A003

WHO Classification 2002: Unfinished Business

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The WHO classification, having evolved from the REAL system, is essentially a list of clinicopathological entities within a histogenetic framework. Multicentre reviews have demonstrated the clinical relevance of this approach and other studies have shown that it can be applied in a diagnostic setting with good inter-observer variation for most categories. Following its rapid acceptance into clinical practice, stimulus for further activity comes in two broad areas.

Firstly, the greater complexity of the WHO classification, in comparison with earlier, primarily morphological systems, demands greater knowledge and technical expertise from the diagnostic pathologist. This is particularly so in a number of well recognized "gray areas" where features of more than one entity overlap in a single case. The clinicopathological approach further requires that the reporting pathologist coordinate morphological, immunophenotypic and genotypic studies – often from different sources and of varying or unspecified quality. This challenge is exacerbated by the increasing tendency towards less invasive diagnostic techniques such as needle core biopsy.

The second area of activity is that of taxonomic development. A number of WHO "entities" are known to be quite heterogeneous both clinically and biologically. In some of these, such as anaplastic large cell lymphoma and extranodal marginal zone lymphoma, the molecular events underlying the disease are becoming better understood, leading to further refinement and subcategorisaton. Thus, the histogenetic classification can be expected to grow organically, by the addition of further hierarchical layers, rather than by radical restructuring. Frustratingly however, some categories such as diffuse large B-cell lymphoma have resisted efforts at meaningful subdivision based upon morphology or immunophenotype alone, and most of the recent insights in this area have come from microarray analysis.

Debate also continues as to whether or not cutaneous or other extranodal lymphomas warrant a separate classification system.

The lack of intrinsic grading within the WHO classification is a further source of criticism. Apart from the grading of follicular lymphoma, we do not yet have pathological prognostic markers to guide us in any individual case. Again, it is the often unexpected findings of microarray analysis, that have been most useful in identifying potential prognostic markers. Although this technique is unlikely to become a mainstream diagnostic tool in the foreseeable future, immunohistochemical surrogates for these markers could soon become an important part of diagnostic practice.

Finally, as we move toward a molecular classification, it remains to be seen whether the histogenetic imperative of the WHO remains.

A005

Zebrafish Genetics Provides New Opportunities in t(8;21)-Mediated Leukaemia

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Insights into the genetic programs that regulate haematopoiesis are being made through the use of a variety of model systems. The zebrafish is one of the more recently adopted vertebrate models, that is highly amenable to developmental and genetic studies. It also has great potential as a cancer model system. Comparison of human and zebrafish genomes reveals conservation of cell cycle genes, oncogenes and tumour suppressor genes. Forward genetic screens in zebrafish, that have been so successful in identifying key molecules in development, can therefore be targeted to these highly conserved cancer pathways. Furthermore, having identified a cancer mutation, enhancer/suppressor screens can be used to identify genes that modify the effects of this mutation. For example, zebrafish with a tumour suppressor gene mutation may be used to find a second gene that, in reduced dosage, prevents the development of cancer and thereby becomes a target for an anti-tumour drug.

We have demonstrated that zebrafish Runx1 (AML1) functions in both blood and vessel development at the haemangioblast level, and contributes to both primitive and definitive haematopoiesis. Furthermore, we have shown that a human RUNX1-CBF2T1 (AML1-ETO) transgene disrupts zebrafish haematopoiesis and is associated with the generation of dysplastic blood cells. The phenotype obtained with transient expression of this oncogenic fusion validates the zebrafish as a model system to study t(8;21)-mediated leukaemogenesis. We are generating a zebrafish transgenic line with inducible RUNX1-CBF2T1 expression for use in a genetic screen to identify molecules that modulate the effects of the fusion protein.

A006

Utilisation of the NOD/SCID Mouse to Study Drug Resistance Mechanisms in Childhood Acute Lymphoblastic Leukaemia

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Multiagent chemotherapy is the most effective modality for the treatment of childhood acute lymphoblastic leukaemia (ALL), the most common paediatric malignancy. While over 70% of children diagnosed with ALL will be cured, relapsed ALL remains one of the most common causes of death from disease in children. The aim of this work was to establish improved experimental model systems that would facilitate: (1) a greater understanding of mechanisms associated with drug resistance and relapse; and (2) the preclinical testing of new anti-leukaemic agents. Primary childhood ALL cells derived from bone marrow biopsies or peripheral blood were xenografted as systemic disease in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Continuous xenografts have been established by inoculating cells harvested from the spleens of engrafted mice into secondary and tertiary recipient mice. Tests performed to date (blast morphology, organ infiltration, immunophenotype, p53 status) indicate that this is a highly relevant model of childhood ALL. Experiments were performed to assess the *in vivo* responses of these xenografts to chemotherapeutic drugs. NOD/SCID mice received 5 million leukaemia cells via tail-vein injection, and engraftment was monitored by dual-colour flow cytometric estimation of the proportion of human $CD45^+$ (pan leukocyte antigen) cells in the peripheral blood. Cohorts of 4 mice were treated with vincristine (0.5 mg/kg q7d x 4 i.p.), methotrexate (5 mg/kg daily x 5 q21d i.p.), dexamethasone (15 mg/kg daily x 5 x 4 i.p.) or saline control when the proportion of human $CD45^+$ cells in the peripheral blood reached 1-5%. Xenografts derived from patients who experienced early relapse and death from disease were relatively resistant to vincristine and dexamethasone ($p<0.05$), but not methotrexate, compared with those derived from long-term survivors. An *in vitro* model system has also been developed in which xenograft cells are placed into short-term culture on a monolayer of the MS-5 mouse bone marrow stromal cell line. This system will be useful to prioritise new drugs for subsequent *in vivo* testing, to test agents that interfere with leukaemia/stromal cell interactions, and to delineate mechanisms associated with resistance to established drugs used in the clinical management of children with ALL.

BB

Preeclampsia and Thrombophilia: Is There a Link?

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Preeclampsia, defined by gestational hypertension and proteinuria, is a multisystem disorder that affects 1 in 20 pregnant women. The exact aetiology of preeclampsia is unknown but it is a multifactorial condition with the cardinal features being utero-placental ischaemia, endothelial dysfunction and activated coagulation. Known maternal risk factors include diabetes, obesity, renal disease and nulliparity. Dekker first reported an association between preeclampsia and the inherited thrombophilias in 1995. The inherited thrombophilias may contribute to the development of preeclampsia by predisposing to utero-placental thrombosis as well as augmenting the enhanced inflammatory response and thus endothelial cell dysfunction. Case control studies investigating the prevalence of inherited thrombophilias in preeclampsia have investigated heterogenous groups of women. These show a 2-fold increased risk of preeclampsia in women with factor V Leiden [OR 2.0 (95%CI 1.6-2.5)] and the prothrombin gene mutation [OR 2.3 (95%CI 1.5-3.6)]. There appears to be no increased risk of preeclampsia in women with the MTHFR polymorphism [OR 1.2 (95%CI 1.0-1.4)] but hyperhomocysteinaemia is associated with a 7-fold increased risk (95%CI 3.0-19.1). There is an increased prevalence of APCR [OR 4.1 (95%CI 1.5-10.9)] in women with preeclampsia. Case control studies have not demonstrated an increased prevalence in deficiencies of the natural anticoagulants- antithrombin, protein C and protein S but the studies have been underpowered.

The type of patients included in case-control studies may be important, as women with severe disease have a more marked maternal response and often more severe utero-placental pathology. Inherited thrombophilias may be of greater significance in this group of patients - FVL [OR 2.5 (95%CI 1.8-3.3)] and PT20210 [OR 2.7 (95%CI 1.4-5.0)] appear to be slightly more prevalent in women with severe preeclampsia. Overall, studies show a small but consistent association between FVL and PT20210 and development preeclampsia. However, not every woman with a thrombophilia will develop preeclampsia and population screening is not useful in predicting risk. The relative importance of a thrombophilia will very much depend on the reason it was initially diagnosed. However, for some women, the presence of a thrombophilia in conjunction with other predisposing risk factors may be enough to tip the balance in favour of development the disorder. It is not yet clear if women with inherited thrombophilias are at increased risk of recurrent preeclampsia or if women with a thrombophilia would benefit from treatment in addition to aspirin to prevent recurrence in a future pregnancy.

A011

Repeated Miscarriage and Intrauterine Growth

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Aims
Review of the association of the acquired thrombophilias and pregnancy complications

Methodology
Literature review and metaanalysis of case-control and cohort studies which relate stillbirth, intrauterine growth restriction and recurrent abortion to the Thrombophilias: Factor V Leiden, Prothrombin and MTHFR mutations or Protein C, S and AT3 deficiencies.

Results
Stillbirth
There is presently no evidence of a relationship between Factor V Leiden, MTHFR or Prothrombin mutations or Protein C deficiency and stillbirth. There is weak evidence of an association of Protein S and AT3 deficiencies and stillbirth.

Intrauterine Growth Restriction
There is no evidence of a relationship between any of the acquired thrombophilias and growth restriction of the fetus.

Recurrent Abortion
There is considerable heterogeneity of reporting which makes conclusions on the relationship of acquired thrombophilias and recurrent abortion very difficult. There is however enough data to raise doubts about any relation of Factor V Leiden, MTHFR and Prothrombin mutations and recurrent abortions.

Conclusion
Clinicians should await further evidence before resorting to mass thrombophilia testing in the conditions discussed.

A013

Molecular Mechanisms of Cell Death

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Cell death by apoptosis is essential to remove unwanted and superfluous cells to maintain tissue homeostasis. As apoptosis plays a fundamental role in maintaining tissue homeostasis, aberrant apoptosis can result in accumulation of harmful cells (such as in cancer), or loss of cells that are necessary for the normal functioning of tissues (such as in neurodegenerative disorders). Our main interest is to decipher the molecular mechanisms that mediate apoptosis and understand what factors regulate the life/death decisions of a cell. Genetic studies in the nematode *Caenorhabditis elegans* has identified EGL-1, CED-3, CED-4 and CED-9 as the main components of the cell death machinery. In mammals, all these proteins are conserved and represented by the BH3-only proteins, caspases, the adaptor Apaf-1, and the Bcl-2 family of proteins, respectively. BH3 only proteins counteract the function of prosurvival Bcl-2 proteins to promote Apaf-1-dependent caspase activation. Caspases are cysteine proteases that upon activation cleave a number of cellular proteins causing cells to undergo apoptosis. In recent years we have been using *Drosophila melanogaster* as a model system to understand cell death regulation. There are many advantages of using *Drosophila* as an experimental system. For example, the genetics of *Drosophila* is well understood, the genome sequence and a large number of mapped mutants are available, and *Drosophila* is amenable to sophisticated genetics to study *in vivo* interactions between various molecules, that is not possible in mammals. In *Drosophila* there are seven caspases, an Apaf-1 homologue (Dark) and two Bcl-2-like proteins (Debcl and Buffy). One of the two Bcl-2 homologues in the fly, Debcl, is a pro-apoptotic protein that functions in a caspase-dependent manner. Ectopic expression of Debcl causes cell death in transgenic flies that is suppressed in animals carrying a mutation in *dark* gene. Overexpression of Buffy suppresses cell death in transgenic flies and causes melanotic tumours, suggesting that ectopic expression of this molecule is oncogenic. We are now using the Debcl and Buffy overexpression phenotypes to screen for genes that genetically modify the function of the fly Bcl-2 proteins. The mammalian counterparts of the regulators of fly Bcl-2 proteins are likely to be useful targets for therapeutic intervention in cancers that arise due to the aberrant expression/function of molecules of the apoptotic machinery.

A014

Apoptosis in Human Disease - Always Present, Sometimes Relevant

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Physiological cell death, often recognised morphologically as "apoptosis", is required for removal of unwanted cells during development, and to maintain constant cell number by balancing mitosis in adults. In addition, alterations in the number of cells undergoing apoptosis have been seen in every disease. This has led to an avalanche of papers on apoptosis, with over ten thousand being published in the last twelve months. To determine the significance of these findings it will be necessary to distinguish in which circumstances dysregulation of apoptosis is a primary cause of disease from those in which it is merely a consequence of the disease process. For example, several pharmaceutical companies are developing caspase inhibitory drugs to reduce apoptosis following ischaemic vascular accidents. The usefulness of these drugs will depend on whether caspases are required for cell death, or whether loss of mitochondrial function will cause the cell to die anyway. Pro-apoptotic drugs have been proposed for the treatment of malignant disease. Whether these drugs will be useful will depend on how quickly cancer cells can be selected that have lost components of the apoptosis effector mechanism.

A015

Prospects for Targeting the Bcl-2 Family of Proteins to Treat Haematological Malignancies

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A key regulator of programmed cell death (apoptosis) is the small cytoplasmic protein, Bcl-2. Bcl-2 was originally discovered because it is overexpressed as the result of the t(14;18) translocation found in many cases of follicular lymphoma. Overexpression of Bcl-2, or its functional homologues, has also been found in other forms of lymphoma and leukaemia. It appears that many physiological and damage signals activate the cell death machinery by inhibiting the pro-survival Bcl-2 proteins. Bcl-2 overexpression may contribute to malignant transformation by promoting the survival of damaged cells. Furthermore, as many chemotherapeutic drugs kill by activating the cell death machinery, Bcl-2 overexpression may also contribute to chemo-resistance. Thus, there is much interest in developing peptide and non-peptide mimics of the BH3-only proteins, a family of proteins that act as direct physiological antagonists of Bcl-2, as novel anti-cancer agents. Recently, progress has been made in our search for such drugs based on our current understanding of the cell death signalling. The potential for discovering novel agents that may form a useful part of the treatment of malignant disease is great but the challenge of discovering drugs that modulate protein interaction(s) is equally enormous. I shall discuss ongoing studies to meet this challenge by exploiting our knowledge of the molecular mechanisms controlling apoptosis as these become clearer.

A016

Understanding the Alloimmune Response in Stem Cell Transplantation

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In allogeneic stem cell transplantation genetic differences between the host and donor lead to vigorous allogeneic T cell responses towards host antigens resulting in graft versus host disease. Until recently the structural basis of these reactions has been a mystery, however the molecular mechanisms of T cell recognition, including major and minor genetic antigens, are now much better understood.

In HLA-mismatched transplant combinations the dominant host antigens comprise major histocompatibility differences at the classical HLA loci. However, in HLA-matched donor-recipient pairs T cell alloreactivity recognises minor histocompatibility differences that are sampled from the repertoire of genome-wide polymorphisms generating peptide antigens that distinguish host or donor cells. Some of these minor antigen differences have been identified in transplanted patients and have been shown to exert weak clinical effects in mis-matched transplant pairs.

The extent of polymorphism in HLA molecules is now known to be enormous. Moreover, the repertoire of minor antigens appears that can induce GVHD also appears very significant despite the existence of immunodominant minor antigens within the hierarchy of potential targets. Thus, even in HLA-matched transplant pairs minor antigen differences are potentially so numerous that pre-clinical genetic matching may not solve the problem of GVHD. In addition HLA matching for all donor-recipient HLA types is likely to be impossible even in very large registries of potential unrelated donors. The long-term solution to elimination of GVHD may require host-specific tolerogenic strategies or improved antigen-specific immunotherapy.

A017

Cytokines and Graft-versus-Host Disease

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Allogeneic bone marrow transplantation (BMT) is the most effective therapy for a number of malignant diseases, including acute and chronic leukemia, myeloma, lymphoma and some solid tumors. Recently, the transplantation of stem cells that are collected from the peripheral blood of G-CSF treated donors (peripheral blood stem cell transplantation (PBSCT)) has become widespread practice. Graft-versus-host disease (GVHD) remains the major limitation of either procedure. Much of the therapeutic potential of BMT relates to the graft-versus-leukemia (GVL) effect, which eradicates host malignancy after BMT and is mediated by donor T and NK cells. The graft-versus-leukemia effect appears to be more potent after PBSCT than BMT, resulting in a reduction in the rate of leukemic relapse and an improvement in disease free survival in those patients at high risk of relapse. Allogeneic PBSCT thus offers a major advance in the management of malignant diseases due rapidity of myeloid and immune reconstitution and augmentation of the GVL effect. Unfortunately, an associated increase in incidence and/or severity of chronic GVHD has detrimental effects on quality of life of PBSCT recipients and necessitates continued follow-up, expensive and poorly effective immunosuppressive therapy. The question now remains whether the mechanisms of cGVHD after PBSCT are sufficiently understood to plan rational intervention and whether further separation of GVHD and GVL may be possible by the use of new growth factors.

We have demonstrated that the treatment of donors with G-CSF does not increase cGVHD per se and indeed appears to be protective. Instead, the increase in cGVHD is associated with escalation of the donor T cell dose. Subsequently, the characteristic hepatic and cutaneous features of cGVHD appear to correlate with TGFβ and this would appear an ideal target for therapeutic inhibition. The combination of G-CSF and Flt-3L receptor agonists (either with the native cytokines, or the synthetic progenipoietins) may be a useful mechanism to escalate stem cell dose (up to 10-fold) in the setting of non-ablative transplantation, haploidentical T-cell depleted transplantation or for the induction of tolerance in the solid organ transplant setting. We have studied this in murine models and have noted that donor stem cell mobilization with progenipoietin-1 (ProGP-1) prevents GVHD to a significantly greater extent than G-CSF. This effect is associated with significant impairment of donor CD4 responses and the prevention of inflammatory cytokine generation and associated gut injury. Of most interest, CD8 mediated cytotoxicity remains intact and the effect of ProGP-1 and G-CSF/FLT-3L on GVL is currently under study. Whilst these manoeuvres promise the further separation of GVHD and GVL, the treatment of established, severe GVHD remains inadequate and requires innovative studies that aim to block additional non-T cell dependent pathways of target tissue destruction and prevent subsequent reactivation of donor T cell anti-host reactivity.

A018

New Directions in Tissue Typing for Stem Cell Transplantation

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Matching of donor and recipients for antigens of the human major histocompatibility complex (MHC) is crucial to reduce the risk of graft-versus-host disease after allogeneic stem cell transplantation. Historically this has involved matching for HLA-A, B and DR determinants, initially serologically, and more recently by molecular methods. Despite the best "6 on 6" match, severe GVHD remains a major problem. Developments in characterisation of loci and polymorphisms of the MHC region and advances in molecular typing techniques have allowed higher resolution DNA typing and matching of a greater number of MHC genes e.g. HLA-Cw, DQA1, DQB1 and DPB1. Large studies from international transplant registries have confirmed that such high resolution 'molecular' matching does reduce the risk of disease relapse, graft failure and GVHD. Recent studies have now extended the sphere of transplant genetics to beyond the MHC region. Several 'minor' histocompatibility antigens e.g. HA-1 are polymorphic and may influence GVHD risk. Also, the pathogenesis of GVHD has now been studied in detail, and many of the key mediators are encoded by polymorphic genes. Work of our group and others has demonstrated that multiple non-HLA immunogenetic polymorphisms influence the risk and severity of acute and chronic GVHD. Genes associated with GVHD include the inflammatory cytokines TNF and IL-6, the anti-inflammatory cytokine IL-10, and the apoptosis gene *Fas.* The molecules encoded by these genes are of central importance in GVHD, and the associated polymorphisms are thought to influence the level or function of the encoded molecules. This novel data has the potential to improve outcome following transplantation: for example by refining choice between multiple HLA-matched donors, tailoring immunosuppression in those at greatest risk, or by allowing targeted prophylactic administration of cytokine blocking therapy. Our knowledge of the genetic determinants of other transplant complications is by comparison in its infancy. Our recent work has shown that polymorphisms in the innate host defence molecule mannose-binding lectin (MBL) strongly influence the risk of major infection post-transplant. This is also of clinical interest as MBL is being developed as a therapeutic product. Most of this immunogenetic data has been studied only in the sibling context. Unrelated donor transplantation carries even higher risks of complications, and international collaborative studies of the genetics of unrelated donor transplantation are in progress.

A021

DIC, Septic Shock, and Natural Anti-Coagulants

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Severe sepsis is a common problem, with a mortality rate of ~30% in Australia and New Zealand, and ~45% of deaths are attributed to sepsis and multiple organ failure. Knowledge about its pathophysiology and potential specific interventions has grown rapidly over the last twenty years, but numerous novel therapies directed against targets such as individual cytokines, eicosanoids, platelet activating factor and activated granulocytes have all had negative clinical trials. Given the 19.4% relative risk reduction reported from a recent large trial of activated Protein-C it is worth reviewing the role of DIC and natural anticoagulants in severe sepsis.

Although DIC can be detected clinically in over one third of patients with severe sepsis, it is uncommon for bleeding to be a major problem. However, DIC also leads to a thrombotic reduction in microcirculatory perfusion, and this may contribute to multiple organ failure. Sepsis is the most common cause of DIC, with the intense expression of cytokines such as IL-1, IL-6, IL-8 and TNF-α leading to increased expression of tissue factor on monocytes, granulocytes and most importantly endothelial cells. The resultant tissue factor-VIIa complex leads to activation of Xa and prothrombin. In addition, there is dysregulation of the natural anti-coagulant system with impaired synthesis and consumption of anti-thrombin III and Protein-C, and cytokine mediated reduction in cofactors such as the glycosaminoglycans, thrombomodulin and Protein-S. Tissue factor pathway inhibitor, another natural anti-coagulant is dysfunctional, and fibrinolysis is suppressed by cytokine mediated release of plasminogen activator inhibitor-1.

Cross talk between the coagulation pathway and inflammation may also contribute to multiple organ failure. Tissue factor-VIIa, Xa and IIa complexes have each been shown to increase the expression of pro-inflammatory cytokines, and activated Protein-C appears to have anti-inflammatory effects on monocytes and granulocytes independent of its anticoagulant activity. Consequently, the natural anti-coagulants may provide a unique therapy for sepsis, both improving organ perfusion and reducing inflammatory damage. Confirmation of the clinical efficacy of activated Protein-C and prospective, randomized studies of other natural anti-coagulants are awaited.

A022

Crisis Management
Massive Bleeding: Antidotes and Haemostatic Agents (DDAVP, FEIBA, Factor VIIa etc)

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Massive bleeding often represents the failure of haemostasis in the context of overdosage or inappropriate doses of anticoagulants or massive transfusion.

Antidotes (neutralising therapy) may be required.

