autologous platelet support for high-dose chemotherapy (HDCT) and autologous peripheral blood progenitor cell transplantation (PBPC) required the development of complementary platelet cryopreservation/reinfusion methodology.

METHODOLOGY: Platelet counts were monitored following stem cell collection and rHuTPO administered in two dosing schedules (1.2mcg/kg D1+D4; 2.4mcg/kg D1) when platelet count had recovered $\geq 100 \times 10^9/L$. Autologous platelets/plasma were collected using modified plateletpheresis (COBE Spectra Version 6.0) as previously described (Kelly C et al, 2000) on either day 12 post-rhTPO or platelet count $\geq 600 \times 10^9/L$. A target of 25×10^{11} platelets was predicted as sufficient to provide autologous support allowing for theoretical loss of 50% of platelet numbers during thawing/reconstitution. Platelets were mixed with saline (0.9%) and dimethyl-sulfoxide (DMSO) at a ratio (16:3:1) and divided into aliquots that facilitated a reinfusion dose of $\geq 2 \times 10^{11}$ platelets post-thaw. Aliquots were rate frozen (1°C/min to -100°C) then stored in liquid nitrogen. Autologous plasma was cryopreserved by rapid cooling in vapour phase liquid nitrogen. Reinfusion of cryopreserved autologous platelets occurred post HDCT/PBPC using a prophylactic trigger of $20 \times 10^9/L$ or in response to any haemorrhagic events. Platelets were rapidly thawed at 37°C, diluted in a PBS/ACD-A wash solution then recovered by centrifugation (930g, 20min, 20°C) and resuspended in autologous plasma (50-100ml) prior to reinfusion.

RESULTS: Seventy-eight cryopreservations have been performed on 24 patients with a median total platelet harvest of 25.74×10^{11} (12.25 - 42.33). 17/24 patients (71%) achieved the target yield of $\geq 25 \times 10^{11}$. Median platelet dose cryopreserved per aliquot was 2.49×10^{11} (0.55 - 6.47). 22/24 patients have undergone HDCT/PBPC with 71 episodes of autologous platelet transfusion. Median pre-thaw transfusion dose was 4.75×10^{11} (1.99-7.82). Median post-thaw washed platelet transfusion dose was 2.61×10^{11} (0.67-6.22) giving a median recovery of 55.7% (16.32-91.31).

CONCLUSIONS: This methodology has successfully combined historical donor platelet cryopreservation techniques with the advances made in apheresis and growth factor technology. We have adapted and standardised procedures to minimise quantitative and qualitative platelet loss whilst maximising in vivo haemostatic capability. Autologous platelet transfusion support is a feasible alternative to homologous platelet transfusion in the prevention and treatment of bleeding in patients undergoing HDCT/PBPC.

High incidence of CD20 negative relapses following Rituximab (Mabthera) therapy for relapsed/refractory aggressive Non-Hodgkin's lymphoma (NHL).

Tey SK*, Kennedy G, PV Marlton, Cull G, Grimmer K, Cobcroft RG, Thomson DB, Gill DS.

Dept. of Oncology/Haem, Princess Alexandra Hospital, Brisbane Q4102

Introduction. CD20 antigen is present in >95% of aggressive NHL. Rituximab, a chimeric anti-CD20 monoclonal antibody has been shown to be effective in the treatment of NHL. Reports of CD20 negative relapses after treatment with Rituximab are rare.

Aim of study. The incidence of CD20 negative relapses following Rituximab therapy in relapsed/refractory aggressive NHL, was investigated.

Methods and materials. A retrospective review of patients who received Rituximab for aggressive NHL was performed. Biopsy was taken at relapse following Rituximab therapy where possible. Samples included lymph node or other tissue biopsy, bone marrow and peripheral blood. Immunophenotyping was performed by immunoperoxidase using L26 (DAKO) in fixed tissue and/or by flow cytometry using anti-CD20 antibody (Immunotech).

Results. Eighteen cases were identified, 8 patients had progressive disease (PD), 3 were in partial remission (PR), 6 were in complete remission (CR) and 1 had minimal residual disease (MRD). Twelve patients relapsed or progressed following Rituximab therapy, 8 patients had repeat biopsy. Six of the 8 patients had at least one site of relapse, which was CD20 negative either by L26 immunoperoxidase or by flow cytometry. Median age of the CD20- relapse patients was 54.9 years (range 38.9 to 71.8), 2 males and 4 females. Five patients had diffuse large B-cell lymphoma (1 HIV+), of which 2 had discordant histology on bone marrow and 1 transformed from follicular centre cell lymphoma. One patient had mantle cell lymphoma. Of the CD20- relapse patients, at the time of rituximab therapy, 3 patients were in CR, one case of each with stable disease, PD, and MRD (PCR +only). Median time to CD20- relapse after last dose of Rituximab was 178 days (range 34 to 367). Four patients had unusual sites of relapse including the chest wall, thigh, trochlear mass and subcutaneous nodules.

Discussion. The high incidence of CD20 negative relapses in relapsed or refractory aggressive NHL would suggest the existence of >1 clone at the time of Rituximab therapy, and/or evolution of new clones with mutation or loss of CD20 antigen. The relapse at unusual sites would support the latter. Even if Rituximab was blocking the CD20 binding sites thereby resulting in apparent CD20 negativity by flow cytometry but positive by immunoperoxidase (L26), the continued survival and growth of these lymphoma cells suggests these lymphoma cells are resistant to Rituximab.

Conclusion. The incidence of CD20 negative relapses in aggressive NHL is unexpectedly high. Lymphoma relapses may occur at new and unusual sites often without relapse at site of original disease. Biopsy should be undertaken at relapse, particularly if re-treatment with Rituximab is under consideration.

Immune Reconstitution Following High-Dose Therapy and Autologous Stem Cell Transplantation: Possible Association with CD34 dose and Immunoglobulin Reconstitution.

ALWatson*, S Farag, A McCutchan, NW Wickham

North Queensland Oncology Service, Townsville General Hospital, Townsville, Queensland.

A prospective study was performed in patients undergoing high dose therapy and autologous stem cell transplantation to determine when immune reconstitution occurs, relationship with the CD34 cell dose and correlation with infections.

Patients who underwent autologous stem cell transplantation were assessed for immune recovery with immunoglobulin levels (IgG, IgA and IgM) and lymphocyte subsets (CD3, CD4 and CD8 T lymphocytes, CD19 B lymphocytes and CD16/56 NK cells) on day+100, then 3 monthly until 2 years post transplant and then 6 monthly or until evidence of recovery.

The study population was 30 patients (female 16, male 14) aged 19-72 years (median 56.5) who underwent autologous stem cell transplantation from August 1998 to February 2001 with a median follow-up of 15 months (range 3-30). Diagnoses included NHL (16 patients), multiple myeloma (8), Hodgkin's disease (4) acute leukaemia (1) and breast cancer (1). Median CD34 count was 4.07×10^6 cells/kg (range $2.18 - 13.5 \times 10^6$). Immunoglobulin reconstitution occurred at a median of 6 months in 23 (76%) patients including 3 patients with continued selective reduction of IgA only. Patients who did not recover normal immunoglobulin levels had a lower CD 34 count (median 3.3×10^6) compared to those who achieved normal levels (median 4.15×10^6). Only 12/30 (40%) of patients achieved a normal CD4 T lymphocyte count at a median of 16.5 months post-transplant. There are 10 patients > 12 months post transplant with subnormal CD4 counts including 7 patients > 2 years post transplant. There was no difference in median CD34 counts between those who achieved a normal CD4 count and those that did not. One patient had delayed CD8 T lymphocyte recovery at 6 months with all other patients having normal CD8 count at day+100. All patients excepting those 3 patients who received post transplant Rituximab had normal CD19 B lymphocyte counts at day+100. All except 1 patient had normal NK cell levels at day+100 or when first tested. NK cell recovery occurred at 12 months in the remaining patient. Three patients had CMV infections (concurrent abscess in 1 patient) at 3, 3 and 8 months post-transplant. There was subsequent CD4 recovery in 2 patients. One patient with no immunoglobulin or CD4 T lymphocyte recovery at 12 months had problems with recurrent infections.

Immune recovery is delayed in many patients who undergo autologous stem cell transplantation however usually is not associated with serious infection. There may be a correlation with CD34 dose and immunoglobulin recovery.