If the bleeding is compounded by heparin, protamine sulphate, which is also anticoagulant in excess, may be used. This compound has limited effect in neutralising low molecular weight heparin to which there is no specific antidote.

Bleeding secondary to fibrinolytic therapy could be treated either with replacement of fibrinogen (Cryoprecipitate) or with anti-fibrinolytic drugs such as the lysine analogues - EACA or tranexamic acid. Most data on the efficacy and safety of these drugs relates to peri-operative bleeding reduction rather than treatment of overdosage however.

Although administration of DDAVP causes a transient rise in factor VIII, von Willebrand factor and enhanced platelet aggregation, its use as an antidote to anti-platelet agents in the management of bleeding is not widely proven.

Bleeding associated with oral anticoagulants often requires both neutralisation with vitamin K and plasma product (II, VII, IX, and X) replacement. The dose and route of vitamin K depends on clinical circumstances as to whether temporary or more longstanding reversal is appropriate. The choice of plasma product, FFP and/or Prothrombin Complex concentrate (PCC) depends not only on the clinical imperative for reversal but also upon the patient's ability to tolerate large intravascular volume infusion and the availability of PCC.

If the bleeding is due to dilution or consumption of cellular and plasma coagulation factors, as in massive transfusion, replacement is required. No 'formula' exists to predict or prevent the occurrence of such bleeding.

More recently multiple anecdotal reports on the use of recombinant factor VIIa as a heroic measure in massive bleeding have been published. This is an off-label indication for the product. Trials yet to be developed and implemented to assess efficacy and define appropriate clinical indications for the product are eagerly awaited.

A023

TTP/HUS and Plasma Exchange Therapy

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Thrombotic thrombocytopenia purpura (TTP) and haemolytic uraemic syndrome (HUS) are of considerable clinical importance because of abrupt onset, fulminant course, and high morbidity and mortality in the absence of early recognition and treatment.

TTP and HUS are multi-system disorders that are characterized by a classic group of symptoms including fever, thrombocytopenia, and microangiopathic haemolytic anaemia. Neurological symptoms and renal abnormalities also occur to varying degrees due to ischaemia as a result of platelet aggregation in the arterial microvasculature.

Both conditions share the same pathophysiology aetiology and may be varied expressions of the same underlying disease process. HUS is more common in children and is characterised by prominent renal involvement whereas neurological abnormalities are more dominant in TTP. TTP occurs more frequently in adults and is associated with pregnancy; diseases such as HIV, cancer, bacterial infection, and vasculitis; bone marrow transplantation; and drugs.

If untreated, HUS-TTP typically follows a progressive course in which irreversible renal failure and death are common outcomes with the mortality rate as high as 90%. During the 1980s plasma exchange using plasma was found to be effective in treatment of the disorders with an 80% response rate and 90% survival. Plasma exchange has been the main form of treatment ever since. Consequently large volume plasma exchange should be initiated promptly even if there is uncertainty about the diagnosis.

Until recently, it was not clear how the treatment works. New understanding of the pathophysiology of TTP-HUS has clarified that issue. Recent evidence suggests that deficiency of a specific plasma protease responsible for the physiological degradation of von Willebrand factor plays an important role in the disease process. Autoantibody production may also play a role. Relapsing disease remains a challenge with questions still to be answered concerning the activating mechanisms and effective long-term therapy.

A025

Minimal Residual Disease

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Minimal residual disease in ALL is important in determining prognosis and identifying molecular relapse and it is coming to be a basic feature of many risk adapted treatment protocols. It may also provide new information on the biology of leukaemia during treatment and also be a surrogate marker for assessing the effects of new therapies. An important issue in measurement and interpretation of MRD levels is methodology, which is at present complex, expensive and subject to error. Approaches to correcting some of these problems will be discussed.

A026

A Polymorphic Mutation in the Cytolytic $P2X_7$ Receptor: Implications for Indolent CLL

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B-CLL follows a variable clinical course with up to half of patients showing an indolent rise in lymphocyte counts which does not require treatment for many years. Moreover, CLL is unique in having a familial incidence nearly three times higher than expected for the general population. Clinical staging (Binet or Rai) gives little information on the rate of disease progression. Lymphocyte doubling time (LDT) has been proposed as a prognostic marker with values > 365 days identifying indolent disease and long survival and values < 365 days associated with progressive disease. We have previously reported on increased frequency in B-CLL of a polymorphic allele (1513A→C) in the $P2X_7$ gene which leads to loss of function of this cytolytic receptor by changing glutamic acid to alanine at amino acid 496 in the carboxyl cytoplasmic domain (Lancet 359, 1114; 2002). In the present study the frequency of the polymorphic allele was compared between normal subjects (n=121), B-cell lymphoma (n=40) and B-CLL (n=68). Allele frequencies were identical in normal (0.14) and lymphoma subjects (0.14) but were significantly increased in B-CLL (0.21; $P<0.05$). In 41 patients with B-CLL the LDT was > 365 days and in 8 patients it was < 365 days. Of 41 patients with indolent disease 18 were heterozygous or homozygous for the polymorphic allele while this polymorphic allele was absent in those patients with LDT < 365 days ($P<0.02$). The expression of CD38 was measured on leukemic $CD5^+$ B-lymphocytes as a marker of progressive disease. The prevalence of the polymorphic allele was not different between those who were CD38 positive (5/13) and those who were CD38 negative (12/35). Our data suggest that inheritance of the polymorphic 1513A→C allele and reduced $P2X_7$ function increases the survival of B-CLL lymphocytes

within the circulation. The higher lymphocyte count with minimal disease leads to earlier diagnosis in those patients with indolent disease. This mechanism involving loss of function mutations in the $P2X_7$ gene may contribute the pathogenesis of indolent CLL in some patients.

A027

Progenitor Cell Viability Assays

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The FDA requires the Cell Manipulation Center to perform full characterization of cell products before issuing the product for clinical use. This characterization includes: safety, purity, potency, identity, and stability. We have been evaluating different methodology for viability determination. We have compared trypan blue, flow analysis using both 7-AAD and Propidium Iodide, and a new instrument, Guava PCA Person.

Cell Analysis System
We have compared these methodologies using bone marrow, and either fresh or previously cryopreserved peripheral blood progenitor cells.

A031

Cancer in Thrombosis. How Hard Should I Look?

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There is convincing evidence that during the first 2 to 3 years the incidence of newly detected cancer in increased among patients with idiopathic venous thromboembolism. However the clinical implications of this finding are uncertain and clinical practice varies from little attention, via low threshold for suspicion of cancer and subsequent testing to extensive testing for cancer in these patients. Recently, a randomized trial was conducted in which patients with idiopathic venous thromboembolism, who were apparently free of cancer were randomized to no further screening and extensive screening program. Unfortunately, the trial had to be interrupted early, and no statistically significant difference in mortality was demonstrated, although in the extensive screening group more cancer were identified in an early stage and only few cancers escaped detection. In a subsequent cost-effectiveness analysis, it was demonstrated that screening with CT (pelvis, abdomen and thorax) and mammography has the potential to be cost-effective. Further studies are needed to establish an effect on the prognosis of patients with idiopathic venous thromboembolism.

A032

Cancer and Thrombosis

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Venous thromboembolism (VTE) in cancer patients is common, occurring in around 15% of patients. It is the second most common cause of death and has a frequent negative impact on quality of life. The reason is multi-factorial and include - stasis of blood flow; from immobility, surgery and extrinsic compression - vessel wall damage; with central lines, chemo-radiotherapy and direct invasion - and changes in blood constituents; such as procoagulant factors and impaired fibrinolysis. The aims of prevention of cancer associated VTE is not only to decrease the rate of VTE but also avoid treatment-related haemorrhage and increase the quality of life. Thrombo-prophylaxis with either oral anticoagulants or low molecular weight heparin (LMWH) produces more than a 50% risk reduction in cancer patients, particularly when given for extended periods after abdominal surgery. The challenges for the initial treatment of cancer related VTE include overcoming the problem of recurrent VTE despite warfarin, decreasing anticoagulant related bleeding, managing anticoagulant interruptions caused by necessary procedures or thrombocytopenia, poor venous access, difficult INR control and heparin antibody development. For these reasons several large randomised trials comparing the safety and efficacy of long term LMWH compared to warfarin in cancer patients have recently been completed and their results are awaited with interest. Most intriguing is the suggestion of the role of anticoagulation as adjuvant cancer treatment to improve survival by reducing tumour growth and rate of metastasis.

A033

Who Needs the New Antithrombotics?

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Two new classes of antithrombotic agents will be available for clinical use soon. First, a new synthetic compound *(Fondaparinux sodium)* which is almost identical to the natural pentasaccharide sequence of heparin, the shortest structure able to catalyse antithrombin-mediated factor Xa inhibition, has already been registered for subcutaneous administration in the US for the indication of prophylaxis following major orthopaedic surgery and is presently under consideration for registration in Europe in this indication. Studies in other indications, including treatment of venous thromboembolism are ongoing. Second, an orally active, synthetic direct antithrombin, ximelagatran, is presently undergoing phase III evaluation in a large spectrum of indications, from prevention to treatment of venous thromboembolism, and prevention of systemic embolization in patients with atrial fibrillation.

In a pooled analysis of 4 studies with more than 2500 patients undergoing major orthopedic surgery in total, fondaparinux was associated with a relative reduction of more than 50% of total venous thromboembolism, compared to standard regimens of low-molecular weight heparin (LMWH) (roughly from 14% with enoxaparin to 7% with fondaparinux), which was statistically highly significant. However, the increased rate of major bleeding (roughly 1% more with fondaparinux compared with enoxaparin) deserves some caution. Fragile patients (e.g. those with underweight, decreased renal function, or any hemorrhagic tendency) should probably not receive the new compound, especially because only a single, fixed dose regimen has been studied during the phase III development of the drug.

Ximelagatran has the potential of replacing both heparin and LMWH but also vitamin-K antagonists (VKA). Its future, however, will depend upon the overall benefit-risk which cannot be evaluated fully at the present time. For sure, being eliminated via the renal route, ximelagatran will be used cautiously in patients with an impaired renal function.

There is no information as to whether one of the two compounds or both can be used in pregnant women. Finally, because the new antithrombotics aim at replacing well established compounds such as unfractionated heparin, LMWH and VKA, cost analysis will also be crucial.

A034

Mechanisms of Haemopoietic Stem Cell Mobilisation

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During mammalian development, haemopoietic stem cells (HSC) migrate from the AGM region to the foetal liver and then to the bone marrow (BM) which remains the main site of haemopoiesis in adults. Although in steady-state conditions the vast majority of HSC reside in the extravascular compartment of the bone marrow (BM), a small proportion of these cells re-circulates in the peripheral blood (PB) before re-homing into the BM. Large numbers of HSC however can be mobilised into the PB following a variety of stimuli including administration of cytokines or myeloablative chemotherapy. HSC mobilised by G-CSF and/or chemotherapy have recently overtaken BM aspirates as a preferred source of HSC for transplantation. Despite widespread use of mobilised HSC (around 30,000 transplants/year worldwide), the molecular mechanisms responsible for the mobilisation of HSC are still poorly understood.

We have recently identified a mechanism that takes place during both G-CSF-induced and chemotherapy-induced mobilisation of HSC. This involves a dramatic expansion of granulocyte precursors in the BM and release of large amounts of neutrophil proteases such as neutrophil elastase and cathepsin G in the extravascular compartment of the BM. In turn, these proteases cleave molecules essential for the retention of HSC in the BM, such as the cell adhesion molecule VCAM-1/CD106 and the chemokine SDF-1/CXCL12 and its receptor CXCR4.

A036

Mobilised Stem Cells for Non-Haemopoietic Diseases – 'Transdifferentiation' of the Haematologist

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There are now many descriptive reports of the conversion of transplanted bone marrow cells to cardiomyocytes, hepatocytes and neurones, so called 'transdifferentiation'. These reports have raised hope that bone marrow cells could one day be used for tissue repair, providing a more palatable alternative to embryonic stem cells. However, many theoretical and practical issues

need to be addressed before haematologists begin treating patients for myocardial infarction, liver failure or degenerative neurological diseases. Can the destiny of a haemopoietic stem cell truly change to a non-haemopoietic cell fate or is there some other stem cell within the bone marrow that generates non-haemopoietic cells? Can the bone marrow cell responsible for 'transdifferentiation' be mobilised into the peripheral blood and if so, what are the best methods of mobilisation? What is the best method of delivering these cells to the damaged tissue? What are the signals that control 'transdifferentiation'? Can we increase the efficiency of 'transdifferentiation' to make it clinically useful? Our laboratory is beginning to study the transcriptional control of 'transdifferentiation' using mouse models of heart and liver damage. SCL is a transcription factor first identified in a case of T-cell ALL. Gene targeting studies have shown that SCL normally functions as a master regulator of haemopoiesis. In particular, SCL expression in the developing embryo is critical for specifying blood development from early mesoderm. We are using SCL-targeted mouse models to address the hypothesis that loss of SCL expression in adult haemopoietic stem cells may enhance 'transdifferentiation' to cardiomyocytes and hepatocytes.

A044

Clinical Research for Clinical Practice

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This is one clinical researcher's attempt to understand how good or bad ideas go on to become good or bad clinical management – illustrated by personal experience with the diagnosis, prevention and treatment of one disease: venous thromboembolism.

The drivers for change include the development of new drugs, devices and diagnostic procedures, the increasing mass of information from clinical trials of new diagnostic, preventive or therapeutic regimens, the way this information is presented, and the way it is understood by clinicians, patients, and health care managers.

At each step towards change there is a new mix of players: commercial, scientific, clinical, regulatory, consumer and government. Each player is driven by motives that are usually complex rather than simple, which are rarely altogether pure, and which may change their emphasis over time. The commercial organisations that develop new drugs and devices must be profitable to survive, but cannot profit without the good science needed to generate improved clinical outcomes. The fundamental need of clinical researchers to keep publishing can temper their altruism and methodological purity. Regulators and governments must now consider the impact of change (however beneficial in clinical terms) on health economics and health care costs. Health units are driven by local considerations of cost and convenience. No clinician can be fully informed on all aspects of their practice range. Nor can they ever be fully immunised against inappropriately weighted advice from experts who are never wholly free of bias, or from information designed specifically to increase market penetration and market share. And, in the consulting room, clinicians and patients are often driven by the dynamics of a doctor-client relationship that calls for certainty of advice when there is none, or overindulges medico-legal considerations.

As a result of these many and complex (and entirely human) interactions, it is no surprise that some 'good ideas' are brought too early into clinical practice while others may never gain clinical credibility, and that some 'bad ideas' become widespread. Examples of how this process has worked for good or bad include the ready use of blood tests to exclude venous thromboembolism or detect thrombophilia, attitudes to the selection and duration of thrombosis prophylaxis after joint surgery, as well as the early adoption of abbreviated heparin treatment and home therapy for venous thrombosis, and pulmonary embolism.

A046

The Myelodisplasia Aplasia Syndrome: Coexistence of Normal and Clonal Hematopoiesis

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Cytogenetic abnormalities and paroxysmal nocturnal haemoglobinuria (PNH) phenotype are frequent findings in patients with marrow failure treated with immunosuppressive therapy (IST). In this study we investigated whether the appearance of clonal haemopoiesis influences patient outcome and survival. 97 patients entered this study and were followed from the onset of the disease for a median follow-up (FU) of 53 months. 93% are alive, 56% achieved a complete remission (transfusion independence and normal blood counts), 30% a partial remission (transfusion independence), and 14% did not respond. Three groups were identified: (A) patients without evidence of emerging clones (71/97); (B) patients who acquired chromosomal abnormalities (13/97); (C) patients who showed low expression of glycosyl phosphatidylinositol anchored proteins (GPI-AP) (PNH phenotype) at presentation or later (16/97). Three patients showed both PIG-AP deficiency and chromosomal abnormalities. The actuarial survival of patients without clonal haemopoiesis (n=71) at 6 years was 95%, for patients with chromosomal abnormalities (n=13), 88%, and for patients with PIG-AP deficiency (n=16), 89%. There was no difference in the probability of becoming transfusion independent in the three groups (93%, 92% and 88% respectively). This study

confirms that cytogenetic abnormalities compatible with myelodisplasia, are a relatively frequent event in patients presenting with cytopenia and treated with IST. The clonal abnormalities coexist with normal hematopoiesis and are compatible with long-term survival in many patients. The role of specific abnormalities such as deletion of chromosome 7 and trisomy 8 will also be discussed.

A053

Gene Expression Profiling in Paediatric Leukaemia

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The most common form of cancer in children is leukaemia. The prognosis for children with acute lymphoblastic leukaemia (ALL) has dramatically improved over the past decades and survival has reached 70-75%. Up to 85% of patients classified as standard risk are projected to be long-term survivors. Current treatment protocols make use of risk-directed therapy with the aim of administering intensified therapy to those patients with a high likelihood of relapse and decreasing toxicity for those patients with a lower risk of relapse, while maintaining high cure rates. The risk stratification for appropriate therapy is based on clinical and laboratory features, including age, white blood count, immunophenotype, DNA index, cytogenetics and early response to induction chemotherapy. However, a substantial number of patients currently classified and treated as standard risk patients continue to relapse.

In order to maximize efficacy of therapy and to minimize toxicity, cancer therapy should be targeted to pathogenetically distinct types of tumour. RNA expression profiles are proving to be very useful clinically. The novel microarray technology not only discriminates cases according to subtypes identified by other methods, e.g. morphology, histochemistry and immunophenotyping, it has the capacity to discover new ones. Three recent studies on paediatric leukaemia demonstrated the power of this technology. They provided new information on the classification and subtypes among paediatric ALL.

Since cell lines provide ideal models for the assessment of potential novel therapies, we determined the gene expression in our panel of established paediatric leukaemia cell lines, comprising ALL of B-lineage and T-cell (T-ALL) phenotype. The study revealed that the lines display the identifying features of distinct ALL subtypes. *HOX11* is a key oncogene in T-ALL. In order to identify downstream functions of the gene we generated stable transfectants of the human cell line PER-117 and assessed their expression patterns by oligonucleotide microarray analysis. Enforced expression of *HOX11* revealed transcriptional stimulation and repression of a large number of genes. We focused on genes highly upregulated and giving concordant results in independent stable clones. Current investigations seek to unravel the differential expression of six genes as a result of *HOX11* expression.

A058

How To Break Bad News – An Interactive Workshop

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Recent research has demonstrated that communication skills of clinicians involved in cancer treatment can be enhanced by training that involves opportunities to interact with simulated patients in a workshop format. Patient outcomes are enhanced when their concerns about diagnosis, treatment, prognosis and potentially threatening procedures are addressed, and realistic hope is maintained. This workshop will feature a simulated patient with newly diagnosed Diffuse Large Cell Non-Hodgkins Lymphoma, and will focus on a consultation in which prognosis and treatment are discussed. Facilitators with psychological and oncological backgrounds will draw on the evidence base underlying performance of this difficult communication task, in guiding participants to explore new strategies.

A064

New Perspectives in Disorders of Iron Metabolism

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Disorders of iron overload and deficiency are prevalent worldwide. Genetic haemochromatosis (GH) is the most common genetic disorder occurring in persons of northern European descent. The disease is characterized by defective regulation of intestinal iron absorption and progressive tissue iron accumulation. Despite the discovery that the HFE gene is defective in the

great majority of patients (85-100% are homozygous for a C282Y mutation), the molecular mechanism underlying GH remains undefined. Recent cloning and preliminary characterization of a variety of iron-binding proteins involved in duodenal absorption, transport, and tissue storage of iron (e.g. DMTI, apical uptake; FPN, basolateral export), have greatly enhanced our understanding of these processes and, in turn, provided new insights into disease states. With regard to the distinction of GH from other causes of iron overload, HFE mutation analysis has greatly enhanced our ability to diagnose GH and to perform family screening. Recent data is suggestive of a role of HFE mutations in other types of liver disease, including chronic hepatitis C, non-alcoholic fatty liver disease and alcoholic liver disease. The role of HFE analysis in screening the general population has yet to be defined.

A067

Haemochromatosis – Have We Changed Our Diagnostic Criteria?

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Hereditary haemochromatosis (HH) is a disorder of iron metabolism characterised by iron overload eventually leading to tissue damage. HFE-related HH is the most common major inherited disorder in Caucasians of Northern European descent. The two common mutations in the HFE gene are C282Y and H63D. In Australians of Northern European origin, the frequency of C282Y homozygosity is approximately 0.5%, C282Y/H63D compound heterozygosity 2%, and simple heterozygosity for C282Y or H63D 33%. There is incomplete penetrance with 50%-75% of C282Y homozygotes displaying evidence of increased iron stores and fewer with clinical manifestations. It is likely that genetic and non-genetic factors account for incomplete penetrance. Genetically predisposed subjects without increased body iron stores over a period of time should not be classified as having haemochromatosis. Optimal management of this subgroup is uncertain. Testing for HFE mutations has had a major impact on diagnosis of HH, but has also caused much anxiety because of uncertainty about the status and management of heterozygotes and homozygotes with incomplete penetrance. While transferrin saturation indicates biochemical expression, a decision to treat is made on the basis of increased body iron stores, reflected in increased serum ferritin concentration. Unfortunately serum ferritin can be falsely raised and a decision to treat requires collateral evidence of iron overload. The role of liver biopsy has diminished and now is used primarily in staging the disease (precirrhotic or cirrhotic). It has been demonstrated that in patients with C282Y homozygosity, cirrhosis can be excluded without a liver biopsy if the serum ferritin is < 1000 ug/L, the aspartate (or alanine) aminotransferase level is normal and there is no hepatomegaly. Liver biopsy can also be used to clarify borderline cases of iron overload and in assessing concurrent disease such as alcoholic liver disease or chronic hepatitis. In summary, a diagnosis of HFE-related HH requires that (a) genetic risk be established by HFE mutation testing, and (b) that increased body iron stores be proven, usually by raised serum ferritin concentration and confirmed by quantitative phlebotomy (or liver biopsy in selected cases).

A068

Screening for Hereditary Haemochromatosis: Unanswered Questions

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Screening for Hereditary Haemochromatosis (HH) has been advocated because the genetic defect (homozygosity for C282Y mutation in the HFE gene) is relatively common, a treatment is readily available, and iron overload may be monitored by a simple test. For these reasons it represents an ideal test case for public health genetics, that is the application of genetic information to prevent disease. However there are a number of unanswered questions that should be addressed prior to the recommendation of screening. In particular, the penetrance of the susceptible genotype is unclear. It has been estimated that the penetrance of iron overload may be as high as 50% but that the clinical sequelae may be as low as 1%. Penetrance of the condition may be modified by other genetic or environmental factors, such as dietary iron intake, alcohol use, reproductive factors and multivitamin use, and these factors are not well established. The natural history of iron overload has not been well studied and it would be unethical to withhold treatment from someone with iron overload based on clinical consensus. In addition, the cost-effectiveness of screening is unclear as this is dependent on the penetrance of disease. Few studies have addressed these questions and the uncertainty regarding penetrance is one of the major reasons for recent U.S. and European consensus statements not supporting population-based genetic screening. These issues, and proposed studies to address them will be discussed.

A073

Qualifications- Not Just for Staff But for Equipment Too!

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The quality of blood components and products offered by ARCBS depends on the use of reliable materiel and equipment. It is important that the materiel and equipment used is of a consistent quality. Apheresis equipment is designated as critical by ARCBS and therefore requires full validation and documentation.

Prior to the acquisition of an apheresis machine, a critical needs analysis is conducted to ensure that it will meet the needs of the organisation and the decision to purchase is made knowing all the issues ahead, and the impacts on each of the departments, apheresis operators and donors.

The validation process allows the performance of the machine to be monitored to ensure it performs as expected throughout all operating ranges, through documented installation and operational qualifications, and functionality studies or process qualification.

The installation qualification/ operational qualification validation component assures the apheresis machine will operate as intended, but without the process qualification its effect on the resulting products would not be known. Process qualification provides documented proof that the machine performs as expected within the manufacturing process. Process qualifications are performed on each intended protocol type, to ensure all collected components meet specification. The process qualification report includes the protocol type, acceptance criteria, raw data, summary of results and conclusion. The number of procedures to be performed is negotiated with the quality control laboratory manager to ensure that a statistically significant number of components are collected and tested. All plasma collected is held in quarantine until approval is given. The validation process allows time for staff training to be conducted, approval of procedures for normal operation, calibration and maintenance, and review of the results of the performance testing.

Once all the required documentation is complete, approval for commissioning is sought from the quality manager and plasma products are released from quarantine.

The assessment and validation process ensures minimal risk to the donor, minimal risk to component, ensures component quality and consistent practice prior to use of the machine in production and release of product to the end user.

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A105

Co-operative Interactions Between Haemopoietic Cells and Osteoblastic Cells Promote Osteoclast Differentiation

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Cells of the osteoblast lineage can form new bone matrix and also act as stromal cells to promote the differentiation of several haemopoietic lineages, including osteoclasts, the latter arising from cells of the monocyte/macrophage lineage. The aim of these studies was to define the cytokine environment in which human OC form, and to determine the contributions of the stromal and haemopoietic elements.

Two experimental systems were used. Osteoclasts were generated when mouse ST-2 cells and human peripheral blood monocytes were co-cultured in the presence of $1,25(OH)_2vitD_3$, dexamethasone and human M-CSF. Species-specific probes were used to identify the murine or human mRNA and cytokine species expressed in the cultures. In addition, co-cultures of purified human monocytes, together with human osteoblast-like cells derived from trabecular bone biopsies of normal individuals, were used to examine events in an all-human system.

In the mouse-human system, ST-2 stromal cells expressed mRNA encoding a repertoire of many known osteoclastogenic factors, including RANK ligand. The ratio of mouse RANKL:osteoprotegerin (OPG) mRNA was found to increase during the co-cultures, consistent with a key role for RANKL in the promotion by stromal cells of OC formation. Analysis of the culture medium showed that the PBMC secreted IL-1, IL-6 and TNF-α protein, only in co-culture with ST-2 cells, during the first few days of osteoclast development. Similarly, prostaglandin E_2 was secreted only in co-cultures. To construct an all-human

system, we first studied the phenotype of cultured human osteoblasts. Cells were subfractionated into STRO-1^{bright} and STRO-1^{dull} populations and the expression of RANKL and OPG mRNA was examined in each population. The results obtained suggest that immature OB are more responsive to pro-osteoclastic stimuli than mature OB, and suggest that the dual functionality of OB in supporting OC formation or forming bone is a function of the maturation state of the this lineage of cells. When human osteoblasts were cultured in the presence of human monocytes, mRNA analysis showed a co-culture dependent increase in the RANKL:OPG mRNA ratio and co-culture dependent secretion of PGE_2 and IL-6.

Together, these data show that monocytes, including OC precursors, provide signals to stromal osteoblast-like cells during human OC development. We propose that this bi-directional signalling is involved in maintenance of the equilibrium between bone resorption and formation.

A106

Effects of the Bone Marrow Microenvironment on Tumor Cell Behavior

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When tumor cells metastasize to the bone marrow, they reside in an environment which is different from that of the primary site, or other soft tissue metastatic sites. It is now clear that in the case of human breast cancer cells, this is associated with a change in their phenotype. The best characterized change is the expression of the tumor peptide PTH-rP, whose expression is increased in the bone marrow microenvironment, but there are also other changes in phenotype that have been described including increased expression of PTH receptors and altered expression of estrogen receptor isoforms. This change in phenotype has important functional consequences in the bone microenvironment. Increased expression of PTH-rP by the breast cancer cells can be stimulated by TGFβ, which is produced in active form as bone resorbs. This leads in turn to a further increase in PTH-rP and even greater bone destruction. This is the basis of the "vicious cycle" hypothesis, and makes bone unique as a site for breast cancer metastasis. Other bone-derived growth factors such IGF-1 also play an important role as stimulators of tumor cell proliferation in this microenvironment. These cellular interactions in the bone marrow microenvironment are also present in other cancers with a proclivity to grow in bone. For example, in osteoblastic metastases which are frequently associated with prostate cancer, inhibition of bone resorption by bisphosphonates causes decreased metastatic growth of the tumor cells in bone, and in myeloma, complete inhibition of bone resorption by the osteoclast inhibitor RANK.Fc leads not only to reduction in osteolysis, but also in tumor burden. There are several other important implications of these observations. Other common metastatic sites such as liver, lung and brain also likely have unique tumor-specific characteristics which select for or alter the behavior of tumor cells that metastasize to these sites. Secondly, if the vicious cycle is interrupted, for example by drugs which inhibit osteoclastic bone resorption, not only will bone loss be reduced but also tumor burden in bone. This important concept has now been defined and confirmed experimentally. Interruption of the vicious cycle by osteoclast inhibitors, by antagonists to PTH-rP, or by inhibitors of TGFβ activity all reduce not only bone lesions associated with cancer metastasis, but also the accumulation of tumor cells in the bone marrow microenvironment.

A107

Progress in the Treatment of Aplastic Anemia

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Treatment of acquired severe aplastic anemia (SAA) is based on allogeneic bone marrow transplantation (BMT) or immunosuppressive therapy (IS). As to HLA identical sibling BMT, there has been a significant reduction of rejection from 18% before 1980 to 11% in 1991-1998, and of grade III-IV acute graft versus host disease (aGvHD) from 20% before 1990 to 6% after 1990 ($p<0.00001$). Both are mainly, but not exclusively, due to the introduction of cyclosporin A (CyA). Chronic GvHD has also been reduced from 30% before 1990 to 7% beyond 1990. Survival is significantly influenced by age also in current transplant era 1994-1999: 80% for patients aged 1-19, 65% for age 20-29 and 48% for age 30-63. This may require changes in the conditioning regimen from the conventional cyclophosphamide (CY) 200 mg/kg to combination of CY, fludarabine, anti-lymphocyte globulin (ALG). The latter has been tested in alternative donor transplants with very encouraging results. ALG remains the most effective single agent for IS therapy of SAA: it has been proven superior to T cell monoclonal antibodies in a prospective trial. The combination of ALG+CyA is the treatment of choice, having been proven superior in randomized trials to CyA alone, ALG alone, CyA+G-CSF. Current 5 year survival of patients receiving ALG+CyA with or without G-CSF is in the 75-90%. Late complications of IS therapy include relapse of aplasia (10-15%), cytogenetic abnormalities (15%), myelodisplasia/leukemia (15%) and paroxysmal nocturnal hemoglobinuria (PNH).

In conclusion, results of transplantation and IS therapy have considerably improved over the past 3 decades, such that over 75% of patients can become long term survivors. Comparison of the two forms of therapy will require studies on comparative quality of life of long term survivors: the impact of complications such as chronic GvHD, transfusion dependence, relapse and dysplasia will need to be carefully evaluated.

A108

Chronic Myeloid Leukaemia – Some Unanswered Questions

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Though much progress has been made in recent years in our understanding of the biology of CML and the recent introduction of imatinib is an extremely exciting development, many questions remain unanswered. Some of the more obvious one are:

1. How is the BCR-ABL gene formed? What is the connection between the BCR-ABL gene and the Ph chromosome?
2. Why do $p190^{BCR-ABL}$ and $p210^{BCR-ABL}$ act differently?
3. What signal transduction pathways are really important in CML?
4. What is the molecular basis of disease progression?
5. What is the best way of predicting survival? Are 9q+ deletions important?
6. What is the mechanism underlying the GvL effect after allo-SCT for CML?
7. Does autografting really prolong life? If so, when?
8. How does imatinib work? Are the molecular remissions durable?
9. Will imatinib alone cure any patient? What is meant by cure?
10. Does imatinib (STI571) affect the most primitive Ph-positive progenitors?
11. What is the commonest mechanism of resistance to imatinib?
12. Can imatinib be combined usefully with other agents? IF so, which?

Some of these questions will be addressed, but probably not answered, in this session.

A109

Is D-dimer Testing Ready for Clinical Application?

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In the past fifteen years, plasma assays of several markers of activation of plasma coagulation and/or fibrinolysis have been made available for clinical use, including D-Dimer (DD), a specific degradation product of crosslinked fibrin, that can be measured using various immunoassays (enzyme-linked immunoassay, ELISA, or latex agglutination tests). Its widespread use has been made possible only in recent years with the development of rapid assays that allowed result delivery within one hour or less after blood sampling. However, the heterogeneity of the assays has raised uncertainty among clinicians and called for rigorous evaluation and standardization of the various tests. Uncertainty was further increased because the usefulness of the test was also found to be dependent upon the populations to which it was applied, due to variations in test specificity.

Nonetheless, a few commercial assays could achieve the following, successive validation steps: 1) technical description; 2) comparison with an established diagnostic standard, and, above all, 3) use in so-called management trials. In such trials, patients are followed-up for 3-6 months after the diagnosis of deep vein thrombosis (DVT) or pulmonary embolism (PE) has been made, and the safety of the diagnostic test or strategy is given by the patients' outcome, i.e. the 3-month thromboembolic risk. The 3-month thromboembolic risk in patients suspected of DVT/PE and a negative DD test has been shown to be 2% or less, which is very similar to that observed in patients with a negative phlebogram, or a negative pulmonary angiogram, or a normal perfusion lung scan.

Additional features of DD testing in patients clinically suspected of DVT/PE include 1) its higher utility for ruling out DVT/PE in younger patients (compared to older individuals), 2) and in outpatients (compared to patients hospitalized); 3) its potential to predict or rule out recurrent DVT/PE.

In conclusion, DD testing has emerged in recent years as a definite aid in the diagnostic approach of venous thromboembolism, since it can safely rule out DVT/PE in a substantial proportion (30% or more) of outpatients clinically suspected, especially if combined with a low (when using less sensitive DD assays) or low-intermediate (highly sensitive tests) clinical probability of having the disease.

A120

Graft-versus-Leukaemia - Present and Future

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The evidence for a graft-versus-leukaemia (GvL) effect in CML is based on the observations that relapse is commoner in recipients of syngeneic transplants, relapse is more frequent in patients who receive T-cell depleted donor marrow and donor lymphocyte infusions are highly effective in reinducing complete cytogenetic and molecular remission in patients who have relapsed after a primary transplant. This concept has had two important implications: it focused on the importance of defining the immunological basis of the GvL effect for exploitation in different circumstances and it laid the foundations for the current interest in reduced intensity allogeneic stem cell transplantation. The antigenic targets for a GvL effect in CML may be leukaemia-specific, lineage specific or allogeneic. Of these, current interest has focused especially on the BCR-ABL peptides, the WT1 transcription factor and minor histocompatibility antigens. For example $p210^{BCR-ABL}$ junction specific peptides presented in conjunction with class I molecules can be detected on the surface of CML cells and BCR-ABL-specific CTLs are present in the blood of CML patients. The WT1 protein is overexpressed on the surface of leukaemia cells and WT1-specific CTLs can specifically kill leukaemia cells in vitro and in an *in vivo* model system. Further definition of the mechanism underlying the GvL effect could prove of great value for the management of patients with leukaemia who are not eligible for conventional transplants.

A121

The Use of Mesenchymal Stem Cells (MSC) to Prevent Graft Vs Host Disease

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We have tested the effect of human mesenchymal stem cells (hMSC) in vitro on PHA and mixed lymphocyte responses (MLR) of T cells. The normal T cell response to PHA (30x10^3 cpm) was suppressed by the addition of hMSC in a dose dependent manner, which was complete (2.5x10^3 cpm) with a T cell/hMSC ratio of 1:1. Similarly MLR was suppressed 100% with at a 1:1 cell ratio. The suppressive effect was seen when using either h-MSC or their supernatant (from a 7 day culture). The addition of lnterleukin-2 (30UI/ml) to T cell cultures decreased hMSC mediated suppression, but did not completely (80% to 50%). Expression of activation markers such as CD38, were down regulated by hMSC on PHA blasts (6% expression vs 41% of cultures without hMSC).

We have also used expanded hMSC in vivo, in the setting of HLA identical sibling marrow transplants. In brief hMSC were grown from a small (40 ml) aliquot of donor marrow and expanded until a dose of 1-2 x10^6/k, at Osiris USA. The cells were then cryopreserved and shipped to the transplant centre in liquid nitrogen: 1-2 x10^6 hMSC/kg were infused 4 hours prior to a conventional HLA identical sibling bone marrow transplant. We have compared 10 adults with advanced hematologic malignancies, undergoing a conventional BMT supplemented with hMSC, with 22 controls concurrent controls. Platelet recovery was faster in hMSC patients (232 vs 104x10^9/l Plt on day +30 and 204 vs 104 x10^9/l on day +50). Acute GvHD was significantly decreased in hMSC patents: it was scored as grade 0,I,II,III,IV respectively in 60%, 24%, 16%, 0%, 0% vs 6%, 46%, 36%, 7%, 0% ($p<0.001$). This was confirmed in a matched pair analysis comparing 31 hMSC patients from 6 centers to 31 patients allografted in Genova or Paris: In particular the 6 months incidence of chronic GvHD was 32 ± 11% versus 67 ± 10% respectively for patients receiving expanded MSC or matched controls. In the log rank test the difference in incidence of aGVHD and CGVHD is significant ($p=0.002$) and ($p=0.02$) respectively. The survival at 6 months was 96 ± 4% versus 68 ± 8% ($p=0.02$) and remained significant different when adjusted for all matching factors in multivariate analysis. No significant difference in relapse was observed. These data suggest that the use of expanded MSC is safe and produces a significant reduction of acute and chronic GvHD. This is in keeping with in vitro data showing immunosuppressive effect of hMSC.

A124

IgG Adsorption in the Treatment of Immune Diseases

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Staph Protein A immune adsorption technology has been in use for IgG depletion in most “First World” countries for more than 15 years. It has evolved over that time to a safe, proven effective mode of IgG depletion and therefore immune disease treatment method.

An example of this is the pivotal trial reported in April, 2001, by Gendreau on behalf of the Prosorba Clinical Trial Group. This study was a randomised double-blind sham-controlled trial of the Prosorba column for treatment of refractory rheumatoid

arthritis in which 41.7% of patients completing all treatments with the Prosorba column met defined response criteria as compared to only 15.6% of sham treated patients. This result was said to compare very well with those being reported for TNF receptor antagonist, widely touted as the "standard of care" in USA. IgA immune adsorption of separate plasma has been used successfully in the vast majority of diseases where IgG is thought to be central to disease pathogenesis.

It is much more selective than simple plasmapheresis, it removes much more IgG more quickly; it avoids the need for plasma replacement with expensive fluids and it requires a smaller number of plasma exchanges to produce and maintain a response. After only a few procedures the cost per litre of processed plasma is lower than with plasma exchange.

There is however a significant "up front" investment required for a dedicated machine, the adsorption columns are relatively expensive, and there are also "biological substances" (Staph Protein A) and therefore require TGA approval.

Why are we not using this technology in Australia?

A131

Prospects for Gene Therapy for Sickle Cell Anemia and Thalassemia

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The severe hemoglobin disorders, sickle cell anemia and β-thalassemia, are among the most common monogenic diseases worldwide and cause significant morbidity and mortality. The development of gene therapy approaches by ex vivo transduction of repopulating hematopoietic stem cells has been a long recognized possibility. Lentiviral vectors based on the human immunodeficiency virus (HIV) have inherent biological advantages over murine oncoretroviral vectors in that the preintegration complex is able to traverse the nuclear membrane allowing genome integration without mitosis. We have assembled a lentiviral vector system based on components of HIV and have been able to achieve high efficiency transduction of cytokine mobilized, peripheral blood cells that establish human hematopoiesis in immunodeficient mice. Clinical observations and studies in transgenic mouse strains suggest that successful treatment of hemoglobin disorders will require 1) expression of a therapeutic globin gene at 20% or more of the level of the endogenous β-globin genes and 2) genetic modification of at least 20% of stem cells. A γ-globin gene lentiviral vector containing regulatory sequences from the locus control region (LCR) corrects the thalassemia phenotype in mice when expressed at relatively high levels but integration position effects result in variable, low and non-therapeutic levels in other mice. Future studies will incorporate larger LCR elements and/or insulators. Our planned approach also includes the incorporation of a drug resistance gene into the vector coupled with cytotoxic drug treatment following infusion of genetically modified cells. We have evaluated the utility of a variant, 0^6-benzylguanine (BG) resistant, methylguanine methyltransferase (MGMT) gene for in vivo selection by the methylating agent, temozolomide (TMZ), and BG. In normal mice, the genetically modified population has been amplified from 5% to more than 90% by serial drug treatment. Amplification of a minority population of MGMT expressing cells has also been achieved in the presence of a majority of thalassemic stem cells without myeloablation resulting in phenotypic correction of the thalassemia phenotype. The variant MGMT gene when linked to the globin cassette resulted in the ability to amplify F cells in mice from levels of less than 1% to levels in the 70-80% range. This progress in addressing the requirements for successful gene therapy of hemoglobin disorders encourages the preliminary formulation of possible clinical protocols as the remaining problems are addressed with additional pre-clinical data.

A132

Reactivation of Fetal Globin Expression as a Therapy for the Hemoglobinopathies

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Sickle cell disease (SCD) and β-thalassemia are the commonest monogenic disorders worldwide. The onset of these diseases is in the first few months of life as the switch from fetal to adult globin synthesis occurs. However, in patients who coinherit a mutation manifesting with persistent high fetal hemoglobin levels (Hereditary Persistence of Fetal Hemoglobin or HPFH), the clinical course of thalassemia or SCD is significantly ameliorated. This observation suggests that treatment strategies capable of re-activating fetal hemoglobin expression after birth should be explored.

Currently, two such strategies are being pursued. The first centres on pharmacological induction of fetal globin using agents such as hydroxyurea, erythropoietin and butyric acid analogues. Clinical benefit has been demonstrated, particularly in the setting of hydroxyurea and sickle cell disease, but concerns about long-term use of these agents in a paediatric population remain. In addition, a significant number of patients are non-responders, emphasising the need for alternate therapies.

The second strategy focuses on gene therapy using the fetal γ-globin genes themselves or alternatively, the transcription factors which up-regulate fetal globin gene expression. The latter approach has a significant potential advantage in SCD in that it would lead to a competitive reduction in the expression of the disease β^S allele.

Our laboratories have focused on the mechanisms of fetal γ-globin gene regulation. We have identified a transcriptional complex, the stage selector protein (SSP) that promotes preferential expression of the γ-globin genes in the fetal erythroid environment. This complex is composed of three subunits: CP2 a ubiquitously expressed factor which interacts with the polymerase complex to facilitate transcription; NF-E4, a tissue-restricted factor which provides the DNA binding properties of the complex and is critical for recruitment of chromatin remodelling factors necessary for gene expression; and ALY, a chaperone factor that enhances the stability of the CP2/NF-E4 interaction.

We have recently examined the functional role of NF-E4 in a transgenic mouse model of hemoglobin switching. Mice overexpressing NF-E4 in the fetal liver were crossed with another transgenic strain carrying the human β-globin locus on a yeast artificial chromosome (YAC). The double transgenics were found to have a delay in the fetal to adult switch in globin subtype during development. This finding provides the first data supporting the potential use of transcription factors in gene therapy for the hemoglobinopathies.

A136

Human Embryonic Stem Cells for Neural Therapy

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Four human Embryonic Stem (ES) cell lines were derived using excess IVF embryos on murine foetal fibroblast feeder layers with added LIF and bFGF in a collaborating IVF clinic in the US and with informed consent for this research use of the excess embryos. These ES cell lines show the accepted human ES cell phenotype, being Oct 4 positive, alkaline phosphatase positive, SSEA-1 negative, SSEA-3 weakly positive and SSEA-4 positive. They have a normal diploid karyotype. They remain fully functional after cryopreservation and thawing. They were included in the cell lines approved for NIH funding by President GW Bush on 9 August 2001 and US$1.6 million of such funding has been awarded to BresaGen.

Because of the established potential for teratoma formation with ES cells and their derivatives, adult and neonatal rat brain transplant models have been developed and demonstrate that teratomas can be produced from murine ES cell derivatives which are not sufficiently differentiated. FAC sorting to remove residual Oct 4 positive cells has not completely eradicated teratoma formation. Primate models, including in-utero CNS transplantation to assess migration and both morphological and functional integration of neuronal precursors, are also available through our collaborator Curt Freed in Denver, Colorado.