Acquired C1 Esterase Inhibitor Deficiency In Waldenstroms Macroglobulinemia- Resolution Of Angioedema After Complete Remission Using FMD Chemotherapy.

Jeremy Wellwood(1)* , Kerry Taylor(1), Susan Wright(1), Mark Bentley(1), Paul Eliadis(2).

1 Mater Adult Hospital, Brisbane.

2 Wesley Medical Centre, Brisbane.

Introduction

Acquired C1 esterase inhibitor deficiency is a rare but well described cause of recurrent angioedema. Indolent lymphoproliferative disorders are an important cause of this acquired syndrome. We present a case of acquired C1 esterase inhibitor deficiency associated with Waldenstroms macroglobulinemia, a rare association with only a few cases being reported.

Case Report

A 58 year old female reported 12 cases of episodic facial angioedema since 1996. 14 months after the initial episode the patient developed mild pancytopenia (Hb 107g/L, WBC 2.4×10^9 /L, Plt 109×10^9 /L). Clinical examination revealed splenomegaly. CT scanning showed the splenomegaly with no evidence of lymphadenopathy. Bone marrow biopsy revealed low level infiltration with a monoclonal population of B lymphocytes (CD19, CD20 and lambda light chain positive; CD 5 negative). An IgM paraprotein of 6g/L was found on serum protein electrophoresis (EPP). Further investigations revealed C4 <55mg/L (Ref 130-780), C1 esterase inhibitor < 30mg/L (Ref 250-550), C1 esterase inhibitor activity <20 U/L (Ref 300-800). A diagnosis of acquired C1 esterase inhibitor deficiency in association with Waldenstroms macroglobulinemia (lymphoplasmacytic lymphoma with an IgM paraprotein) was made. Danazol 200mg orally BD was commenced.

After a period of observation and a course of rituximab for increasing splenomegaly, the patient had continued episodes of angioedema and evidence of disease progression. Treatment with four cycles of fludarabine, mitoxantrone and dexamethasone (FMD) achieved a complete response in the lymphoma (normal bone marrow, spleen size and serum EPP). This was paralleled by normalisation of the C1 esterase inhibitor level and activity (See Table 1). No further episodes of angioedema occurred and danazol was able to be ceased.

	Pre-treatment	After 2ndFMD	After 4th FMD
C1 esterase inhibitor level, mg/L (Ref 250-550mg/L)	80	220	265
C1 esterase inhibitor functional activity, U/L (Ref 300-800 U/L)	80	305	580

Conclusion

Lymphoproliferative disorders are a common cause of acquired C1 esterase inhibitor deficiency and should be considered in the investigation of recurrent angioedema. The typically delayed presentation of the lymphoma with respect to the initial presentation of angioedema is highlighted. Our case reinforces the principle that the most effective way to treat acquired C1 esterase inhibitor deficiency is to treat the underlying disorder.

A proportion of quiescent leukaemic progenitors in CML survive culture in the presence of STI571 and retain clonogenic potential.

DL White*, S Bresatz, AB Lyons & TP Hughes.

Melissa White Laboratory. Hanson Centre for Cancer Research, IMVS, Adelaide. SA.

Chronic myeloid leukaemia (CML) is a haemopoietic stem cell disease characterised by the presence of the Ph chromosome which encodes a fusion protein, BCR/ABL, with increased tyrosine kinase and anti-apoptotic activity resulting in deregulated CML growth. Given the high proliferative potential of these cells it might be expected that cell-cycle specific chemotherapeutic regimens would prove successful in disease eradication. However, early relapses are seen following intensive chemotherapy, suggesting the presence of a quiescent leukaemic population. We have taken two approaches to isolate quiescent leukaemic cells; using Hoechst 33342 and Rhodamine 123 (Rh/Ho) to isolate G₀/G₁ cells; and using the cytoplasmic dye CFSE to FACS sort divided and undivided cells. Combining both approaches in stroma free cytokine dependent culture allows assessment of the proliferative potential and sensitivity to STI571 of the different subsets. Using FISH for BCR/ABL on FACS sorted Rh/Ho CD34⁺ dull/dull cells we have demonstrated the existence of a quiescent leukaemic population (median 33% BCR/ABL⁺/Range 10-46%) in 12 chronic phase CML patients examined to date. These CD34⁺ Rh/Ho dull/dull cells demonstrate high proliferative response to haemopoietic growth factors and give rise to approximately double the number of CFU-GM colonies as CD34⁺ cells over a 21 day period. The addition of STI571 to these primitive CML cells downregulates, but does not abrogate their high proliferative responsiveness. Using CFSE we have demonstrated that CD34⁺ CML cells (but not normal CD34⁺ cells) undergo up to 3 divisions over a 4 day period, in the absence of exogenous growth factors (factor independent growth). The addition of STI571 to CML cells removes their factor independence and commits the majority (>80%) of the quiescent cells to apoptosis in the absence of division.

Importantly, approximately 5-15% of undivided leukaemic cells survive in culture with STI571 (1 μ M) and exhibit marked clonal expansion if growth factors are subsequently added. These "STI insensitive" cells regain their sensitivity to STI571 when induced to cycle, suggesting that they are not intrinsically STI-resistant, rather that their sensitivity is cell cycle dependent. We conclude that a primitive quiescent population of CML cells exists that is relatively insensitive to STI571 and is a potential reservoir of CML cells capable of surviving and undergoing clonal evolution.

Hospital Based Blood Product Support and the Implications of TGA Guidelines and Donor Safety.

Katrina Williams *(1), Sonia Tepes (1), Cathryn Kelly (1), Diana Moore (1), Donna Ryan (1), April Fitzsimmons (1), Jennifer Bourne (1), Kelly Atkinson (1), Chris Leon (1), Alayne Reid (1) Ann Franks (1), Robyn Rodwell (2), Kerry Taylor (3).

1. Registered Nurses Mater Hospital Brisbane Cindy Sheu Apheresis Unit.

2. Haematology Chief Scientist, Pathology Mater Hospital

3. Clinical Haematologist Mater Hospital Brisbane

Blood product support from the Australian Red Cross Blood Transfusion Service (ARCBTS) is required routinely for patients with haematological malignancies. Hospital based blood component support complements this service by utilising healthy normal donors who undergo plateletpheresis and leukopheresis for relatives and friends. Such instances include provision of allogeneic peripheral blood stem cells and donor lymphocytes, and meeting the emergency demand for supplies of platelets and granulocytes.

We reviewed normal donor procedures at the Mater Hospital in the Cindy Sheu apheresis unit has been uneventful. 372 donor procedures have been undertaken in the last 5 years. 198 of these supplied emergency platelets to Mater inpatients. 118 procedures were leukopheresis for granulocyte collection and donation. The other 56 comprise allogeneic stem cell and donor lymphocyte donations and research mononuclear cell donations. 249 out of 372 procedures (66.9%) were free of toxicity outlined by Australia and New Zealand Apheresis Association (ANZAA). The most common toxicities were access flow problems in 12% of donors and mild citrate reactions in 8% of donors. Seven procedures were aborted (1.8%) due to poor venous access and 3 (0.8%) were aborted due to citrate toxicity unresponsive to intervention. 97.5% of the procedures were performed using peripheral access. Central venous access was necessary for two allogeneic stem cell donations by one donor.

Donor safety was preserved in this group by a thorough screening process. A questionnaire derived from the ARCBTS was developed identifying risk to not only recipient but also donor. ECG monitoring and chest x-rays were also employed when cytokines were administered to donors.

Recent amendments in the Therapeutic Goods Association guidelines for blood banking will have implications for hospital generated blood products. Protection of the recipient of these products is accomplished from two angles - Donor screening and product quality control. The Cindy Sheu apheresis unit uses an access data bank to record all product yields. Trends are tracked and reported to ANZAA on a monthly basis. Prior to administration of a stem cell product;

Yields are calculated,

Viability measured pre and post thaw

Product and donor blood is cultured for bacteria and

Viral serology is checked.

An Orientation for Patients and Health Care Professionals to Histocompatibility Typing and Allogeneic Stem Cell and Bone Marrow Donor Searches.