The primary therapeutic target is neuronal cell therapy for Parkinson's Disease (PD), because proofs of principle exist using human foetal CNS cell transplants, and because immunological matching appears unnecessary as a result of the immunological privilege bestowed by the blood/brain barrier. The unilateral 6-OH dopamine lesioned rat model for PD has been established in Adelaide and Athens Georgia and partial correction of amphetamine induced rotational behaviour achieved with both murine foetal ventral mesencephalon cells (positive control) and murine ES cell derived neuronal precursors.

Murine, primate and human ES cell neuronal differentiation has been achieved using a conditioned medium from human HepG2 hepatoma cell lines with added retinoic acid and FGF-2. Tyrosine Hydroxylase positive pre-dopaminergic neural precursors have been developed. Transplantation of human precursors into the unlesioned rat has shown engraftment. Transplantation into the lesioned rat model is imminent.

A137

Neural Stem Cells – Molecular Mechanisms of Migration in the Brain

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It has been a long-standing dogma that the brain had no capacity to regenerate in the mature adult. This dogma has now been rejected and it is acknowledged that a limited number of cells located in discrete areas of the adult brain are capable of division and differentiation long after the completion of embryonic development. The subventricular zone is one such area where neural stem cells divide and migrate towards the olfactory bulb (organ for smell). In the adult mouse brain this migration occurs along a defined anatomical pathway known as the rostral migratory stream (RMS). We aim to understand the molecular cues that coordinate cell migration of neural stem cells in the adult brain. With this knowledge it may be possible to direct the migration of these and other stem cells to areas of damage in the adult brain and thus provide an invaluable therapeutic tool to

treat a myriad of brain injury processes. The molecular guidance mechanisms discovered may also apply to other stem cell populations and make this relevant to tissue engineering in general.

A138

Treatment of Osteochondral Defects of the Knee

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This paper discusses the currently available methods of treating osteochondral defects particularly of the knee. Osteochondral defects of the knee are usually a result of osteochondritis dissecans and trauma. Large chondral or osteochondral defects particularly if greater than 1 centimetre in area are associated with a relatively poor natural history due to progressive degenerative change. Articular cartilage has a very limited capacity for repair or regeneration. Fibrocartilage tissue will form over exposed bone if the underlying bone marrow is stimulated by mechanical means such as drilling or abrasion. Fibrocartilage however, has inferior properties to articular cartilage and long-term success has not been achieved with these methods.

Resurfacing chondral and osteochondral defects with true articular cartilage is therefore the aim. Small autologous osteochondral grafts (mosaicplasty) is one method of achieving this although there are some problems with donor site morbidity and the development of sub-chondral cysts. Autologous chondrocyte transplantation uses tissue engineering techniques to produce an articular like cartilage for implantation to cover chondral and osteochondral defects. It is a two-stage technique. The first stage involves harvesting of articular cartilage so that the chondrocytes can be extracted and cultured. The second stage involves implantation of the cultured chondrocytes into the chondral or osteochondral defect. The technique and early results are discussed.

A139

Mesenchymal Stem Cells: Regeneration of Skeletal Tissue

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The recent impetus in stem cell research has highlighted the potential use of bone marrow mesenchymal stem cells (MSCs), for tissue regeneration. While little is known about their properties *in situ*, MSCs are thought to possess a "plasticity" that allows them to differentiate into a variety of different stromal cell types including bone, fat and cartilage, and other lineages such as muscle cells and astrocytes. However, studies have shown that only a minor proportion of MSCs clonal cell lines can maintain a primitive multi-potential phenotype following *ex vivo* expansion. Moreover, with successive subculture, progeny of MSCs display a diminished capacity to proliferate and differentiate into various tissues such as bone. This has hampered the use of MSCs in the development of cellular therapies, in particular for skeletal tissue regeneration. We have recently developed an immunoselection protocol to purify human MSCs populations directly from bone marrow aspirates, based on their expression of the pre-osteoprogenitor marker, STRO-1 and the vascular/smooth muscle antigens, CD106. This has allowed us to identify the *in vivo* properties of purified human MSCs. One distinguishing feature of freshly isolated MSCs is their expression of telomerase activity which is rapidly decreased *in vitro*. The absence of telomerase activity in *ex vivo* expanded MSCs leads to shortening of chromosomal telomeres and eventually to cellular senescense. Recently, we and others have found that enforced expression of telomerase activity in cultured human MSCs significantly prolongs their normal life span *in vitro* and greatly enhances their potential to form bone *in vivo*. The increased osteogenic capacity of telomerase expressing MSCs also correlated with a significant elevation in the numbers of cells expressing the STRO-1 antigen, indicating a maintenance of stem cell populations following *ex vivo* expansion. Understanding the properties of stem cells *in situ* will help elucidate the fundamental conditions necessary to maintain and expand primitive MSC populations *ex vivo*, in order to effectively direct and enhance their developmental potential for a range of tissue engineering and gene therapy strategies.

B003 **P42**

Post-Partum Thrombotic Thrombocytopenic Purpura (TTP) in a Term Patient with Type IIa von Willebrand Disease (vWD) after DDAVP Infusion

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TTP is a rare but potentially fatal complication of pregnancy. We report a case of post partum TTP in a patient with type IIa vWD receiving DDAVP at delivery. We speculate that the DDAVP may have contributed to the TTP.

A 30 year old female with vWD presented in her first pregnancy. Prepartum testing suggested type IIa vWD with disproportionately low vWF:Ristocetin cofactor activity (vWF:RcoF = 0.29 U/ml) and absence of the high molecular weight multimers. Given a lack of significant bleeding in her past history, DDAVP was planned for the time of delivery. No formal platelet aggregometry or trial of DDAVP was performed.

She presented in labour at term with a platelet count (Plt) of 82 $X10^9$. The blood film was otherwise unremarkable. Blood pressure and urinalysis were normal. Immediately after vaginal delivery she received 24 micrograms of DDAVP intravenously. This dose was repeated 12 hourly for three further doses.

36 hours post delivery she was pale and lethargic. Examination revealed no evidence of bleeding or sepsis. Investigations revealed Plt 25, haemoglobin 60 g/l, bilirubin 23, normal coagulation and renal function, lactate dehydrogenase 1200 and haptoglobin <0.6 g/L. The blood film showed mild to moderate red cell fragmentation. The direct antiglobulin test was negative. A presumptive diagnosis of TTP was made.

Therapeutic plasma exchange was commenced. One daily blood volume was exchanged for cryosupernatant or fresh frozen plasma. Plasmapheresis continued daily for 2 weeks and then second daily for 1 week. The LDH, haptoglobin and Plt had normalised by day 5, 7 and 10 respectively. There has been no recurrence of TTP off treatment.

The association of vWD Type IIa and TTP has not to our knowledge been described. Current knowledge links TTP with a deficiency of the ADAMTS-13 vWF protease, creating an excess of ultra-large multimers of vWF. Recent reports have shown that the vWF protease progressively decreases in normal pregnancies. DDAVP causes an increase in release of vWF and has use in preventing the drop in vWF post partum. We speculate that the increased levels of vWF after DDAVP along with the reduced levels of the vWF cleaving protease may have combined to increase the likelihood of TTP.

Ristocetin induced platelet aggregation to exclude Type IIb vWD was normal. Type IIb vWD has similarities to TTP in terms of the mechanism of thrombocytopenia. Both conditions cause platelet agglutination due to abnormal vWF with high affinity for platelets. Whether our patient has a Type IIb phenotype which is not sensitive to ristocetin but has a greater affinity for platelets is uncertain.

In this case monitoring of factor VIII and vWF post partum to assess the need for DDAVP would have been more appropriate. Whether this would have reduced the risk of TTP is uncertain. Caution in the use of DDAVP and avoidance of excessive doses appears warranted in similar cases of Type IIa vWD in pregnancy.

B004

A Rapid Paediatric PBSC Collection Protocol

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The first PBSC collection at Princess Margaret Hospital in Perth was in March 1995. Since then 59 procedures have been undertaken on the Baxter CS3000 / 3000*plus* apheresis machine. Initial collections were carried out on the Haematology ward using similar protocols to the Granulocyte or Plasmapheresis procedures of the preceding 13 years. Initial protocols were quite conservative and required multiple apheresis episodes. A review of the 31 procedures on 11 patients for the period until January 1997 highlighted some areas where there could be an improvement. Namely the timing and number apheresis procedures required. The areas of extracorporeal volume loss, hypocalcaemia and access/flow rates were also examined. The following protocol for the collection of Peripheral Blood Stem Cells (PBSC) was developed in collaboration with the Intensive Care Unit with the aim of addressing these issues and therefore providing a single rapid apheresis event with maximum patient safeguards.

Apheresis is commenced the day following the attainment of a peripheral blood CD34 count of 20 x 10^9/L. An apheresis target is set at 5 blood volumes or 20 L which ever is less. The use of the Granulocyte separation chamber allowing greater flow rates rather than the Small Volume Separation Container Holder. Patient access is generally via a radial arterial cannula with a venous return in the same arm. Patients are monitored for blood pressure, pulse and Oxygen saturation. Patient chemistries in particular Ca^{++}, K^+ and Hb are performed on a Bayer 865 blood gas analyser at 20 minute intervals. Patients under 25 kg have a modified anticoagulant regime incorporating a heparin/ACD mix at a ratio of 25:1 and the machine is primed with allogeneic Packed Cells. In addition to this a slow calcium gluconate infusion is commenced.

Since the introduction of this protocol in July 1997 26 paediatric patients varying in weight from 6 to 70kg who have mobilised have been apheresed. The collections have yielded 7.6 x 10^6 CD34 cells/kg (median), 18.3 x 10^6 CD34 cells/kg (mean) and a range of 1.0 to 194.3 x 10^6 CD34 cells/kg.

B005 **P43**

Bone Turnover Studies in Patients with Idiopathic Myelofibrosis and Osteosclerosis

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Although 80% of patients with idiopathic myelofibrosis (MF) may present with some degree of osteosclerosis (OS), characterisation of the skeletal pathology remains poorly defined. In this report we describe serum biochemistry, non-invasive markers of bone turnover, tetracycline-labelled bone histomorphometry and bone densitometry data of four men (aged 48-73 yrs) with biopsy-proven MF and OS. Three were newly diagnosed; the other was diagnosed two years earlier and was treated with hydroxyurea for thrombocytosis. None had received any agents known to affect bone metabolism.

Clinical features of OS included chest wall tenderness and metaphyseal bone pain, radiological evidence of trabecular coarsening with sclerosis, and increased technetium uptake in the chest wall and metaphyses of the long bones on radionucleotide bone scans. The serum biochemistry and most plasma cytokine (TGF-β, lL-6, lGF-1, lGF-2) concentrations were normal; the exception was TNF-α, which was raised in three of the four patients. Markers of bone formation (including serum alkaline phosphatase and serum bone Gla-protein concentrations) and markers of bone resorption (including serum cross-linked carboxy-telopeptide concentrations and urinary deoxypyridinoline excretion rates) were significantly elevated in all men. On average, the markers of bone turnover were increased by 1.8-3.6 fold above normal. MRI examination showed features of new bone formation and granulation tissue.

Bone densitometry studies showed significantly elevated measurements in the lumbar spine region in all four patients, but only two had similar results in the femoral neck region. All four patients had significantly elevated histomorphometric indices of bone formation and resorption and higher percent cancellous bone volume results. None had evidence of mineralisation defect. Longitudinal studies in two patients showed progressive changes over 2-4 years.

Our studies have not shown any significant changes in serum biochemistry, plasma cytokines or growth factors to delineate the mechanism responsible for OS, suggesting that more sensitive and specific cellular assays are required. We plan to extend our study to include patients with MF but without clinical features of OS and also evaluate the role of novel cytokines such as osteoprotegerin (OPG) and OPG ligand which may regulate the osteoblast and osteoclast interactions in this syndrome.

B008 **P38**

The Favourable Effect of Aciclovir and Valaciclovir on CMV Infection and Disease in Allogeneic Bone Marrow Transplant Recipients; A Single Institution's Experience Over 20 Years

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Early published series have shown an incidence of CMV end organ disease in approximately 20% patients, with a mortality rate in excess of 90%. A retrospective analysis was made for CMV infection and disease of all patients receiving allogeneic bone marrow transplantation between 1980 and the end of 2001 at a single institution. There were 249 allografts performed in this period.

The analysis was divided into the 3 periods, 1980-1985 with no available therapy, 1986-1993 reflecting the introduction of high dose intravenous and oral aciclovir prophylaxis and 1995-end 2001 reflecting the introduction of high dose valaciclovir prophylaxis.

In the first period 2 of 5 (40%) patients developed CMV disease both developing interstitial pneumonitis, 1 dying of CMV disease.

In the second period 4 of 73 (5.4%) patients developed CMV infection all developing CMV disease, 3 deceased of CMV disease (4.1%).

In the third period 8 of 171 (4.7%) patients developed CMV infection, 8 progressing to CMV disease, 7 dying (4.1%).

There was an increased incidence of reported CMV viraemia in the third period due to the introduction of PCR technology, including qualitative and quantitative PCR to monitor CMV infection, but a lower proportion progressed to CMV disease, in part attributable to pre-emptive ganciclovir therapy.

Campath 1G or 1H was used from 1991 in unrelated and high risk related transplants with no adverse impact on the rate of CMV infection, disease or survival.

Aciclovir and valaciclovir have been effective in our experience in reducing the incidence of CMV end organ disease, but when end organ disease did develop the mortality rate has remained high (11 of 14 patients) despite ganciclovir and other therapy.

B009 **P08**

Single Centre Outcome Using a Liposomal Daunorubicin (Daunoxome) Based Regimen for the Treatment of Acute Myeloid Leukaemia

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A phase III study was carried out in 1999/2000 under the auspices of the International Oncology Study Group utilising Liposomal Daunorubicin 60mgs/m^2 versus 120mg/m^2 days 1-3 and cytarabine 1gm/m^2 bd days 1-4 as induction for de novo acute myeloid leukaemia (AML). Consolidation was with 2 and 3 days of respective agents as above.

All patients to age 70 were eligible. Twelve patients were treated at Royal Perth Hospital (RPH) on this protocol with a median age of 54.7 years (range 17-69), but the trial was closed prematurely due to withdrawal of sponsor support.

Complete remission was seen in 9 of 12 patients (75%). Disease free survival (DFS) was a median of 148 days and overall survival (OS) a median of 344 days.

These figures were compared to a historical control group treated at RPH with the Australian Leukaemia Lymphoma Group M7 protocol utilising idarubicin and high dose cytarabine in induction and consolidation (n=16). The DFS was a median of 995 days in the M7 study (p=0.006) and the OS was a median of 1100 days for M7 protocol compared with 344 days of IOSG protocol (p=0.19).

An analysis was undertaken to look at the impact of bone marrow transplantation as fewer transplants were done in the IOSG protocol group due to increased median age, (54.7 years for IOSG, 46.9 years for M7). No significant difference was found in the median OS for those in the IOSG group who had a transplant (379 days) compared with those who did not have a transplant (269 days). For those transplanted subsequent to the M7 protocol OS is 60% at 2000 days whereas those not transplanted had a median OS of 433 days (p=NS) whereas the median OS for the IOSG group was 379 days for those transplanted compared with 344 days for those not transplanted (p=NS).

The IOSG protocol was tolerated well but proved inferior in terms of survival compared with ALLG M7 study. Liposomal daunorubicin has the potential, because of it's diminished cardiotoxicity, to permit higher doses and treatment of older patients with AML, but future studies with liposomal daunorubicin should incorporate high dose cytarabine.

B010 **P61**

Immunomodulatory Effects of Post-Storage Leuco-Depleted Plasma on Lymphocyte Proliferation *In Vitro*

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Prior blood transfusions have been documented to improve the success of renal allografts and have been associated with increased postoperative infections and possible cancer reoccurrence. Pre-storage leuco-depletion has been shown to reduce the tumor growth promoting effect of stored blood, while post-storage leuco-depletion did not. These data suggest that leucocyte/platelet products are released during storage, and following transfusion may modulate the recipient's immune system.

To investigate this hypothesis, blood was collected from 5 consenting adults and stored for 30 days at 4^0C. Plasma was then removed and mixed with fresh packed cells from the same donors. Both fresh plasma + PBMC and post-storage leuco-depleted (plasma) + PBMC were cultured with phytohaemagglutinin at 37^0C in 5% CO_2. At 72 hours, cultures were pulsed with ^{3}Hthy for 18 hours and lymphocyte proliferation determined using a cell harvester and liquid scintillation counter.

Upregulation of the activation inducer molecule CD69 (involved in lymphocyte signal transduction) and the interleukin-2α receptor (IL-2αR) was also determined using multiparameter flow cytometry.

T-cell proliferation was inhibited in a dose-dependent manner in cell cultures exposed to post-storage leuco-depleted plasma.

CD69 and IL-2αR upregulation by T-cells was inhibited in a dose-dependent manner in cell cultures exposed to post-storage leuco-depleted plasma.

The decrease in T-cell proliferation together with the inhibition of CD69 and IL-2αR upregulation in the presence of stored plasma, may explain the clinical findings in post-storage leuco-depleted transfused patients.

B014

Bacterial Screening of Umbilical Cord Blood Units: Practical Issues about Sensitivity and Delayed Testing

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Quality assurance of cord blood (CB) units banked for clinical transplantation includes bacterial screening. Often Cord Blood Banks are not located in close proximity to a Good Manufacturing Practice-accredited microbiology laboratory resulting in time-delays for screening. The automated bacterial culture system, BacTAlert (bioMérieux) is commonly used for bacterial screening of blood products. This system reputedly detects one viable bacterium in the original sample inoculum. The objectives of this study were to determine: 1) the sensitivity of the BacTAlert system for CB screening; 2) the effect of delayed inoculation and delayed entry of samples into the BacTAlert; and 3) the recovery of bacteria from cryopreserved samples.

CB buffy coat cells were mixed with cryopreservation solution according to standard procedures and then spiked with *Staphylococcus epidermidis* (ATCC 12228) or *Escherichia coli* (clinical isolate) at 2, 20, 200 or 2,000 colony forming units (CFU)/ml. Spiked-CB (0.5 ml) was aliquoted into vials and later inoculated into paediatric BacTAlert bottles at day 1, 4 and 7; or inoculated directly (day 0) into replicate paediatric BacTAlert bottles; or cryopreserved in liquid nitrogen. Quantitation of bacteria was determined by CFU counts on horse blood agar (HBA) plates.

Sensitivity studies showed that 50% (2 of 4) samples spiked with 2 CFU/ml *E. coli* were detected, which is within the sampling error limit of a 0.5 ml sample used for inoculation of the bottles. Replicate cryopreserved samples gave 100% detection by BacTAlert or HBA plates. Samples spiked with >5 CFU/ml of either *E. coli* or *S. epidermidis* gave positive detection of all samples (n = 14 and 16, respectively). Delayed entry into the incubator of BacTAlert bottles inoculated at day 0 and then stored at room temperature (RT) showed that *E. coli* and *S. epidermidis* remained viable up to 7 days. In contrast, delayed inoculation of spiked samples into BacTAlert bottles showed variable results, depending on the bacterial strain and dose. At doses >20 CFU/ml, *S. epidermidis* remained viable in all sample vials stored at RT for up to 7 days (n = 8). At doses <20 CFU/ml the detection of *S. epidermidis* in spiked samples stored for more than 24 hours at RT was inconsistent. *E. coli* was considerably more sensitive and lost viability within 24 hours, except at doses >2,000 CFU/ml. Both *E. coli* and *S. epidermidis* could be readily recovered from thawed cryopreserved spiked samples, even at low doses of bacteria.

For the bacterial strains used in this study 1) the BacTAlert system can detect one viable bacterium in the original sample; 2) bacterial screening samples are best inoculated into BacTAlert bottles at the time of sample preparation; 3) up to 7 days delayed entry into the incubator of BacTAlert bottles inoculated at the time of sample preparation did not compromise bacterial detection and 4) bacteria can be reliably recovered from cryopreserved CB samples. This study provided a useful approach for the validation of an automated bacterial culture system for bacterial screening of CB.

B015 **P37**

Unrelated Cord Blood as a Backup for Unrelated Bone Marrow Transplantation – an Additional Role for Cord Blood

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Stem cell transplantation is a proven treatment for leukemia. When a suitable related donor is unavailable, stem cells can be sourced from unrelated donors (bone marrow (MUD) or cord blood (UCB)). We report the use of a rapidly identified UCB donation following failure to proceed with a planned MUD transplant. A male patient, diagnosed with AML at age 10 years in 10/2000, was induced with Idarubicin, Ara-C and thioguinine on ANZCCSG Study II, achieved complete remission (CR) and proceeded to autologous transplant after high dose melphalan. BM relapse occurred 12 months from diagnosis. CR was re-induced using fludarabine and Ara-C (FLAG). No suitable family donor was identified and search of unrelated donor registries identified a MUD. Conditioning consisted of TBI, Cyclophosphamide, Thiotepa and ATG. $CD34^+$ selection was planned but during initial RBC depletion on a Cobe Spectra, 90% of stem cells were lost. A micro-leak was identified in the Gambro bag at the connection between the port and the bag. The salvaged cells were considered inadequate for engraftment. Access to the

original donor might have been possible but with a minimum delay of 10-14 days. Search of Cord Blood Banks identified a number of 5/6 HLA (A, B, DR) matched UCB donations in overseas and 4/6 matched donations in local Banks. Since conditioning was completed and the patient aplastic, timing was considered an issue, and UCB was pursued locally. Urgent confirmatory molecular typing was performed, a 4/6 matched unit selected and transplant performed 6 days after the original date for MUD transplant. The nucleated cell dose was $5.75x10^7$/kg and $CD34^+$ was $0.31x10^6$/kg (post-thaw). GVHD prevention included Cyclosporine, steroids and methotrexate. Engrafted (NP>$0.5x10^9$/l) occurred by day 23 and platelets>$20x10^9$/l by d34 after UCB transplant. GVHD grade II of skin developed by d29 and resolved rapidly with prednisolone. He was discharged on d34, steroids were ceased on d44 and blood count was normal by d51. He remains fully engrafted and in remission 4 months after CB transplant. This report highlights the accessibility and versatility of UCB and indicates its suitability as a back-up stem cell source.