Katrina Williams*(1), Sonia Tepes (1), Ann Franks (1), Mark Bentley (2), Susan Wright (2), Kerry Taylor (2),

1. Registered Nurses Cindy Sheu Apheresis Unit, Mater Hospitals, South Brisbane, Australia

2. Clinical Haematologists Cancer Services, Mater Hospitals, South Brisbane, Australia

The use of allogeneic donors has expanded recently due to changes in treatment modalities in the transplant setting and the introduction of the practice of cord blood banking. These two factors have exposed a larger patient population to the concepts of histocompatibility and the search processes involved in identifying an appropriate donor. The Leukaemia Foundation in Queensland has identified that requests for information on donor searches are commonly made by patients and their families. The Mater Adult Cancer Services have always allocated one member of the nursing staff to track its patients' donor searches. This ensures the search is completed in a timely fashion and follows most direct pathway to a donor. Staff turnover and leave in our unit has necessitated implementation of an ongoing teaching program to allow education of new staff and continuing updates. To aid this endeavour a teaching tool has been developed. This simple computer program outlines the following concepts;

Basic biology of the major histocompatibility complex (MHC)

Organising DNA collection, storage and transport

Donor search tracking technique

Search pathway " Dos and Don'ts"

An information leaflet is also used to help patients follow their own searches. It comprises a glossary of terms, simple statistics synthesised from previous donor searches and some basic biology use to determine if someone is a compatible match.

Erythrocyte Sedimentation Rate in a Subject with High Haematocrit: A Single Case Report

N Woodland*, K Cordatos, W Hung, A Reuben, L Holley

University of Technology, Sydney, NSW, 2007

Erythrocyte sedimentation rate [ESR] is commonly used in clinical laboratories as a non-specific indicator of disease. While several factors contribute to ESR, the two most important are plasma fibrinogen concentration [FC] and haematocrit. The influence of anaemia is well documented, however high haematocrit values are rarely considered when clinically evaluating ESR.

The aim of this study was to investigate a borderline ESR for an adult female with a haematocrit of 50%.

A full blood count was performed using a Coulter StkS haematology analyser, FC was determined using the Clauss assay and the blood film was examined. Four samples were prepared for ESR determination: at native haematocrit [50%]; at manipulated [by the addition of native plasma] haematocrit of 40% [average haematocrit for adult female]; and washed erythrocytes suspended in saline [9.0g/L] and adjusted to haematocrits of 40% and 50%. These four volumes were diluted 1:4 with 3.8% sodium citrate according to the Westergren technique and drawn into ESR tubes on a rack in a temperature-controlled room [22±1°C]. ESR readings were taken after one hour.

FC was 5.6g/L [normal range (NR) 1.5-4.0g/L], erythrocyte indices were within NRs and erythrocyte morphology appeared normal. ESR [native, 50% haematocrit] was 24mm/hour [NR 0-19mm/hour]; ESR [40% haematocrit in plasma] was 49mm/hour; ESR [50% haematocrit in saline] was 1mm/hour; ESR [40% haematocrit in saline] was 2mm/hour.

Absence of plasma proteins resulted in very low ESR values. For the samples containing plasma proteins, ESR values were significantly higher, with the 40% haematocrit sample clearly outside the NR. High FC is known to contribute to high ESR. Results indicated a slightly raised ESR for the native blood sample, and a very elevated ESR for the sample adjusted to haematocrit of 40%.

These results suggest that despite the high FC there was a lowering effect on ESR by the very high haematocrit. However, when the haematocrit was adjusted to a more appropriate value for an adult female, a high ESR, which was consistent with the high FC and may be indicative of disease, was apparent. This supports claims that high haematocrit values should be considered when interpreting ESR.

The Erythrocyte Sedimentation Rate: Determination of a Normal Range in the Elderly

Petkovic, D (1,2), Woodland, N* (1)

(1) University of Technology, Sydney; (2) Sydney Diagnostic Services Pathology

The erythrocyte sedimentation rate (ESR) is a common, non-specific indicator of disease. Reference ranges for individuals aged 17-50 years are well established. However, ranges for individuals over 60 years are controversial. Increased disease in the elderly and changes in haematocrit and plasma protein concentrations, which occur in healthy elderly individuals, influence ESR. Thus, it is difficult to establish a normal reference range.

The aim of this study was to determine the reference range for ESR in elderly individuals. Two hundred haematologically normal subjects aged 60-95 years, with normal liver function tests and electrolytes and no evidence of disease, were selected. To compare ESR values, another 200 clinically abnormal subjects in the same age group and a further 200 normal subjects aged 18-59 years, were analysed. Each group had equal numbers of males and females. ESRs were performed on blood collected in EDTA tubes and analysed by the Starrsed automated ESR analyser, which utilises the Westergren method.

Results suggest that ESR up to 21 mm/hour in elderly males and up to 24 mm/hour in elderly females should be considered normal. Classification of these individuals into three age groups ie 60-69 years (48 males, 43 females), 70-79 years (37 males, 38 females) and 80+ years (15 males, 19 females) showed that for males ESR was significantly higher for the 80+ group. However, as only a small number of individuals were analysed in the 80+ group, analysis of larger numbers is recommended. The females showed no difference in ESR values between the three age groups.

For both males and females, the clinically abnormal elderly individuals had a much higher mean ESR than the clinically normal elderly individuals. The clinically normal males below 60 years had a lower ESR mean than the clinically normal females, with values up to 14 mm for males and 19 mm for females. Both of these upper limits were lower than those determined in the clinically normal elderly individuals. This indicates that the ESR does increase with age and is higher for females than males.

This study suggests that both age and gender affect ESR and that values obtained from younger individuals should not be accepted as the normal range for elderly individuals.

Abnormal vBeta T cell populations in rheumatoid arthritis (RA) patients are not altered by haemopoietic stem cell transplantation (HSCT) despite increased thymic output.

J.Moore*(1), J.Snowden (1), V.Nink (1), D.Ma (1), S.Milliken (1), F.Ponchel (2), J.Issacs (2) on behalf of the Australian Rheumatoid Transplant Study Group.

1 Haematology and Immunology Depts, St. Vincents Hospital, Sydney.

2 Molecular Medicine Dept, University of Leeds, UK.

Recent studies demonstrating reduced thymic output and the presence of oligoclonal expansions of T cells have suggested that RA patients suffer from a defect in T cell homeostasis which may have a role to play in the disease process. Our group have completed a trial of HSCT in patients with severe RA resulting in significant remissions followed by recurrence of disease. One hypothesis of the action of HSCT is that "re-education" of the immune system occurs through the induction of tolerance by the passage of new stem cells through the thymus post high dose immunosuppression. Thus far, there has been no evidence to justify this hypothesis. We assessed these abnormal T cell populations by measurement of CD4 vBeta antigens using flow cytometry. We also measured thymic output by a competitive PCR measurement of thymic re-excision circles(TREC). In this way we could measure the extent of re-education of the immune system post HSCT. RA patients (n=30) had a vBeta measurement performed pre HSCT and 1.5, 6 months post HSCT. In comparison to normals (n=16) there was significant (p < 0.05) expansion of CD4 vBeta 2, 3, 7 and 12 in the RA patients pre HSCT, consistent with other studies. However following HSCT, there was no significant reduction in any of the vBeta populations in either the CD34 selected or unmanipulated groups suggesting that abnormal clones of T cells were resistant to HSCT or alternatively there were no naive T cells produced post HSCT. TREC analysis in 13 patients however revealed that 9/13 had an increased thymic output post HSCT suggesting that despite the generation of new T cells, they are still able to escape tolerance and continue to express expanded T cells clones after an autologous HSCT. These results suggest that autologous HSCT does not result in re-education of abnormal T cell populations in RA despite the generation of naive T cells. It is possible that the stem cell or thymus is intrinsically abnormal in RA resulting in this lack of correction with an autologous graft which would be consistent with the clinical results.

Loss of Heterozygosity (LOH) of MHC, beta-2 microglobulin (beta-2 m) and MEMO-1 Genes in Acute Lymphoblastic Leukaemia and Breast Cancer

CRE McEvoy, AA Morley* and FA Firgaira

Department of Haematology & Genetic Pathology, Flinders University and Medical Centre, Adelaide, South Australia

Objectives: As loss of expression of histocompatibility proteins may be a mechanism whereby tumours evade immune surveillance, we determined the frequency and nature of LOH mutations affecting the MHC genomic region and the beta-2 m and MEMO-1 genes in acute lymphoblastic leukaemia and breast cancer.