B018 **P46**

Report of a RSV Outbreak in an Adult Haematology Ward and a Review of the Literature

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Respiratory syncytial virus (RSV) is an enveloped RNA paramyxovirus that can be community or nosocomially acquired. In healthy adults, it usually causes uncomplicated upper respiratory tract infections. RSV is the most frequent respiratory virus in the adult immunocompromised. Infection typically begins as an upper respiratory tract infection that can rapidly progress to pneumonia and acute lung injury in up to 80% of the immunocompromised. Mortality rates of 30-100% in this group depending on the treatment used, time to initiation of treatment, myelosuppression status and presence of GVHD at the time of developing lower respiratory tract infection (LRTI) have been reported.

We report on an outbreak of RSV in six patients in an adult haematology ward. Four of the six patients had been undergoing chemotherapy at the time of developing infection (1 ALL, 1 AML, 1 BL and HIV +, 1 autologous PBSCT for transformed CLL). The fifth patient had been a frequent visitor to the ward for follow-ups. The sixth case occurred in the setting of post-allogeneic stem cell transplant (for Richter's transformation of CLL) in a private hospital on the same campus.

Diagnosis of RSV was made from positive DFA from nasopharyngeal aspirate and broncho-alveolar lavage in all but one patient (positive NPA only). Serological diagnosis was not considered useful. Other pathogens eg PCP and aspergillus were excluded.

Nebulised Ribavirin was given to five of the six patients and intravenous Ribavirin to patient 4, who only had upper respiratory involvement. Intravenous gammaglobulin was also used in some. One patient required assisted ventilation (the index case). Two patients died.

Early reports noted high rates of progression of upper to lower respiratory tract infection, with fears of subsequent high mortality leading to a policy of delaying chemotherapy in patients who tested NPA positive prior to planned therapy. More recent papers have refuted these findings. The Cochrane review of nebulised ribavirin in children has also failed to note a significant survival advantage for the therapy. However there is a trend in favour of survival advantage in 160 infants, shorter ventilation periods and hospitalisation. Our limited anecdotal experience and 2/5 mortality in those with LRTI has impressed us of both the seriousness of the infection and the utility of the treatment.

B019 **P04**

Myeloid Cytokines During Induction Therapy of APL - A Cautionary Tale

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G-CSF is widely used in the supportive care of haematologic malignancies. We report two cases of acute promyelocytic leukaemia (APL) where early administration of myeloid growth factor support was associated with recrudescence of DIC/fibrinolysis.

Case 1
A 38-year-old woman was diagnosed with APL, following presentation with thrombocytopenia and coagulopathy. Bone marrow examination was confirmatory and molecular analysis demonstrated the PML-RARα fusion transcript. Treatment was

commenced with ATRA (80 mg/d) and Idarubicin (12 mg/m^2 on days 2, 4, 6 and 8). G-CSF was given on day 10 of chemotherapy for neutropenic fever with resultant increment in WCC of 0.3 to 4.9 x 10^9/L and neutrophils, 0 to 1.0 x 10^9/L over 24 hours. Accompanying this was re-emergence of fibrinolysis and DIC (Fibrinogen < 0.1 g/L, reduced α-2-antiplasmin, borderline-low plasminogen). The d-dimer rose from 10 to 91 mg/L. She was treated with supportive therapy including epsilon aminocaproic acid with slow resolution of the coagulopathy. She achieved cytogenetic remission without additional treatment by day 35.

Case 2
A 41-year-old female was diagnosed with APL in 1988. She was treated with Ara-c (3g on day 1 followed by 1.5 g x 4) and methylprednisolone (100 mg x 5 days). Laboratory evidence of coagulopathy (increase in fibrin split products (FSP) to 640 [NR < 10]) developed by day 9. The APTT and PT remained normal. This settled completely by day 10. GM-CSF was started at 0.12 mg/m^2 on Day 8, by which time the WCC was < 0.1 x 10^9/L. There was a recrudescence of the coagulopathy on day 9 of the GM-CSF therapy with a rise in FSP to 40 and fall in fibrinogen. This was accompanied by clinically significant bleeding. Cytogenetic remission was achieved by day 35.

The coagulopathy of APL is complex, however, thought to involve activation of the coagulation cascade, proteolysis and fibrinolysis. The leukaemic promyelocytes have both procoagulant activity on the cell membrane and proteolytic activity in the granules. Procoagulant mediators consist of tissue factor, cancer procoagulant and cytokines such as IL-1, TNFα and Vascular Permeability Factor (VPF). Release of plasminogen activators from the leukaemic promyelocytes result in activation of plasminogen and initiation of fibrinogenolysis. Release of elastase results in inactivation of α-2-antiplasmin. This report highlights the potential risk of re-activating persistent APL cells if myeloid cytokines are administered too early. We recommend that these should be used with extreme caution in this condition.

B022 **P06**

Hyperdiploidy in Childhood Acute Lymphoblastic Leukaemia

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In the period 01/01/97-30/04/02, 133 children with ALL had cytogenetic analysis at diagnosis at RBH. Karyotypes were obtained for 116 (87.2%) of these; 48 (41.3%) were hyperdiploid: 10(8.6%) with 47-50 chromosomes and 40 (34.5%) with 51-68. Two patients had karyotypes in the tetraploid range. The most common trisomies seen were 10 and 21 in the 47-50 group and 4,6,14,17,18 and 21, tetrasomy 21 and an extra copy of the X in the 51-68 group. Structural abnormalities were found in 25% of the 47-50 group and in 35% of the 51-68 group. Interphase FISH using centromere probes for the most frequently detected trisomies in this series was performed on those patients with no metaphases at diagnosis and a DNA index∃ 1.1. This yielded useful information in all 10 cases. Patients have been followed at day 14 and day 28-30 post induction using FISH to monitor levels of the abnormal clone. This study shows the value of interphase FISH in detecting hyperdiploidy in ALL and utilizing the DNA index as a guide to which probes to use.

B023

Constitutive Association of Calmodulin with Positively Charged Residues in the Juxtamembrane Region of PECAM-1

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Recent studies have demonstrated that a cluster of highly conserved charged amino acid residues in the juxtamembrane region of PECAM-1 is required for stabilisation of PECAM-1 at the lateral junctions of PECAM-1-expressing cells. We hypothesised that these highly charged residues may be important in cytoskeletal reorganisation via the direct recruitment of signaling molecules or cytoskeletal components. One of the candidate molecules we investigated was calmodulin.

Reciprocal co-immunoprecipitation studies revealed that cytosolic calmodulin is constitutively associated with PECAM-1 in resting, thrombin activated and aggregated human platelets. Synthetic overlapping peptides encompassing the entire human PECAM-1 cytoplasmic domain were used to screen for their ability to associate with purified bovine calmodulin. Calmodulin was found to interact specifically with a PECAM-1 peptide (594-619 C595A). In order to narrow down the consensus sequences required for calmodulin binding, synthetic peptides derived from five different regions within the cytoplasmic domain of PECAM-1 were screened for their ability to bind to calmodulin derived from platelet lysates and with purified bovine calmodulin. Our studies demonstrate that calmodulin directly interacts with a PECAM-1 peptide (594-604 C595A) containing the sequences ^{594}KAFYLRKAKAK^{604}. This calmodulin:PECAM-1 peptide interaction is potentiated by the addition of calcium ions. Furthermore, this membrane proximal portion of PECAM-1 is evolutionary conserved across all

species and the helical representation of positively charged residues is analogous to other calmodulin binding IQ motifs. Taken together these results suggest that this highly charged cluster of amino acids in the PECAM-1 cytoplasmic domain directly interacts with calmodulin, and this novel interaction may regulate PECAM-1's involvement in a variety of biological processes.

B029 **P07**

Leukaemia Presenting as Skin Lesions: A Case of Myeloid/NK Cell Leukaemia

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Myeloid/NK cell leukaemia is a rare form of acute leukaemia that may mimic morphological and immunophenotypic features of acute promyelocytic leukaemia, especially the microgranular variant. Up to 50% of patients with myeloid/NK leukaemia present with disseminated intravascular coagulopathy, however they are resistant to ATRA therapy. Cytogenetic analysis differentiates the two diseases with absence of the APML translocations in myeloid/NK neoplasms. Patients frequently present with extramedullary disease and follow an aggressive clinical course.

We report a case of a 28 year old with a typical presentation. In the four months prior to diagnosis, she had complained of raised bluish-red dermal nodules over trunk and legs increasing in size. A breast lump biopsy had revealed an "atypical lymphoid infiltrate" but the lesion was diagnosed elsewhere as a juvenile haemangioma. At presentation to RNSH hospital, laboratory features of disseminated intravascular coagulopathy, a spontaneous splenic haematoma, and pancytopenia with reniform blasts raised the possibility of APML. Bone marrow biopsy revealed morphological features similar to those previously described in myeloid/NK cell leukaemia, with a dense infiltrate of bizarre, pleomorphic blast cells with marked nuclear folding and invagination, some reniform shapes and occasional azurophilic granular inclusions. Immunophenotyping of blasts showed co-expression of myeloid and NK cell markers: $CD34^-$, $HLA\text{-}DR^+$, $CD33^+$, $CD13^-$, $CD56^+$, $CD16^-$, MPO^-. Cytogenetic analysis revealed complex clonal rearrangements including del(6)(q21); -12 and add(19)(p13). Del (6q) is the most common finding in NK cell leukaemia. Skin biopsy confirmed leukaemia cutis.

The patient has responded well to intensive acute myeloid leukaemia therapy without ATRA, however, given the poor prognosis described in the literature, an allogeneic transplant in first CR is planned.

B030 **P15**

Rapid Response of Resistant Primary Hypereosinophilia Syndrome to Imatinib Mesylate

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Primary hypereosinophilic syndrome is a rare clinical syndrome characterised by peripheral blood and marrow eosinophilia which commonly causes significant end organ damage. We report a case of a 57-year-old man with primary hypereosinophilic syndrome with resistance to standard treatment who rapidly responded to low dose Imatinib Mesylate (Glivec).

The patient presented in November 2001 with myalgia, rash, lethargy, malaise and peripheral neuropathy. Cardiac echo revealed a laminar left ventricular thrombus characteristic of Loefflers endocarditis, and sclerosis of the aortic and mitral valves. The absolute eosinophil count was persistently between 5.3 and 9.0 x 10^9/L. No cytogenetic abnormality was detected and PCR for the common bcr/abl mutations were negative. Secondary causes of eosinophilia were excluded.

The patient was warfarinised. The eosinophil count remained elevated despite therapy with Prednisone, Hydroxyurea and Interferon 2α either as single agents or in combination. In May 2002, the patient was on a combination of prednisone 25mg/day, hydroxyurea 500mg/day and interferon 6 million units/day. His absolute eosinophil count ranged between 2.3 and 3.9 and he had progressive neurological symptoms. He had significant side effects from treatment: diabetes and hypertension on prednisone; pancytopenia and neutropenic sepsis on hydroxyurea; profound lethargy, depression and myalgias on interferon.

Imatinib Mesylate was commenced 100mg/day and the absolute eosinophil count fell to normal within one week.

The mechanism of action of Imatinib in hypereosinophilia is unclear. The mechanism is clearly not through the bcr/abl tyrosine kinase inhibition that is the target in chronic myeloid leukaemia, nor does there appear to be expression of the other known targets (c-KIT and PDGF receptor) in hypereosinophilia. However, in a subset of primary hypereosinophilia, pathogenesis appears to be related to a clonal proliferation of T cells producing IL5. IL-5/IL-5 receptor interaction activates Lyn tyrosine kinase and JAK2 tyrosine kinase in eosinophil stimulation. It is possible that imatinib interferes with the activity of these tyrosine kinases in IL5 stimulated eosinophils or that there are other as yet unknown targets.

B031

Detection of Anti-idiotypic T Cells in Patients with Myeloma using Idiotype-Derived Immunodominant Peptide Tetramers

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Direct evidence that anti-idiotypic T cells exist in the blood of patients with myeloma has remained controversial. It has been difficult to demonstrate the existence of such cells *in vitro* and the response to idiotypic vaccination *in vivo* has been minimal. Thus it has been suggested that these cells may have either become tolerised or deleted. The aim of these studies was to search for idiotype-specific T cells using customised tetramers prepared from immunodominant idiotype-derived peptides of patients known to have expanded T cell clones. From a cohort of 22 patients we identified 6 patients who had expanded T cell clones determined by expression of the TCRVβ repertoire who were also HLA-A2, IgG paraprotein and still alive. Bone marrow plasma cells from these 6 patients were flow sorted to >95% purity (CD138+ and CD38++), mRNA was extracted and cDNA prepared. Immunoglobulin heavy and light chain sequences were amplified by PCR using family-specific forward primers derived from the 5' end of FWR1 of V_H, V_κ and V_λ segments. The reverse primer was designed to target the first exon of the IgG constant region common to all 4 subclasses. These are the minimal set of primers required to amplify all functional V genes. PCR products were sequenced and real time PCR was also used to confirm clonality of PCR products in 2 patients who amplified more than one sequence. Variable sequences were translated and examined for nanomer and decamer peptides capable of binding to MHC class I molecules using two independent web-based algorithms (BIMAS and SYFPEITHI). Peptide binding prediction scores were divided into 3 grades based on scores of known high affinity antigens. There were a total of 20 high scoring peptides from the 6 patients though only 2 patients had high scoring CDR peptides and only one scored highly with both algorithms (BIMAS = 1443 and SYFPEITH = 22). High scoring FWR peptides (n=7) were not considered, as T cells specific for these peptides would be deleted by central tolerance. High scoring CDR peptides from 2 patients were synthesised and used to prepare iTAg^{TM} MHC Tetramers (HLA-A*0201) conjugated with phycoerythrin (PE). Tetramers and anti-CD8 FITC were incubated with blood cells from each patient and 3 other HLA-A2+ patients. A small population of CD8+ cells, reacting only with each patient-specific tetramer was observed (<1% of CD8 cells) but only after cells were preincubated at 37^0C for 4 h. An iTAg^{TM} MHC CM VPP65 – NLVPMVATV tetramer used as a control and detected a population of CMV reactive CD3+ cells in the blood of patients known to be CMV+ (n=4; mean= 2.9% of CD3 cells). Thus immunodominant peptides from the CDR of the idiotype are not common in patients with myeloma but anti-idiotypic CD8+ cytotoxic cells specific for immunodominant idiotypic epitopes are present in the blood of patients with myeloma and offer some potential for future immunotherapy strategies.

B032

B7+ T Cells in Myeloma: An Acquired Marker of Prior Chronic Antigen Presentation and Unresponsiveness

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Reversing T cell unresponsiveness may be a key factor in the development of immunotherapy strategies for patients with myeloma. The fate of T cells (anergy, apoptosis or a productive immune response) is determined by the expression of the B7 family of costimulatory factors (esp CD80 and CD86) on antigen presenting cells (APC) and the binding of B7 to counter receptors CD28 and CD152 on T cells. Recent murine studies have shown that T cells acquire CD80 from APC after CD28 ligation rather than by endogenous upregulation. Murine CD80+ memory T cells undergo apoptosis in the presence of high levels of antigen and naive CD80+ T cells are capable of acting as APC, but it is also apparent that T cell:T cell ligation induces anergy and unresponsiveness to antigen rechallenge. We have determined that B7+ T cells (CD80+ or CD86+) are present in 30-33% of patients with myeloma (n=45) and their presence is associated with stable disease. B7+ T cells were not present in the blood of a group of aged-matched controls (n=10). T cells expressing the highest levels of CD80 tended to be CD4 cells (13 of 16) and those expressing high CD86 tended to be CD8 cells (11 of 13). The number of B7+ T cells was stable in longitudinal studies over 12 months. More than 99% of the CD80+ T cells were memory cells (CD45RO+) and polyclonal as no restricted TCR Vβ repertoire was detected (n=3). After affinity purification on MACS beads (n=5 patients), CD80 mRNA expression was present in CD14+ monocytes (with 8-35% CD80+ antigen) but CD80mRNA was either not detectable in CD3+ cells (with 10-37% CD80+ antigen) or at trace levels. This was confirmed by real time PCR. CD80 antigen expression could be upregulated on B cells but not T cells by incubating with huCD40LT + IL-2 (n=6). CD80 antigen on T cells was eluted rather than internalised during *in vitro* culture for 48h. As similar results were obtained with CD86, we conclude that the B7 antigens are acquired by T cells of patients with myeloma rather than being endogenously upregulated. This study demonstrates that B7+ CD45RO+ T cells are common in patients with myeloma but not in age-matched controls. These cells constitute a population of post antigen presentation and unresponsive memory T cells which result from chronic antigenic stimulation. Whether such cells have specificity to a myeloma tumour antigen requires further investigation.

B033

Immature Human Osteoblasts express the Osteoclast Differentiating Factor, RANKL, in Response to Osteotropic Factors

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Normal human bone-derived cells (NHBC) are a mixed population of osteoblast (OB)-like cells, separable on the basis of their stage of osteogenic differentiation using the cell surface markers STRO-1 and alkaline phosphatase (AP). We compared the cell surface expression of STRO-1 and AP with that of molecules central to osteoclast (OC) formation, namely RANKL and OPG, to examine the dual functionality of the human osteoblastic stromal cell.

NHBC were sorted, using fluorescence activated cell sorting (FACS), into STRO-1^{bright} and STRO-1^{dull} populations. The expression of RANKL and OPG mRNA, as well as the cell surface RANKL expression, was examined in each population in response to $1,25(OH)_2$vitaminD_3 (vitD_3) and dexamethasone (DEX). STRO-1^{bright} and STRO-1^{dull} populations were both positive for RANKL mRNA and cell surface RANKL expression. While STRO-1 positivity did not correlate with the basal level of RANKL or OPG mRNA, vitD_3 treatment of these populations resulted in an increase in the RANKL:OPG mRNA ratio to a greater extent in the STRO-1^{bright} cells than the STRO-1^{dull} cells. This suggests a greater sensitivity of immature OB to pro-osteoclastogenic factors. Furthermore, vitD_3 treatment increased the percentage of immature (STRO-1^+/AP^-, STRO-1^+/AP^+) populations over a culture period of 6 days. DEX treatment alone had little effect on the expression of RANKL, downregulated OPG mRNA, and had a profound effect on the maturation of unfractionated NHBC, as indicated by an increase in the percentage of cells expressing AP. The RANKL:OPG mRNA ratio associated negatively with an increase in the overall maturation of the NHBC cultures in response to DEX. To investigate the effect of hemopoietic cells on human osteoblasts, human PBMC were incubated in co-culture with NHBC. Using the fluorescent dye, CFSE, to label NHBC, we found that human PBMC promoted the proliferation and maintenance of an immature NHBC phenotype. Our findings are consistent with the hypothesis that immature OB are more responsive to pro-osteoclastogenic stimuli than mature OB, and that some of these signals derive from the hemopoietic element in bone. Our data suggest that the dual functionality of OB in supporting OC formation or forming bone is a function of the maturation state of the same lineage of cells.

B035 **P35**

Prospective Randomized Trial Comparing Donor Morbidity between G-CSF Stimulated Bone Marrow and Peripheral Blood Stem Cell Collections Used for Allogeneic Stem Cell Transplantation: Increased Pain at Donation Site with Bone Marrow Harvests Although No Increased Delay in Return to Normal Activity

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Background
Relatively few studies have investigated the effects of stem cell collection on donor morbidity in allogeneic stem cell transplantation. In particular, very few comparisons between the source of stem cells and donor outcomes have been reported.

Aims
To compare donor morbidity after G-CSF stimulated bone marrow versus peripheral blood stem cell collections for use in allogeneic stem cell transplantation.

Methods
59 consecutive donors involved in a prospective randomized study comparing G-CSF stimulated bone marrow versus peripheral blood stem cells for use in allogeneic stem cell transplantation were included. G-CSF was administered at 10mcg/kg/SC/day to all donors for 5 days prior to stem cell collection, which was performed via standard aphoresis using a COBE-spectra for peripheral blood collections, and via bilateral posterior superior iliac crest aspiration under general anaesthesia for bone marrow collections. Donors were subsequently given a questionnaire to complete at 28days, 3months, 12months and 24months post stem cell collection. The questionnaire asked donors to comment on the presence or absence of fatigue, pain at donation site, lower back pain, difficulty with walking, light-headedness and fainting, sleeping difficulties, bleeding and any other symptoms at each time point. Donors were also asked to estimate the time taken to return to normal activity post stem cell donation.

Results
A total of 30 bone marrow and 29 peripheral stem cell donors were included. Response rates to questionnaires varied between 93% of donors at 28days and 57% at 24months. There was no significant difference between the number of responses received from each group at any of the time points studied. Post donation, bone marrow donors experienced significantly more localized pain at the collection site compared to peripheral blood stem cell donors (20/25 vs 5/27; p=<0.00001). This

difference persisted at 3months follow-up (8/20 vs 0/21; p=<0.005), though was lost on 12month review (2/20 vs 0/22; p=0.42). No other significant difference between any symptoms experienced by the 2 groups was noted at any other time point. Time taken to return to normal activity after bone marrow vs peripheral blood stem cell collection was also no different between the 2 groups (mean 20days vs 18days respectively; p=0.4). 14/22 and 15/26 responders had returned to normal activity within 7 days of bone marrow vs peripheral blood stem cell collection respectively (p=0.9). To date, 2 donor deaths have occurred, both within the peripheral blood stem cell donor group. One donor died from an acute myocardial infarct at 23months and the second from a ruptured berry aneurysm at 28months post stem cell donation respectively.

Conclusions
Donation of G-CSF stimulated bone marrow for allogeneic stem cell transplantation is associated with significantly more localized pain at the donation site compared to peripheral blood stem cell donation. Although this difference persists for 3-12 months post donation, it is not associated with any increased delay in returning to normal activity. No serious long-term adverse effects related to stem cell donation have been noted to date.