Methods: Paired normal/tumour DNA was analysed for LOH at chromosome 6 using 10 microsatellite markers (5 within the MHC), 2 markers flanking the beta-2 m gene and one marker within the MEMO-1 "minimum region of deletion". Markers were amplified by PCR and fluorescent product was analysed on an ABI373 DNA sequencer using GenescanTM software. Peak height ratios for marker alleles were determined with a 25% reduction in a tumour marker peak height being considered as indicating LOH.

Results: The table below shows LOH frequencies for the MHC (LOH of at least one of the 5 markers), beta-2 m and MEMO-1. The leukaemic LOH frequency within the MHC was double that for breast cancer. In contrast, LOH of the beta-2 m and MEMO-1 genes was more frequent in the breast cancer cohort. In all cases, the frequency differences were statistically significant (Fishers exact test). If we assume that LOH at either of these three genomic regions leads to abnormal HLA class I expression, then we can predict the total frequency of such samples. These results are remarkably similar (see column 4). The majority (14/19) of MHC LOH events in leukaemia were associated with loss of an entire copy of chromosome 6 whereas the majority (8/10) of events in breast cancer were generally local deletion or recombination.

	MHC	beta-2 m	MEMO-1	Predicted total abnormal HLA class I
Leukaemia	0.34 (19/56)	0.04 (2/55)	0.12 (5/42)	44%
Breast Cancer	0.17 (10/58)	0.28 (16/57)	0.33 (11/33)	53%

Conclusion: This study has made a direct comparison of LOH frequencies and genotypes for the MHC genomic region and beta-2 m and MEMO-1 genes between a haematogenous neoplasm and a solid tumour. The contrasting results suggest alternative mechanisms of HLA class I loss/down regulation between the two cancer types.

Granulocyte-colony stimulating factor induced mobilization of blood "lymphoid" CD123^{hi} DC is associated with downregulation of surface L selectin

*+S. Vuckovic, +M. Kim, +D. Khalil, +G. V. Crosbie, +A. M McGee +N. Williams, +L. Brown, #K. Williams, #C. Kelly, #R. Rodwell, +G.R. Hill, #S. Wright, #K.A. Taylor, +D.N.J. Hart.

+Mater Medical Research Institute, Aubigny Place, Raymond Terrace, South Brisbane, Queensland, 4101, Australia
#Mater Misericordiae Hospital, Stanley Street, South Brisbane, Queensland, 4101, Australia

Recent studies have shown that granulocyte-colony stimulating factor (G-CSF) produces profound changes in peripheral blood dendritic cell (DC) counts (Blood 1999;93:728-36) and preferentially increases "lymphoid" (CD123^{hi}) DC over myeloid (CD11c⁺) DC in normal stem cell donors (Blood 2000;95:2484-90, J Immun 2000;165:566-72). CD123^{hi} DC are found near high endothelial venules, and are postulated to migrate directly from blood into the lymphoid tissues. We have been studying factors that influence blood DC counts (Blood 2001;98:140-45) and hypothesized that G-CSF may change blood DC adhesion phenotype, thereby permitting these cells to accumulate in blood. Therefore, we examined the effect of G-CSF on the expression of CD49d and CD62L on blood DC of normal donors and patients with multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL), undergoing stem cell mobilization. Normal donors (n=5) were treated with G-CSF (16 mg/kg/day for 4-5 days) and MM (n=9) and NHL (n=8) patients with cyclophosphamide(CY)/G-CSF (CY 4g/m² day 1, G-CSF 30µg/kg/day 9-12 from day 1). Myeloid DC were identified as lin-HLA-DR+CD11c+ and "lymphoid" DC as lin-HLA-DR+CD123^{hi} cells. Blood DC counts were analysed before G-CSF or CY treatment, on the day of stem cell harvest and sequentially after apheresis (days 4-23 in normal donors and days 13-45 in patients). DC were analysed for expression of adhesion molecules before G-CSF or CY treatment and on the day of stem cell harvest. In normal donors, G-CSF treatment increased the number of CD123^{hi} DC from a mean of 4.1 x 10⁶/L to 22.9 x 10⁶/L on the day of stem cell harvest. CY/G-CSF treatment increased the number of CD123^{hi} DC from a pre-mobilization mean of 1.1 x 10⁶/L to 7.5 x 10⁶/L on stem cell harvest in MM patients, but only modestly in NHL patients (mean of 3.4 x 10⁶/L pre-mobilization and mean of 7.8 x 10⁶ /L on stem cell harvest). G-CSF and CY/G-CSF treatment of normal donors, MM and NHL patients had minimal effect on the number of CD11c⁺ DC at all time points. After treatment with G-CSF, CD123^{hi} DC downregulated CD62L in normal donors (mean CD123^{hi} DC positive: pre-mobilisation 66%, stem cell harvest 57%) and in MM patients (pre-mobilisation 51%, stem cell harvest 29%) and upregulated CD62L in NHL patients (pre-mobilisation 15% stem cells harvest 72%). CD49d expression did not change on CD123^{hi} DC and neither CD62L nor CD49d was altered on CD11c⁺ DC. The downregulation of CD62L on CD123^{hi} DC appeared to correlate with the increase in blood CD123^{hi} DC number. Thus, the blood CD123^{hi} DC increase in G-CSF treated stem cell donors may result from altered migration due to of cell surface CD62L-selectin downregulation.