B037

The Major Transforming Protein of Epstein Barr Virus -LMP1, Upregulates IL-13 Expression in Hodgkin's and Burkitt's Lymphoma Cell Lines

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IL-13 is an important immunoregulatory cytokine produced mainly by activated Th2 cells. It upregulates CD23 and MHC class II expression on B cells and promotes B cell differentiation while inhibiting the expression of pro-inflammatory cytokines by down-regulating macrophage activity. Recently, IL-13 expression has been detected in the malignant Reed-Sternberg cells of Hodgkin's lymphoma and it has been shown to be an autocrine growth factor for these cells. The mechanism by which upregulation of IL-13 expression in H-RS cells occurs is unknown. Epstein-Barr virus has been implicated in the pathogenesis of HD. Its major oncogenic protein- latent membrane protein (LMP1), has been shown to induce the expression of IL-10, another cytokine which has been implicated in lymphomagenesis. In order to determine whether LMP1 is capable of upregulating IL-13 in lymphoma cell lines, we transfected a HD cell line (L428) and a Burkitt's lymphoma cell line (P3HR-1) with plasmids encoding the wild-type (WT) and two truncated versions (del-10 and CΔ55) of the LMP1 protein. L428 is an EBV negative HD cell line, which constitutively expresses IL-13. P3HR-1 contains a transformation defective EBV strain, which is unable to produce LMP1, and does not express IL-13. Both cell lines were transfected using electroporation and IL-10 and IL-13 expression was measured by specific ELISAs. Our results show that both the WT and del-10 LMP1 proteins induce IL13 and IL10 expression in the HD cell line L428, and to a lesser degree in the Burkitt's line P3HR-1. In both cell lines, transfection with the WT LMP1 protein resulted in significantly higher IL-10 and IL-13 expression than the del-10 LMP1 compared to mock-transfected controls. Transfection with the CΔ55 LMP1 variant failed to induce IL-10 or IL-13 expression in either cell line, compared to mock-transfected controls.

Our results show that the major transforming protein of EBV is capable of upregulating expression of an important immunoregulatory cytokine which has been implicated in the pathogenesis of HD. Moreover, failure of the CΔ55 LMP1 variant to induce cytokine expression in our cell lines suggests that this upregulation is mediated by the nuclear transcription factor NF-κβ. As LMP1 expression is often detected in the H-RS cells in HD, this may be an important mechanism by which dysregulation of IL-13 expression occurs, thus contributing to the pathogenesis of EBV- associated lymphomas.

B039 **P16**

Myelodysplasia (Refractory Anaemia with Ring Sideroblasts [RARS]) Occurring in a Patient with CML Treated with Imatinib

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We report a 42-year-old man who developed myelodysplastic syndrome (MDS), associated with ring sideroblasts and new cytogenetic abnormalities, after 8 months of therapy with imatinib (STI-571; Glivec) for chronic myeloid leukaemia (CML) in blast crisis. At diagnosis in 1995, the patient had the standard Philadelphia chromosome (Ph) [46,XY, t(9;22)(q34;q11)] as the sole cytogenetic abnormality. No donor was found for an allogeneic transplant. Treatment included hydroxyurea, interferon, melphalan, cytarabine, palliative splenic irradiation, and FLAG. Treatment repeatedly normalised the white cell count but was only partly effective in controlling the spleen size. Five years after diagnosis blast crisis was confirmed; there were no additional cytogenetic abnormalities. Imatinib was commenced with good effect initially. There was marked reduction in spleen size and normalisation of the blood count. However neutropenia developed after 8 months. At this time the bone marrow was hypercellular with marked erythroid hyperplasia and dyserythropoiesis including 10% ring sideroblasts, findings indicative of a diagnosis of MDS of the RARS subtype. Twenty-two cells underwent cytogenetic analysis. Of these, 18

showed only the standard Ph that had been present at diagnosis. The other 4 cells had the Ph as well as loss of chromosome 13 with additional chromosomal material on the short arm of chromosome 18 (p11.3). Myelodysplasia in the setting of CML is rare. That it developed in a patient receiving imatinib raises the question of the role of this new drug in its development.

B040

Flt3-L Inhibits the Proliferation of Megakaryocytes Isolated from CD34$^+$ Peripheral Blood Progenitor Cells

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Chemotherapy-induced thrombocytopenia is a major risk factor in cancer treatment. The transfusion of autologous *ex vivo* expanded megakaryocyte (Mk) progenitor cells offers a feasible approach to ameliorate or even abrogate post-transplant thrombocytopenia. The optimal cytokine combination for the production of Mk progenitor cells from mobilised peripheral blood progenitor cells (mPBPC) has yet to be defined. A two-level fractional factorial experiment (2^{7-3}_{IV}) was designed to determine the main factors that have either positive or negative interactions with MGDF on the production of Mk progenitor cells. mPBPC (from patients undergoing autologous stem cell transplants) were CD34$^+$ MACS enriched, and incubated for 7 days in serum free medium along with various cytokines shown to be responsible for the regulation of megakaryocytopoiesis and platelet production (GM-CSF, IL-3, IL-6, IL-11, SCF, Epo, Flt3-L and MGDF). Nucleated cell count (NCC) and the immunophenotype of cells before and after cytokine incubation was determined by three-colour flow cytometry using a panel of monoclonal antibodies to distinguish between committed and primitive progenitors (CD41a/CD71/CD33, CD61/CD38/CD34). Of the 7 cytokines tested in combination with MGDF, the factors that amplify production of total CD61$^+$, CD61$^+$CD34$^+$ and total CD41a$^+$ are IL-3 ($p<0.002$), SCF ($p<0.001$) and GM-CSF ($p<0.05$). Interestingly, Flt3-L had an inhibitory effect on the production of total CD61$^+$ cells ($p<0.05$), CD61$^+$CD34$^+$ cells ($p<0.03$) and total CD41a$^+$ cells ($p<0.01$). Based on these results, MACS enriched, CD34$^+$ mPBPC were then incubated for 7 days in serum free medium with either MGDF+IL-3+SCF+GM-CSF (4GF) or MGDF+IL-3+SCF+GM-CSF+Flt3-L (5GF). NCC and immunophenotype of cells before and after cytokine incubation was determined as described above. Results are shown below and expressed as the mean fold expansion±standard deviation. Expansion = (Output-Input)/Input.

	4GF	5GF
Total NCC	12.15±3.12	8±2.74
Total CD61$^+$	47.63±27.68	30.02±18.20
CD61$^+$CD34$^+$	43.33±8.31	26.48±8.57
Total CD41a$^+$	95.75±122.26	49.97±49.74

Mk differentiation, but not proliferation, was observed in cultures in the presence of MGDF alone (data not shown). MGDF must be combined with other cytokines to achieve the proliferation of MK progenitors. In conclusion, the cytokines that amplify production of Mk progenitor cells from MACS enriched, CD34$^+$ mPBPC in combination with MGDF were IL-3, SCF and GM-CSF. Furthermore, the addition of Flt3-L to the 4GF combination had an inhibitory effect on the production/proliferation of Mk progenitor cells, when compared to that of the 4GF combination. Overall, factorial experimental design analysis has provided an appropriate statistical model for understanding the complex nature of cytokine interactions that regulate *in vitro* production of haematopoietic progeny.

B043

Favourable Results With Peripheral Blood Stem Cell Mobilisation in Patients with Chronic Myeloid Leukaemia (CML) on Imatinib Mesylate During Cytogenetic Remission – Experience from St Vincent's Hospital and St George Hospital, Sydney

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Treatment recommendations for Chronic Myeloid Leukaemia (CML) are still evolving, especially with the advent of new drugs such as Imatinib Mesylate (STI 571). In view of the scarcity of data on durability of response to Imatinib and the limited treatment options for patients diagnosed after the age of 55 or who lack a suitable donor, we decided to study the feasibility of peripheral blood stem cell (PBSC) collection in patients who attained cytogenetic remission with Imatinib.

To date we have studied seven patients in various phases of CML who attained complete cytogenetic remission with Imatinib. The protocol involved cessation of Imatinib for a week, followed by daily injections of G-CSF (Filgrastim, 10ug/k/d) for up to 5 days. The table summarises the patient characteristics:

Sex	Age (av/range, yrs)	% Ph chromosome at Dx (range)	CML status at commencement of STI	Duration of STI (av/range, mos.)
M:5, F:2	54.3 (48-59)	82.5%-100%	CP1:4, CP2:2, BP:2	9.2 (4-14)

Two patients had received hydroxyurea and interferon and one patient had hydroxyurea only. One second chronic phase patient had a major cytogenetic response with Interferon and had an autologous transplant. Two of the seven patients had 100% Philadelphia chromosome in their marrow at diagnosis and the rest had between 82.5% and 90%. PBSC collection was successful in six patients with an average absolute CD 34+ cell yield of 1643.3 x 10^3/ml or 4.9 x 10^6 CD34+ cells per kilogram body weight. One patient failed to mobilise with growth factor alone or a combination of growth factor with cyclophosphamide. She was heavily pretreated with chemotherapy for lymphoid blast crisis before commencing Imatinib. The average volume of blood processed was 17.2 litres per patient. Three patients who were naïve to hydroxyurea and interferon achieved very high CD 34+ cell yield of 5.7, 5.8 and 8.7 x10^6/kg body weight, in contrary to the other three. Two patients required two apheresis sessions to achieve a yield of 1.7 x 10^6/kg and 2.3 x 10^6/kg body weight respectively. Progenitor culture assays were performed to examine the proliferative potential of the CD34+ progenitors.

This study shows that successful collection of sufficient CD34+ cells can be performed after cessation of Imatinib. In view of the preliminary data on colony assays, the function of these CD34+ cells require further investigations.

B044 **P27**

Diagnosis and Monitoring of Mantle Cell Lymphoma: Use of Molecular, Cytogenetic and Flow Cytometric Assays

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Mantle Cell Lymphoma (MCL) represents a distinct subtype of non-Hodgkin's lymphoma (NHL) characterised by t(11;14) resulting in the deregulation of cyclin D1, a cell cycle regulator. Historically, distinction between MCL and other NHL entities such as atypical chronic lymphocytic leukemia (CLL) has proved difficult, relying on morphological and immunophenotypic profiles. Here, we report the combined use of cytogenetic, immunophenotypic and novel molecular assays for the improved diagnosis and sensitive monitoring of disease in MCL.

A 57-year-old male presented with mild splenomegaly and an elevated lymphocyte count. Initial immunophenotyping was consistent with a diagnosis of atypical CLL and the patient subsequently underwent 5 cycles of fludarabine with minimal haematological response.

Peripheral blood and bone marrow samples were subsequently analysed using combined flow cytometric, molecular and cytogenetic assays including expression of the CD79a monoclonal antibody by flow cytometry and detection of t(11;14) by fluorescence in situ hybridisation. Nested PCR for the detection of t(11;14) in the major translocation cluster (MTC) and competitive and real-time PCR techniques were also used to detect and quantitate the level of cyclin D1 expression in diagnostic and follow up samples.

The diagnostic sample demonstrated strong expression of CD79a by flow cytometry consistent with a diagnosis of MCL. One hundred interphase cells at diagnosis were scored for the presence of t(11;14) with 94 /100 cells exhibiting dual fused signals indicative of the translocation. Competitive PCR analysis demonstrated distinct overexpression of cyclin D1 in all samples compared to normal haematopoietic cells despite a negative result for the nested PCR using a single set of primers within the MTC. Real-time PCR revealed an elevated normalised level of cyclin D1 expression compared to normal controls. Diagnosis was subsequently altered to MCL based on these laboratory findings and treatment was altered to CHOP and CHOP/Mabthera with a marked haematological response.

Mantle cell lymphoma is an aggressive disease with a poor prognosis and historically has been a difficult disease to classify. We conclude the combined use of flow cytometric, cytogenetic and molecular assays, enables a more accurate diagnosis and sensitive monitoring of MCL in our patients with the potential for quantitation of minimal residual disease using real-time PCR.

B045

Measurement of Patient and Carer Reactions to Reinfusion of Cryopreserved Haematopoietic Stem Cells (HSC)

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Historically, significant morbidities have been associated with the reinfusion of HSC during stem cell transplantation and are primarily attributed to the presence of DMSO and cell lysis products in the graft. Commonly reported side effects include nausea, vomiting, flushing, breathlessness in addition to the characteristic "dead oyster" odour emitted by the patient. The utilisation of post-thaw stem cell washing is an increasing practice, however, the extra processing of cells needs to be balanced against the fact that morbidity has already significantly lowered by reductions in the reinfusion volume (achieved through improved harvesting, mobilising and processing techniques) and improved patient pre-medication. Thus we conducted a study of patient and carer responses following HSC reinfusion in order to determine whether there is a clinical need for the further amelioration of reinfusion side effects.

A Visual Analog Scale Quality of Life (VASQOL) survey and nursing documentation forms were developed for this study to assess symptoms associated with the reinfusion of cryopreserved HSC. As the majority of our reinfusions are performed in the outpatient setting, we have also studied the side effects experienced by family members/carers and nursing staff. The VASQOL surveys were self-completed by the patient, the nurse and the carer at intervals over a 24 hour period. The most significant side effect noted by patients was an offensive taste that was experienced by all recipients with several rating it "the worst they could imagine". This peaked in the first 6 hours post-reinfusion and had returned to normal by 24 hours. Nausea, shortness of breath and flushing were generally rated as not significant by patients, indicating that pre-medication is appropriate. Family members / carers were seriously affected by the intensity of the dimethylsulphide odour with the majority rating it as "the worst they could imagine". Nurses were also affected by the smell, but not to the same degree as family members. Response intensities were related to the reinfusion volume.

This study has lead us to initiate a clinical trial of reinfusing washed HSC for patients with a reinfusion volume of >50mls. This will reduce the amount of reinfused DMSO and hopefully diminish the intensity of the dimethylsulphide odour and taste that adversely affects both the patient and their primary carer(s).

B046

Leukapheresis for *ex vivo* Dendritic Cell Culture for an Immunotherapy Trial

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Mononuclear cells were collected by leukapheresis with the purpose of culturing dendritic cells to be loaded with a recombinant vaccine consisting of the tumour antigen, Mucin 1, linked to mannan (Mannan-MUC1). The cultured cells were used in a Phase I clinical trial of adoptive immunotherapy of patients with MUC1 expressing adenocarcinoma. Leukapheresis was performed using the Haemonetics MCS plus.

Selection criteria included diagnosis of adenocarcinoma with tumour cells positive for Mucin 1 and suitable peripheral venous access for leukapheresis. A total of 10 patients participated in the trial and underwent three leukapheresis procedures. Patients were unstimulated and had leukapheresis on weeks 1, 5, and 9.

The MCS plus peripheral blood stem cell (PBSC) protocol was modified to reduce red blood cell contamination of the end product, and to maximise mononuclear and dendritic cell yield as determined by flow cytometric analysis. Extra plasma was collected for processing and culturing of cells.

The collection protocol allowed a maximum of 2.5 hours machine run time and resulted in 3.3 - 5.1 litres of whole blood being processed, with a product volume of 20 - 42 mL and 100 mL of plasma collected. The ACD-A ratio was 1:10. The WBC yield ranged between 1.30 - 7.0 x 10^9, with minimal red cell and platelet contamination. White blood cell yields were determined by FBE analysis using the "Cell Dyn 2000" analyser. Product results included WBC (leukapheresis) yields between 1.3 x 10^9 - 6.7 x 10^9, WBC yield after washing 1.1 x 10^9 – 5.0 x 10^9 and the viable cell yield after 6 days culture of 2.5 x 10^7 – 22 x 10^7, being the dendritic cell / vaccine preparation. There were no adverse events in patients following injection of these cells i.d. and s.c.

There were only minor complications at leukapheresis, consisting of one vasovagal episode, mild citrate toxicity, and one procedure was aborted due to failed cannulation.

This collection protocol gave reproducible and high yield dendritic cell harvests from single collections in unstimulated donors for adoptive immunotherapy.

B047

Granulocyte Transfusion Post Liver Transplant

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We report an unusual indication for granulocyte transfusion in a patient with probable flucloxacillin induced hepatitis complicated by aplastic anaemia, requiring urgent liver transplantation.

This previously well 40-year-old woman developed anaemia whilst awaiting liver transplantation and was found to have pure red cell aplasia on bone marrow biopsy. She subsequently developed severe pancytopenia associated with an aplastic bone marrow. It was thought that liver transplantation was contraindicated, as immunosuppression for aplastic anaemia would increase the risk of post liver transplant infection substantially, with a high risk of mortality. She was found to have a histocompatible sibling, who was being assessed for potential haemopoietic stem cell transplantation.

Post liver transplantation, granulocyte transfusions were collected and transfused from a G-CSF and dexamethasone stimulated unrelated donor. Granulocytes were collected on three subsequent days from a single donor who tolerated the collections well, without complication. The Haemonetics MCS plus was used for collection of cells with product volume ranging between 342 mls - 439 mls and neutrophil counts between 37.63 $x10^9$- 45.63 x 10^9 cells.

The patient was successfully transplanted without neutropenic sepsis. Following the restoration of liver function, the effects of immunosuppression (cyclosporin and prednisolone) and G-CSF, her blood counts improved and aplasia resolved after only three granulocyte transfusions.

The recipient's neutrophil count pre-granulocyte transfusion was 0.14 x 10^9/L, following the first granulocyte transfusion, the neutrophil count increased to 1.11 x 10^9/L. Similar increments were seen on the following days, but the neutrophils rose spontaneously to 24.6 x 10^9/L by day 4 post transplantation.

Although the neutrophil count recovered, as a result the effects of immunosuppression and restoration of liver function, the improved neutrophil count from the granulocyte transfusions may have served to reduce potential sepsis in the vulnerable post operative period.

B048 **P62**

Managing the Introduction of Automated Component Extraction at ARCBS-SA

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Introduction
Process change within the production environment of the Blood Service must be managed to ensure continued product quality. Where this change involves the introduction of new equipment, rigorous quality parameters must be met. The introduction of automated component extraction was a planned process in ARCBS-SA, however, only during the evaluation process were major issues identified which ultimately required creative solutions.

Method
An impact analysis was performed prior to the introduction of Terumo Automated Component Extractors (T-ACE's) into Processing, Inventory and Distribution (PID) in ARCBS-SA. A change control plan was developed to address these impacts in PID. Evaluation commenced and, because of the identification of major organizational issues, an Organizational Consultative Change Committee (OCCC) was assembled to manage these impacts.

The evaluation stage of the T-ACE implementation process suggested that a holding period of eighteen (18) hours prior to processing would optimise quality of blood components intended for the preparation of platelets. The decision to introduce this process required revision of working hours to optimise the collection, processing and testing of blood components

Results
Within PID the changes needed were as follows:

- Refit of the Processing area to adequately accommodate the introduction of six (6) T-ACE's. A plan for the area was designed in consultation with the Facilities Department and approved by TGA.
- Development of new Standard Operating Procedures (SOPs).
- Training and assessment of staff in the new SOPs.
- A communication plan was developed for our End Users for notification of new components, Buffy Coat Poor Red Blood Cells and 4 unit Platelet Pools suspended in T-Sol.
- Departments impacted upon by this process change were identified and a team formed to communicate proposed changes to procedures.

The OCCC comprised both management representatives and staff representatives from the areas most affected by the change. Following consultation staff were rostered over six (6) days between the hours of 8.00am Monday to 4.00 pm Saturday. While other staff were required to work their standard daily hours rostered for duty between 7.00 am and 11.00 pm Monday to Friday.

Conclusion
The introduction of new technology involves careful consideration of all stakeholders' needs. It is necessary to develop a detailed project plan with constant review as the evaluation progresses to identify further changes throughout the process that may have significant organizational impact.

B049

Protocol for the Acute Management of Chemotherapy-induced Neutropenic Sepsis

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Neutropenic sepsis is the most common life-threatening complication of chemotherapy. Mortality rates are increased if there are excessive delays in the administration of broad-spectrum antibiotics (BSA). Whilst guidelines offer a variety of antibiotic alternatives, they strongly advocate rapid initiation of the first dose.

In an ideal world, patients presenting to a hospital Emergency Department would all receive treatment from someone knowledgeable in the management of oncological emergencies. In the same ideal world, pathology and radiology results would be available immediately. As this is not always possible in real life, management protocols are developed for common clinical scenarios.

In this study we report the development and implementation of a new Neutropenic Sepsis management protocol, designed to improve clinical outcomes by providing a framework for rapid assessment and initiation of BSA to patients fulfilling inclusion criteria. Between August 1998 and June 2000, there were 65 entries from 56 patients.

The treatment protocol was limited to patients having received chemotherapy within the previous three weeks. Patients had to exhibit one major clinical criterion for neutropenic sepsis or two minor criteria. The minor criteria were chosen as non-specific symptoms not infrequently associated with sepsis. Patients were triaged to be seen within thirty minutes by a Medical Officer. If this was not possible, Emergency Department nursing staff were approved to establish intravenous access, perform routine investigations and administer the first dose of antibiotics.

Adherence to the protocol, time to first dose of BSA and clinical outcomes were assessed. Ninety eight percent (98%) of patients fulfilled protocol entry criteria. All appropriate investigations were performed on 100% of patients, 82% of whom received a first dose of BSA within 60 minutes (median - 30 minutes). Of protocol entries, 71% had an Absolute Neutrophil Count (ANC) less than 1.0 x 10^9/L and continued BSA. In this group (n=46): median days until afebrile were 3; median days until ANC recovery >1 x10^9/L were 3; and median days until discharge were 5 days. There were 2 deaths (3.0% of protocol entries) and only one reversible antibiotic reaction.

The positive outcomes of this study were a high adherence to the protocol, early BSA administration, minimal BSA toxicity, and acceptable patient outcomes. The protocol will continue at our institution and may be relevant to other hospitals.

B050

***In Vitro* Transdifferentiation of Human Bone Marrow Stem Cells into Dopaminergic Neuronal Cells**

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Bone marrow stem cells (BMSC) have been well known as the restrict precursors of circulating blood cells. However, this long held concept has been challenged by a series of exciting observations mainly in animal models. A subpopulation of BMSC has been shown to generate a variety of non-heamatopoietic tissues, such as bone, muscle and neural cells. Neuronal degenerative disorders, such as stroke and Parkinson's disease are characterized by a loss of dopaminergic neurons. Conventional therapies have been ineffective. Transplantation based cellular therapy has been investigated with the aim to restore functional neurons. The aim of this study is to investigate the transdifferentiation of human BMSC into functional neural cells. BM samples from individuals were collected after consent. $CD34^-/14^-/45^-$/Glycophorin-A^- BM cells were isolated by gradient centrifugation followed by plastic adherence or immunomagnetic beads. The cells were grown in DMEM based culture medium supplemented with or without fetal calf serum. After several passages, cells were induced for neuronal differentiation using dimethylsulfoxide and butylated hydroxyanisole or cytokines. Following the treatment, fibroblast like

BM cells morphologically changed to neural like cells, having refractile cell bodies and extended long processes. Immunofluorescence study of these differentiated cells showed a declined expression for BM stromal markers (CD44, CD29) and exhibited a neuronal phenotype, expressing neuron-specific enolase (NSE), NeuN, neurofilament-M (NF-M), MAP2, β-tubulin III and tau. Furthermore, these neural marker positive cells also stained positive for neurotransmitters (tyrosine hydroxylase and γ-aminobutyric acid). The neuronal differentiation was confirmed by Western blotting study (NSE^+) and RT-PCR technique ($Nestin^+$, $NF\text{-}M^+$, $MAP2^+$). Work is in progress to elucidate and optimise this transdifferentiation process.

B051 **P26**

Non Hodgkins Lymphoma, Hospital-based Cancer Registry

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The South Australian State Cancer Registry has collected information on non-Hodgkin's lymphoma, and has reported time trends in incidence and mortality, since 1977. Meanwhile, hospital-based cancer registries in South Australian teaching hospitals have maintained more detailed clinical data collections that have included items not covered by the State registry, such as stage, grade, other prognostic indicators, treatments, and treatment outcomes.

The Queen Elizabeth Hospital has a longstanding hospital-based registry. The data have been used to show patterns of care and outcomes of care according to relevant clinical characteristics for non-Hodgkin's lymphoma and other cancers. This is regarded by the Hospital as an important contributor to quality assurance activity. Detailed evaluation can be made with Hospital registry data of time trends in treatment outcomes by chemotherapy protocol and other treatment modalities.

In this paper, trends in clinical management and outcomes of non-Hodgkin's lymphoma are presented to illustrate the utility of a hospital-based cancer registry. Particular attention is given in the paper to outcomes among older patients.

B057

The Structural and Functional Characteristics of Gpibα in a Bernard Soulier Syndrome Patient

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Bernard Soulier Syndrome (BSS) is a rare, autosomal recessive bleeding disorder characterized by thrombocytopenia, prolonged bleeding time and circulating giant platelets. Underlying this disorder is a defect in the platelet glycoprotein (GP) Ib-IX complex. Molecular characterization of individuals with BSS has identified mutations in the genes of the various components of the GPIb-IX complex, GPIbα, GPIbβ and IX, responsible for the expressed phenotype.

We describe here the genetic basis of the disorder in a BSS patient. Direct sequencing of the GPIbα gene revealed that the patient was homozygous for the presence of a novel mutation (C→T) resulting in a L^{99}→P substitution. Furthermore, the patient was homozygous for the HPA-2b dimorphism (T^{145}/M). The GPIbβ and IX genes were normal.

Flow cytometry analysis of the patient's platelets demonstrated that surface expression of GPIbα could be detected only with the monoclonal antibodies WM23 and not Ak2. Platelet aggregometry experiments indicated that the BSS platelets were aggregating in the presence of ADP and collagen but no ristocetin-induced aggregation could be detected. Furthermore, the GPIbα expressed on the surface of the patient's platelets was approximately 40 kDa smaller than that on the normal platelets when detected by Western blotting. The combination of the above results suggests that the BSS GPIbα lacks the N-terminal domain containing the Ak2 and von Wille Brand factor (vWF) binding sites. This lack of GPIbα N-terminus in the BSS patient could be the result of a higher degree of susceptibility to the action of platelet derived proteolytic enzymes.

Constructs containing the dimorphism and the BSS mutation, in combination or alone, were expressed on the surface of CHO cells already expressing GPIbβ and GPIX. Utilization of these cells in vWF binding assays demonstrated that while the L^{99}→P substitution alone significantly reduced ristocetin induced vWF binding, when combined with the HPA-2b dimorphism, it resulted in the reduction of vWF binding to almost negligible levels.

In conclusion, we have characterized the contribution of the genetic changes in this BSS patient to the clinical picture of the disease. To our knowledge, this is the first BSS case in which mutations have not affected the expression of the GPIb-IX complex but its structure/function.

B063 **P14**

Rapidly Fatal Acute Myeloid Leukaemia after Treatment for Primary Mediastinal Malignant Teratoma

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Aim
The association of haematological disease and germ cell tumours has been recognised since 1985. However there are only about twenty reported cases world wide of acute myeloid leukaemia (AML) occurring either simultaneously or following primary mediastinal germ cell tumour. Patients are generally young males with endodermal sinus tumour or malignant teratoma of the mediastinum and present with pancytopaenia and organomegaly. The prognosis is poor with either death before treatment, chemotherapy resistant disease or short-lived remissions.

Methods
We describe a case of rapidly fatal AML occurring six months after initial diagnosis of primary mediastinal malignant teratoma treated with two cycles of bleomycin, etoposide and cisplatin (BEP) chemotherapy and surgical resection.

Results
A 23-year-old male presented in August 1999 with a two-week history of cough, fever, weight loss and pleuritic chest pain. Imaging revealed a large mediastinal mass and no other site of disease. αfetoprotein was markedly elevated 2480 μg/L and mediastinal biopsy demonstrated malignant teratoma. The patient received two cycles of BEP chemotherapy which were poorly tolerated and complicated by pneumonia. Due to poor tumour response he proceeded to surgical resection following which αfetoprotein was negative. High dose chemotherapy with peripheral blood stem cell transplantation was planned but the patient refused further treatment. Five weeks later he represented with fever, sweats, arthralgias and hepatosplenomegaly. A peripheral blood film demonstrated anaemia, thrombocytopaenia and numerous basophilic blasts with prominent cytoplasmic vacuolation and a CD45, CD33, CD34 and CD11c positive immunophenotype. Cytogenetics revealed a complex hyperdiploid karyotype including tetrasomy 8, trisomies 9, 12, 15 and 19, monosomy 21 and 5q deletion. Despite therapy for spontaneous tumour lysis syndrome the patient deteriorated rapidly and died within hours of presentation.

Conclusions
There are three possible mechanisms for the association of mediastinal GCT and AML. Given the low incidence of each disease chance occurrence is extremely unlikely. Therapy related leukaemia is also unlikely since it occurs 5-7 years after alkylating agents and the median time to diagnosis of haematologic neoplasia following GCT, in the largest case series, is six months. However secondary AML is associated with chromosome 5 deletions, which our patient demonstrated. Overall the most likely explanation is that the association occurs as a consequence of multipotential differentiation of malignant germ cells.

B064 **P29**

Isolated Splenic Diffuse Large B Cell Lymphoma Presenting as Refractory Autoimmune Haemolytic Anaemia

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Aim
Less than 1% of non-hodgkin's lymphomas (NHL) are isolated to the spleen. The presenting features are left upper quadrant pain, weight loss, fevers, night sweats and splenomegaly. Autoimmune haemolytic anaemia (AIHA) is due to auto-antibody mediated peripheral red cell destruction. It may be primary or secondary to a variety of diseases including NHL. There are only a small number of reported cases of AIHA in association with primary splenic lymphoma. In each of these cases there were additional presenting features of the underlying lymphoma.

Methods
We present the first reported case of AIHA refractory to medical treatment, in which occult diffuse large B cell lymphoma isolated to the spleen was identified incidentally following splenectomy.

Results
A 65-year-old lady presented with severe dyspnoea and lethargy. Her blood film demonstrated anaemia with haemoglobin 81 g/L, numerous spherocytes, nucleated red blood cells and marked polychromasia. The reticulocyte count was 326 x 10E9/L, bilirubin 56 U/L and lactate dehydrogenase 3920 U/L. Direct antiglobulin test was strongly positive for C3d only. In view of the presence of large numbers of circulating erythroblasts bone marrow was examined confirming AIHA and excluding an erythroblastic leukaemia. Physical examination and CT scanning failed to demonstrate any lymphadenopathy or organomegaly to suggest an underlying lymphoproliferative disorder. Treatment was commenced with prednisolone 1mg/Kg/day but failed to

produce any response after two weeks. Two doses of vincristine 2mg were given, again with no response and the patient proceeded to splenectomy. On histological examination of the spleen there was evidence of diffuse large B cell lymphoma confined to the splenic tissue. The patient was subsequently treated with six cycles of CHOP chemotherapy. The haemolysis improved after splenectomy and resolved after two cycles of chemotherapy. The patient is now six months post chemotherapy with no evidence of recurrent lymphoma or haemolysis.

Conclusions
This patient's AIHA was refractory to treatment until her occult splenic lymphoma was removed. The case demonstrates the importance of considering an underlying lymphoproliferative disorder in patients with AIHA, even in the absence of macroscopic disease. The prognosis is improved for both NHL and AIHA by early diagnosis and management.

B065 **P30**

Lymphomatoid Granulomatosis Presenting as Progressive Neurological Dysfunction

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Aim
Lymphomatoid granulomatosis is a rare extranodal lymphoproliferative disease, which is angiocentric and angiodestructive. Lesions comprise Epstein Barr virus positive B cells admixed with reactive T cells. The lung is the most common site of disease however other organs including brain, kidney, liver and skin may also be involved. There are a few reported cases of disease confined to the central nervous system and occasionally CNS disease may precede pulmonary involvement by years. Treatment is controversial and the prognosis is generally poor with a mortality of 65-90%.

Methods
We present a case of lymphomatoid granulomatosis presenting as progressive neurological dysfunction unresponsive to steroids. Asymptomatic pulmonary involvement occurred several months later leading to a tissue diagnosis.

Results
A 56-year-old man presented with progressive cranial nerve dysfunction and ataxia over a two-month period. At the time of presentation he had gait ataxia, a right facial lower motor neuron lesion, impaired corneal reflex, sensory disturbance in the upper two branches of the trigeminal nerve and a depressed gag reflex on the right. CT scan of the head was normal but MRI scan showed abnormality in the right pons and cerebellar peduncle on T2 flair images. Examination of the CSF was normal. High dose methylprednisolone was given for presumptive demyelination, without any clinical response. Subsequently a lesion appeared on a routine chest xray and the patient underwent a percutaneous biopsy. Biopsy was consistent with lymphomatoid granulomatosis. Treatment with CHOP chemotherapy and high dose methotrexate was commenced and there was no further progression of disease. Repeat MRI scanning after three cycles of chemotherapy demonstrated radiological improvement. After five cycles of chemotherapy the patient developed presumptive pneumocystis carinii pneumonia which settled with cotrimoxazole therapy. No further chemotherapy was given. Three months after ceasing treatment the patient is radiologically and clinically stable but with persistent neurological dysfunction.

Conclusions
This case demonstrates the clinical heterogeneity of lymphomatoid granulomatosis and its unpredictable response to therapy. The diagnosis of lymphomatoid granulomatosis may be elusive, particularly in the absence of pulmonary disease. However it should be considered in all cases of neurological abnormality, which have failed to respond to standard therapy, especially if radiological abnormalities are present on the chest xray.

B067

Role of Graft-Facilitatory Cells in Human Engraftment in the NOD-SCID Mouse Model

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Aim
We used the NOD-SCID mouse model to study factors that effect engraftment of human cord blood (CB) stem cells. Previous work showed that non-engrafting $CD34^-$ filler cells must be co-transplanted with unexpanded CB $CD34^+$ cells to detect human cells in the NOD-SCID mouse.

Methodology

MACS enriched CB $CD34^+$ cells were incubated for 7 days with 20 ng/ml SCF, 20 ng/ml FLT3 Ligand & 20 ng/ml MGDF in serum free media. NOD-SCID mice were transplanted after 250-cGy irradiation by IV injection with CB $CD34^+$ cells, with or without irradiated $CD34^-$ filler cells. Mice were sacrificed 6 weeks post transplant; bone marrow (BM) and spleen were analysed by flow cytometry for human engraftment.

Results

The level of human engraftment is significantly reduced when more than 10^7 filler cells are co-transplanted with unexpanded $CD34^+$ cells (below).

Input cells transplanted	Number of irradiated filler cells co-transplanted		
	5-10 x 10^6	10-20 x 10^6	>20 x 10^6
	Percentage Human $CD45^+$ cells		
10^5 Unexpanded $CD34^+$	19.9±5.7 (n=16)	7.5±2.5 (n=54)	Not done
10^5 Expanded $CD34^+$	14.9±2.8 (n=83)	7.3±1.1 (n=161)	3.3±1.1 (n=123)

We then transplanted mice with 10^7 irradiated filler cells and increasing numbers of unmanipulated or expanded CB $CD34^+$ cells. Again this failed increase engraftment in mice transplanted with unexpanded CB $CD34^+$ input cells, however human engraftment increased when mice were transplanted with increasing numbers of expanded CB $CD34^+$ input cells.

We then tested the effect of increasing numbers of irradiated filler cells on the levels of human engraftment in mice transplanted with unexpanded and expanded $CD34^+$ cells and found that expanded cells don't require filler cells for engraftment. We investigated the phenotypic makeup of irradiated filler cells and expanded $CD34^+$ cells and could not define a cell type that could potentially explain the graft facilitatory effect. We performed experiments to determine if the graft facilitatory effect could be due to cytokine release comparing engraftment levels in mice transplanted with unexpanded $CD34^+$ cells±filler cells immediately after irradiation or 48 hours later. Filler cells facilitate engraftment when mice are transplanted soon after irradiation but are not required when transplantation is delayed for 48 hrs, suggesting that engraftment of unexpanded $CD34^+$ cells is facilitated by cytokines.

Conclusions

Unexpanded and expanded CB $CD34^+$ cells have different requirements for engraftment in the NOD-SCID mouse. Possibly, expanded cells generate cells or factors that facilitate engraftment. Definition of the underlying mechanism for this phenomenon may lead to strategies to facilitate engraftment for transplant recipients.

B068 **P63**

Effect of Preparation and Storage Conditions on Activation Potential of Platelets

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Platelets collected by apheresis typically display a different basal activation status to those prepared from pooled buffy coats. This may be due to the collection process, method of production, or storage medium. This study aimed to determine whether the basal activation status affected the potential for activation and aggregation under the influence of different agonists in vitro.

To determine whether the basal activation status of apheresis and buffy coat platelets was in fact different, eight samples of each were assessed for expression of CD62P, an activation dependent platelet marker, by flow cytometry. The expression of CD62P was found to be significantly higher in buffy coat platelet concentrates than in platelets prepared by apheresis. To assess whether the difference in the basal activation status led to a difference in the ability of platelets to respond to agonists, buffy coat and apheresis platelets were activated with ADP and assessed by flow cytometry for CD62P expression. Apheresis platelets displayed a four-fold increase in the percentage of platelets expressing CD62P following ADP activation compared to the basal level. Buffy coat platelets displayed only a two-fold increase in the percentage of CD62P expression.

To assess whether the decreased responsiveness of buffy coat platelets to agonists was due to the storage medium, buffy coat platelets were resuspended in plasma and incubated at 37°C. The basal and ADP stimulated activation status was then measured. Following resuspension in plasma, buffy coat platelets displayed a decreased expression of CD62P under basal conditions. The level of expression of CD62P following stimulation with ADP was decreased compared to the response of buffy coat platelets in TSol. This demonstrated that despite a lower basal activation level being observed there was no increase in the potential for buffy coat platelets to express CD62p in response to ADP in vitro.

Apheresis and buffy coat platelets were also tested functionally by aggregometry to measure the ability of platelets to change shape in the presence of ADP. Apheresis platelets displayed a significantly higher response than buffy coat platelets on day one of storage. The response decreased over the storage period with both buffy coat and apheresis platelets displaying an

equal response on day five of storage. When the buffy coat platelets were restored to plasma the aggregation response was equivalent to that of apheresis platelets suggesting that platelet function is restored on return to physiological conditions.

B069

Defects in SDF-1-Mediated Signalling in Pre-B Acute Lymphoblastic Leukemia

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Stromal derived factor-1 (SDF-1) is a member of the CXC chemokine family. Unlike other chemokines, SDF-1 is constitutively expressed at high levels by a number of tissues including bone marrow stromal cells, lymph nodes, spleen and liver. It binds a single receptor, CXCR4, which is a seven transmembrane, G protein coupled receptor expressed primarily on hemopoietic cells. SDF-1 and CXCR4 are essential for the development of lymphopoiesis, which is completely absent in knockout mice. The homing of normal hemopoietic progenitors and acute lymphocytic leukemia (ALL) cells to the bone marrow is dependent on CXCR4 expression by these cells and SDF-1 production by bone marrow stromal cells. In addition SDF-1 is involved in stroma dependent survival of ALL cells in vitro. We have examined the response of 15 cases of pre-B ALL to SDF-1 and compared it to that of normal B cell progenitors at comparable stages of maturation. Three cases (20%) failed to demonstrate an increase in adhesion to bone marrow stromal layers following SDF-1 stimulation. All three cases expressed normal beta 1 integrin and CXCR4 levels. In one case beta 1 integrins could not be activated by other means including stimulating antibody and PMA stimulation, suggesting that the failure to respond may be due to defective integrins. In the other two cases integrin function was normal, suggesting a defect in CXCR4 itself or in the signalling pathway. SDF-1 was able to induce calcium fluxes in both these cases suggesting that CXCR4 was normal. Despite this these cases did not demonstrate a chemotactic response to SDF-1. These data suggest that abnormal responses to SDF-1 occur in a significant percentage of pre-B ALL cases. Considering the pivotal role SDF-1 and CXCR4 play in pre-B cell biology, defective CXCR4 signalling is likely to have implications for the progression of this disease.

B070

CD44v6 Expression Results in Increased Aggressiveness of Acute Lymphoblastic Leukemia in NOD/SCID Mice

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CD44 is a transmembrane glycoprotein expressed on all hemopoietic cells. It can be expressed in a diverse range of isoforms, many of which arise from alternate mRNA splicing. Isoforms containing variant exon 6 have been implicated in disease progression in a number of solid tumors including osteosarcoma, diffuse type gastric and pancreatic cancer and with metastasis formation in non-small cell lung cancer and gastric cancer. In the hemopoietic system it has been associated with poor prognosis in non-Hodgkin's lymphoma and myeloma and acute myeloid leukemia. We have examined CD44 variant expression by RT-PCR and southern blotting in 12 cases of acute lymphoblastic leukemia (ALL). We observed the expression of variant exons 3, 6 and 10 in the majority of cases. Other variant exons were not detected. High levels of CD44v6 expression correlated with a shorter complete remission in these patients. Using a NOD/SCID mouse model CD44v6 and CD44v3 expression were inversely correlated with the time to engraftment of the patient samples in the mice. We transduced the CD44 negative pre-B ALL cell line, NALM6, with CD44 lacking any variant exons (CD44H) and CD44 containing variant exon 6 (CD44v6). Mice receiving NALM6 cells expressing CD44v6 (NALM6.CD44v6) demonstrated greater infiltration of the bone marrow (54±15%) compared to that observed in mice receiving NALM6 alone (36±16%) or NALM6 transfected with CD44H (NALM6.CD44H, 21±7%). Greater leukemic cell infiltration of the liver and spleen was also observed in mice receiving NALM6.CD44v6 cells. Expression of CD44 or CD44v6 did not result in increased *in vitro* proliferation compared to the parental NALM6 cells. Nor did it alter the adhesion of these cells to bone marrow stromal components including hyaluronic acid, fibronectin, collagen, bone marrow fibroblasts, Dexter stroma or the osteoblastic cell lines U2OS or SAOS-2. However NALM6 cells expressing CD44v6 were less likely to localise to the endosteal region of the bone marrow in non-irradiated NOD/SCID mice (41.5±1.5% for NALM6.CD44v6 vs 66.0±5.6 for NALM6.CD44H $p<0.01$). Reduced endosteal localisation is consistent with the localisation of more mature rapidly dividing hemopoietic progenitors. We hypothesise that CD44v6 permits adhesion to ligands not bound by CD44H which results in altered localisation of these cells in the bone marrow. This altered localisation in turn results in increased proliferation and/or survival of these cells in the bone marrow and facilitates spread to secondary organs. CD44v6 expression may be useful as a prognostic indicator in acute lymphoblastic leukemia.

B072

RANK Ligand (RANKL) is Expressed by Human Myeloma Cells: Direct Support of Osteoclast Formation and Activation by Human Myeloma Cells

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Multiple myeloma (MM) is an incurable B cell malignancy able to mediate massive destruction of the axial skeleton. It is characterised by the presence of a monoclonal population of end-stage myeloma plasma cells (MPC), which localise to sites in the bone marrow in close proximity to the bone trabecular. The precise mechanisms responsible for the observed bone pathology remain unclear; nevertheless, it is generally accepted that it is due to MM cell-mediated disruption of the normal equilibrium between bone formation by osteoblasts (OB) and bone resorption by the multinucleated osteoclasts (OC). The aim of this study was to examine the involvement of the newly defined TNF-ligand family member, RANKL and its naturally occurring antagonist, osteoprotegerin (OPG) in MM biology. We found that most human MM cell lines tested expressed RANKL at both the mRNA and protein level. RANKL expression was also detected by flow cytometry in MM patient-derived BM mononuclear cells, including purified $CD38^{+++}CD45^{+}$ and $CD38^{+++}CD45^{-}$ MPC subpopulations, using two independent anti-RANKL antibodies. Co-culture of fluorescence-sorted patient-derived $CD38^{+++}CD45^{+}$ and $CD38^{+++}CD45^{-}$ MPC with adherent human peripheral blood mononuclear cells, resulted in the formation of multinucleate, tartrate-resistant acid phosphatase (TRAP)-positive OC-like cells capable of forming resorption lacunae on slices of dentine. Our data suggest that RANKL expression by MPC confers on them the ability to participate directly in the formation of OC. Moreover, high expression of membrane-associated RANKL by $CD38^{+++}$ cells is associated with the presence of radiological lesions in multiple bones in individuals with MM, suggesting that myeloma-expressed RANKL has a direct role in the formation of focal osteolytic lesions characteristic of this disease.