Polyclonal B-cell EBV associated Lymphoproliferative Disorder in a patient with treated Hodgkin's disease.

Dr TB Keng (1)*, Dr S Wright(1), Dr D Norris (2), Dr K Taylor (1)

(1) Haematology Department, Mater Misericordiae Hospital, Queensland 4101.

(2) Pathology Department, Mater Misericordiae Hospital, Queensland 4101.

Epstein-Barr virus (EBV)-associated B-cell lymphoproliferative disorder (LPD) is a well described disorder occurring in patients with congenital immuno-deficiency and in post-transplant patients. To our knowledge, it has not been described in patient with Hodgkin's disease.

Here, we report a case with EBV-associated B-cell LPD occurring 12 years after last therapy for Hodgkin's disease.

A 53 year old lady, first presented in 1966 with Hodgkin's disease involving bilateral cervical and mediastinal lymph nodes. Involved field radiotherapy was initially given. Subsequently, she was treated with abdominal radiotherapy for para-aortic lymph node involvement.

In 1970, her disease relapsed in the mediastinum with concomitant pericardial involvement. She was given mediastinal radiotherapy and cardiac irradiation. She then remained well until 1989 when she relapsed. She received 6 cycles of MOPP chemotherapy after which she achieved her third complete remission.

In February 2001, she noticed transient cervical lymphadenopathy associated with URTI symptoms. Chest X-ray and CT scan of the neck, chest and abdomen/pelvis showed extensive nodular infiltrates in the lungs but no lymphadenopathy or hepato-splenomegaly. Bone marrow biopsy was normal. Serum cryptococcal antigen, ANA, ENA, ANCA were negative. Her EBV IgG was positive and IgM was non-reactive. The total IgG level was normal but levels of IgG 2, 3, 4 subclasses were low.

FNA of lung nodule and trans-bronchial biopsy were non-diagnostic and no organisms were seen microscopically or grown in cultures. She proceeded to have an open lung biopsy which revealed a nodular lymphoid aggregation. No Reed-Sternberg cells were identified. The immuno-peroxidase stains showed that even though the dominant infiltrate making up the nodule was T-cells (CD3+, CD5+), the cytologically abnormal cells were B cell (CD20+, CD 79+). Kappa/lambda stains showed polyclonal staining. Immunoglobulin and T cell receptor gene rearrangement were polyclonal on PCR. However, EBER-ISH is strongly positive which is consistent with an EBV-associated LPD.

Polyclonal/oligoclonal EBV associated LPD have been seen predominantly in immuno-suppressed patients, either post-transplant or in patients with congenital immuno-deficiency. Hodgkin's disease is known to be associated with continuing immunological deficiency or perturbation related to the underlying disease and/or its treatment. This might predispose to EBV-associated LPD post-Hodgkin's disease. A single report had identified EBV in two post-Hodgkin's disease aggressive non-Hodgkin's lymphoma, however, polyclonal EBV-associated LPD post-Hodgkin's disease has not been previously described.