B073

Zoledronate is Anabolic for Human Osteoblast-Like Cells

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Bisphosphonates (BP) exhibit high affinity for the hydroxyapatite mineral in bone and are selectively taken up and adsorbed to mineral surfaces at sites of increased bone turnover. BPs are potent inhibitors of osteoclast-mediated bone resorption and are used extensively for the treatment of skeletal diseases. BPs inhibit bone resorption by acting directly on osteoclasts (OC) and indirectly *via* effects on osteoblasts (OB). The newer nitrogen-containing BPs have been shown to inhibit the mevalonate pathway, with wide ranging cellular effects that include induction of apoptosis in osteoclasts and inhibition of osteoclast formation by osteoblasts. Zoledronate (ZOL), represents a new generation, nitrogen-containing BP and is the most potent inhibitor of bone resorption currently in use. In this study, we used an established model of OB-differentiation to investigate the effect of ZOL on the molecular expression and cellular phenotype of cultured human osteoblast-like cells. Human OB-like cells, derived from explants of posterior iliac crest and proximal femoral trabecular bone, were cultured in the presence of ZOL at concentrations ranging from 0.05 to 50μM. At concentrations up to 5 μM, ZOL was strongly cytostatic, blocking cell replication in S and G2/M phase. At higher concentrations, ZOL dose-dependently induced cell death in each of the human OB cultures tested. Using dual colour FACS and CFSE staining, it was shown that cells expressing high levels of the stromal stem cell marker, STRO-1, were more susceptible to ZOL-induced cytostasis and cell death, consistent with the greater proliferative potential of this population. Associated with this loss of $STRO\text{-}1^{+}$ cells was a concomitant increase in the proportion of cells, which exhibited a more differentiated phenotype. Molecular analysis revealed that the expression of the bone-associated matrix proteins bone sialoprotein and osteocalcin were significantly elevated in cultures treated with ZOL. Consistent with these observations, *in vitro* mineralisation studies showed that ZOL enhanced mineral formation in cultures from all donors, at concentrations in the range of 5–25 μM. Our studies therefore show that in addition to its effects on OC, ZOL also has direct effects on the proliferation and survival of OB-like cells *in vitro*. Our observations support the notion that ZOL is anabolic in bone by increasing the proportion of differentiated OB and enhancing the bone-forming activities of these cells.

B074 **P32**

Perivascular Localization of Postnatal Mesenchymal Stem Cells in Bone Marrow and Dental Pulp

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We have previously identified adult bone marrow and dental pulp stem cells by their capacity to regenerate bone and its haematopoietic supportive stroma, and dentin-pulp-like structures in vivo, respectively. The maintenance and regulation of these stem cells is tightly controlled by the local micronenvironment, however, little is known about the anatomical niches that these cells occupy. To identify mesenchymal stem cell niches, we isolated bone marrow stromal stem cells (BMSSCs) and dental pulp stem cells (DPSCs) by immunoselection using the antibody, STRO-1 that recognises an antigen on stromal elements and perivascular cells in bone marrow and dental pulp tissue. Freshly isolated STRO-1 positive BMSSCs and DPSCs were tested for expression of the vascular antigens known to be expressed by endothelial cells (Factor VIII-related antigen, E-selectin) smooth muscle cells and pericytes (alpha-smooth muscle actin, MUC-18 (CD146) and pericyte-associated antigen, 3G5) by immunohistochemistry, FACS and/or immunomagnetic bead selection. Both BMSSCs and DPSCs lacked expression of Factor VIII-related antigen and E-Selectin but were found to be positive for alpha-smooth muscle actin and MUC-18, while only DPSCs expressed 3G5. The finding that BMSSCs and DPSCs reside in perivascular niche, regardless of their diverse ontogeny and developmental potentials, may have further implications in understanding the factors that regulate the formation of mineralized matrices and other associated connective tissues. Furthermore, the data implicates pericytes of other tissues as the source of stem cells that are induced to form mineralised tissue either by addition of exogenous factors or through disease processes.

B075

The von Willebrand Factor Reducing Activity of Thrombospondin-1 is Located in the Calcium-binding/C-globular Domain and Requires a Free Thiol at Position 974

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Plasma von Willebrand factor (vWF) is a multimeric protein that mediates adhesion of platelets to sites of vascular injury, however only the very large vWF multimers are effective in promoting platelet adhesion in flowing blood. Both thrombotic and bleeding disorders are characterized by altered vWF multimer size. The multimeric size of vWF can be controlled by the glycoprotein, thrombospondin-1 (TSP1), which facilitates reduction of the disulfide bonds that hold vWF multimers together. TSP1 and TSP2 are members of a family of structurally related extracellular glycoproteins and are composed of three identical disulfide-linked 150-kDa monomers. Overlapping fragments of TSP1 and TSP2 incorporating combinations of domains that span the entire molecules were produced in insect cells and examined for vWF reductase activity. vWF reductase activity was present in the Ca^{2+}-binding repeats and C-globular domain of TSP1, but not of TSP2. Alkylation of Cys 974 in the C-terminal construct ablated vWF reductase activity. These results imply that the reductase function of TSP1 centers on Cys 974 in the C-globular domain. The localization of its functional domain impacts on the development of TSP1 as an antithrombotic drug.

B076 **P39**

Viral Transduction of Human Myeloid Leukemia Cells – Potential Application in Adoptive Immunotherapy

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Leukaemic cells rarely induce a significant T-cell response in vitro despite sufficient levels of HLA class I and II expression. This may be due to the absence of costimulatory molecules such as CD80 which are important for the generation of a primary immune response. Recent studies suggest that increasing the CD80 expression on tumour cells increases their ability to stimulate T-cells. Upregulating CD80 expression on leukaemic cells may lead to successful generation of leukemia-specific cytotoxic T-cells. This approach has potential for use in adoptive immunotherapy after allogeneic stem cell transplantation.

Only 5/23 AML samples studied showed detectable levels of CD80-positive leukaemic cells by flow cytometry (<5.3%). Cytokine treatment induced CD80 expression in 8/13 AML (0.16-31.6%, mean 6.4%, n=13). Retroviral transfection system derived from Moloney murine leukemia virus was not consistently effective in transducing human myeloid leukaemia cells with CD80 (0.89-29.0%, mean 8.3%, n=15), possibly due to lack of blast cell proliferation. We therefore assessed a third generation lentiviral vector system and adenovirus encoding enhanced green fluorescent protein (GFP) for their capacity to transduce myeloid leukaemia. Following infection (14 AML, 2 CML) 2.5-68% (mean 37.9%) of cells expressed GFP. The expression was sustained for more than a week, however maximum expression levels were not obtained until day 3. We used a chimeric Ad5f35 vector, derived from adenovirus type 5 genome that has been modified with a shorter-shafted fiber protein from Ad35, which has been shown to transduce committed hematopoietic cells and primitive progenitor subsets effectively. Following infection (23 AML, 3 CML) 10.8-92.0% (mean 59.0%) of cells expressed GFP. The maximum infection levels were obtained 24h after infection and a significant

proportion of cells still showed expression of GFP 7 days later. We are currently assessing the ability of Ad5f35 vector to deliver other genes to myeloid leukaemic cells, such as human IL-2, IL-12, CD40L, and CD80.

Transduction with Ad5f35-CD80 has potential to improve the antigen presenting function of leukaemic cells and facilitate the generation of leukemia reactive T-cells in autologous and allogeneic settings.

B079

Detection of Change in Gene Expression Profiles by cDNA Microarray in Patients with Ph+ Leukaemias Treated with STI571

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The cDNA Microarray technology can be used to analyse the differential expression of thousands of genes between 2 samples (ie: test and control) simultaneously. This technique is particularly useful for identifying unique genes associated with a particular disease state and investigation of genes affected by drug treatment. The aim of our study is to optimise the cDNA microarray protocol and to investigate gene expression profiles of Ph+ leukaemias treated with STI571. We have established an optimised microarray and statistical data analysis protocol to analyse up to 19000 genes simultaneously. In this preliminary report, we show the use of this method to study the effects of drug treatment on the gene expression profile of a 53-year-old patient with Ph+, c-ALLA+ and CD19+ acute lymphoblastic leukaemia. Blood samples were taken before treatment (blast count $60X10^9$/L), and on 3 consecutive days after STI571 treatment was initiated. The drop in PB blast cell count was 51, 20 and 9 $X10^9$/L respectively. Blast cells were analysed using 19K cDNA microarrays by comparing pre-treatment sample with the 3 treated samples. Microarray data generated from these experiments was statistically analysed using GeneSpring. Using data filtering parameters (standard error, p-value and signal quality factors) we limited our data set of 19K to a group of 4000 'good quality signal genes'. To focus our analysis, we selected 424 genes of differential expression that were at a minimum of 2 fold up- or down-regulated in at least 1 of the 3 treatment samples. Within this grouping, we noted that several tyrosine kinases (including the v-abl gene) were down-regulated during treatment confirming that this assay was capable of detecting the effects of STI571 treatment. We also identified a down-regulation of several cell signalling genes including Ras, Protein Kinase C and Phosphoinositide-3-kinase, which participate in pathways of cell proliferation. Of interest, we detected an unexpected increased expression of several genes including lymphotoxin beta receptor, oncogenic T-cell leukaemia 1A, and coagulation factor II. Analysis is ongoing and we plan to focus on selected genes and conduct molecular and phenotypic experiments to aid our understanding of drug treatment effects. We also plan to conduct microarray analyses on other Ph+ leukaemic patients undertaking STI571 treatment.

B081 **P33**

Serpin Expression in Human Haemopoietic and Peripheral Blood Cells

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Cells of haemopoietic origin produce a variety of potent proteases which are involved in phagocytosis, inflammatory response and apoptosis. Recent evidence has implicated proteases in the mobilisation of haemopoietic progenitors and mutations in the elastase gene have been shown to cause congenital neutropenia. Although the proteases of haemopoietic cells have been well described the regulation of their activity in the bone marrow environment is poorly understood. Several members of the intracellular serpin family are known to be produced by haemopoietic cells but there is no comprehensive description of their relationship to lineage or level of differentiation. We have therefore developed a semi-quantitative PCR approach to measuring the expression of a panel of intracellular serpins in haemopoietic progenitors, cells committed to different lineages and mature peripheral blood cells.

Our results show that the most abundant protease inhibitor in all compartments is the monocyte/neutrophil elastase inhibitor. The plasma serpin, α1-antitrypsin is also prominently expressed in most cells. Expression of most of the other serpins appears to be lineage or differentiation stage specific. These data point to the importance of elastase regulation in haemopoiesis. They will also serve as basis for further exploration of the role of serpin/protease balance in developing blood cells.

B083

Platelet Cryopreservation and Residual Platelets after Blood Cell Harvesting

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Blood cell harvests for subsequent autologous use in patients undergoing high dose chemotherapy are commonly contaminated with high numbers of platelets (mean $1.77 \pm 1.14 \times 10^{11}$ platelets). We have developed and applied technologies where highly variable products can be routinely processed in an automated and closed manner using an automated blood component machine (Optipress II, Baxter Healthcare).

These collections also produced a platelet rich fraction which was discarded, representing a mean platelet depletion of 52% of the starting product (n=79). Thus, given that we collect a mean of $1.01 \pm 0.84 \times 10^{11}$ platelets as a by-product of blood stem cell collection, these represent an opportunity to provide patients with autologous platelets. We investigated a modified platelet cryopreservation protocol, and report platelet increments in a patient otherwise refractory to allogeneic platelets.

Briefly this approach is based upon transferring a platelet collection to a dry triple pack using a sterile welding machine (Terumo), adjusting the ACD-A concentration by adding 4.5 ml per 100ml, and producing a 10 ml platelet concentrate by centrifuging at 1250*g* for 15 minutes. This volume was taken to 45ml with further autologous plasma. A further 45 ml of autologous plasma was slowly added containing 10% dimethyl sulfoxide prior to rate-control freezing and storing in vapour phase liquid nitrogen. This resulted in 90 ml platelet products containing 5% DMSO which were designed for rapid infusion without washing by bedside thawing.

Remarkably, when these products have been thawed we achieved a mean recovery of 85% (±9.7, n=7) with an average platelet dose of 1.01×10^{11}. Furthermore when infused these products were able to result in an increment in a patient who was refractory to HLA matched platelet products (Table 1). These results suggest that this cryopreservation protocol would be suitable for application to otherwise discarded platelets collected as a contaminant in blood stem cell collections.

Platelet dose infused $x10^{11}$	Peripheral platelet count $x10^9$/l prior to infusion	Peripheral platelet count $x10^9$/l after infusion
0.47	10	13
0.96	9	16
0.56	9	15
0.93	4	26
2.59	6	24
2.11	6	17
1.51	11	25

Table 1. Platelet increments after thawing and infusion. Platelet dose represents the number of platelets infused after thawing.

B085

Salvage Therapy with DT-PACE for Multiple Myeloma Patients Prior to Mini-Allogeneic Transplantation

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Aim
DT-PACE (dexamethasone, thalidomide, cisplatin, adriamycin, cyclophosphamide and etoposide) has previously been reported to be beneficial in patients with relapsed/refractory multiple myeloma. We now report on the therapeutic benefit of DT-PACE as a useful salvage regimen prior to mini-allogeneic transplantation (MAT).

Methods
Five patients with advanced multiple myeloma were salvaged with 2 courses of DT-PACE prior to undergoing MAT. All patients had previously received >= 6 courses of combination chemotherapy (including PCAB, PCM and VAD), three patients had previously undertaken a single autologous transplant, 1 patient had undertaken 2 autologous transplants, and the 5th patient had previously undertaken an allogeneic transplant 9 years prior. DT-PACE consists of dexamethasone (40 mg/d x 4) and thalidomide (400 mg/d) with a 4-day, continuous infusion of cisplatin (10 mg/m^2/d), cyclophosphamide (400 mg/m^2/d), doxorubicin (10 mg/m^2/d), and etoposide (40 mg/m^2/ d). There was a 4-week interval between courses. Conditioning comprised of fludarabine 30mg/m^2 on days –7 to –3 and melphalan 140mg/m^2 on day –2. GVHD prophylaxis consisted of cyclosporin and ganciclovir was given for CMV prophylaxis.

Results
After two courses of DT-PACE, the serum paraprotein decreased from pretreatment levels of 2.0, 5.8, 9.1 and 9.6g/L to undetectable levels in 4 patients (patient 5 had light chain disease). Pretreatment plasma cell concentrations in bone marrow of 90%, 80%, 90%, 20% and 95% decreased to <5%, 3%, 15%, 2% and 45% respectively, after DT-PACE and to <5% in all patients after MAT. Bence-Jones protein levels fell from pretreatment levels of 0.01, 0.24, 2.82, 0 and 0.21 g/day to undetectable levels in four patients and 0.14g/day in patient 5 after DT-PACE. This regimen was very well tolerated. The

average number of days to neutrophil recovery (>0.5 x 10^9/L) ranged from 12 to 15. Complications associated with DT-PACE therapy included febrile neutropenia in three of the five patients (with no life-threatening infections). All five patients underwent MAT without any major acute complications. Two patients subsequently suffered from Graft Versus Host Disease. Four patients remain alive and in complete remission at 19, 9, 4 and 2 months respectively. The remaining patient died of suspected bacterial meningitis 15 months after MAT.

Conclusion
DT-PACE showed excellent efficacy as a salvage regimen in preparation for mini-allogeneic transplantation in this group of patients with heavily pretreated MM.

B088 **P02**

BCR-ABL Fusion Gene is Associated with Prednisolone Resistance in Children with Acute Lymphoblastic Leukaemia

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Aim
Sensitivity of leukaemic cells to corticosteroids has emerged as an important prognostic factor in ALL. The t(9:22) translocation, resulting in BCR-ABL fusion gene, is commonly found in B-lineage acute lymphoblastic leukaemia (ALL) and is known to be an independent poor prognostic factor for long term disease free survival. We studied the association between presence of BCR-ABL fusion gene and in-vitro prednisolone resistance of leukaemic blasts in children with B-lineage ALL at diagnosis.

Methods
A total of 23 children (range 1 to 16 years, median age: 12 years) with B-lineage ALL were included in the study. All cases were recruited at diagnosis. Lineage was determined by flow-cytometry and the presence of BCR-ABL fusion gene by RT-PCR. In-vitro resistance of leukaemic blasts to prednisolone was measured by the short-term colorimetric methyl-thiazol-tetrazolium (MTT) assay and it was done prior to starting any treatment.

Results
A median LD50 value for prednisolone in BCR-ABL positive children (n=7) was 1.6 mg/ml (range: 0.25-5.0 mg/ml) and that of BCR-ABL negative children (n=16) was 0.35 mg/ml (range: 0.062-1.0 mg/ml). The median LD50 value for prednisolone differs significantly between BCR-ABL positive and negative groups ($P<0.005$).

Interpretation and Conclusion
This in-vitro study shows that leukaemic blasts of BCR-ABL positive children with ALL are about four-fold resistant to prednisolone as compared to blasts from BCR-ABL negative children suggesting that one of the reasons for the poor prognosis of BCR-ABL positive ALL could be a lower sensitivity corticosteroids.

B091

Predictive Factors for Impaired Progenitor Cell Mobilization in Patients with Lymphoproliferative Disorders Treated with Fludarabine-based Chemotherapy

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Prior fludarabine (Famp) therapy may impair subsequent progenitor cell (PBSC) mobilization. Famp-based therapy is being increasingly used clinically for treatment of lymphoproliferative disorders. Younger patients (<65 yrs) in particular may benefit from autologous transplantation, however there is little data to predict how previous Famp may affect their ability to mobilize PBSC.

Methods
From 2/96 - 4/02 we have made 42 PBSC mobilization attempts in 33 patients (pts); median 1/pt (range 1 – 3). Diagnosis was CLL or variants in 7, follicular NHL in 21, and other indolent lymphoproliferative disorders in 5. Median age was 52 yrs (34 – 65), 61% male. Marrow was involved in 72%. Median number of prior therapies was 1 (0 – 6).

Results

Combination therapy using Famp 25 mg/m^2/d x 3 was given in 32 cases; with cyclophosphamide (cyclo) 250 mg/m^2/d x 3 in 24 pts, mitozantrone 10 mg/m^2 x 1 in 6, and both cyclo 200 mg/m^2/d x 3 and mitozantrone 8 mg/m^2 x 1 in 2. Six pts were treated with Famp alone (25 mg/m^2/d x 5). Three patients received more than one regimen containing Famp. Overall, 87% attained an objective disease response to Famp-based therapy. At mobilization, the median cumulative prior Famp dose was 300 mg/m^2 (75 – 1450). Only 12 of 33 pts (36%) had successful PBSC collection (≥ 2.0 x 10^6 CD34+/kg) with the first mobilization. Ultimately 52% attained this total CD34+ cell dose. Mobilization used either (1) G-CSF (G; median 10 μg/kg/d, range 5 – 16) alone (n = 8, 2 success) [median CD34+ cells/kg = 0.62 (0 – 4.49)], or Cyclo (1.5 – 4 g/m^2) followed by G (median 10 μg/kg/d, range 5 – 14) (n = 19, 6 success) [median CD34+ cells/kg = 1.36 (0 – 26.48)]. Successful mobilization was not associated with any of; gender, diagnosis, prior cumulative Famp dose, method of mobilization, time from last Famp to mobilization, or No. prior therapies (P > 0.4 in all). Successfully mobilized pts were significantly younger (median 44.5 vs 55 yrs; P = 0.003) and 62.5% of pts aged ≤ 50 yrs were successfully mobilized (vs 22% of pts > 50 yrs; P = 0.002). Pts successfully mobilized tended to have received a higher G dose (P = 0.078) and had greater time from diagnosis to mobilization (median 69 vs 34 mths, P = 0.09).

Conclusion
The only factor definitely associated with successful PBSC mobilization was age ≤ 50 yrs. In this setting we recommend the initial use of growth factor alone for mobilization to potentially spare further cyclo-related morbidity and improve scheduling efficiency. These pts are an appropriate target group to consider the combined use of G and stem-cell factor.

B093

Equivalent Overall Survival for Adult Matched Unrelated Donor Transplants Compared to HLA-Matched Sibling Transplants for Acute Leukaemias and Chronic Myeloid Leukaemia – An ABMTRR Report

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Between the years 1992 and 2001, 310 first matched unrelated donor transplants for leukaemia (ALL 61, AML 114, CML 135) were performed for adults (aged 15+) in Australia and New Zealand. We sought to retrospectively compare the outcome of such transplants with equal sized control groups of sibling transplants, matched with the subjects for demographic and disease characteristics.

For ALL, overall survival probability (OS) at 5 years post transplant and transplant related mortality (TRM) were not significantly different for MUD and sibling recipients allografted during or beyond first complete remission (CR1). However, in multivariate analysis, the significant independent risk factors for poorer OS were matched sibling allograft, male recipient, recipients age 35 and above and marrow as stem cell source. There was a significantly higher incidence of relapse at 2 years among sibling transplant recipients as compared to MUD transplant recipients (68% versus 35%, p=0.003). We hypothesise that there may be a more intense graft-versus-leukaemia effect in MUD allografts and more extramedullary relapse among male transplant recipients.

For AML, OS at 5 years, TRM and incidence of relapse were not significantly different for MUD and sibling recipients allografted during or beyond CR1. In multivariate analysis, the only significant independent risk factor for poorer OS was disease stage past CR1 at the time of transplantation.

For CML, OS at 5 years for patients allografted during first chronic phase (CP1) was lower for MUD transplants (58% vs 80%, p=0.004). OS at 5 years for patients allografted beyond CP1 was not significantly different for MUD and sibling transplants (26% vs 31%, p=0.09). In multivariate analysis, the significant independent risk factors affecting OS were MUD allograft and disease stage beyond CP1. In this analysis recipient age did not have a significant impact on OS. There was a significantly higher TRM among MUD transplant recipients (29% vs 13%, p=0.001).). There was no significant difference in terms of incidence of relapse. The inferior OS with MUD transplantation in CML may be due to a longer delay before transplantation as reported in other studies.

In conclusion, this registry data indicates that MUD transplants should be considered early in patients who might benefit from allogeneic transplant and do not have a suitable sibling donor.

B096 **P28**

Detection of Bcl-2 Protein by Flow Cytometry in Lymphoma

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Aim
To evaluate the role of analysing Bcl-2 protein expression by flow cytometry for the diagnostic evaluation of patients with lymphoma.

Method
Thirty-six patients were tested by flow cytometry for Bcl-2 protein expression. Twenty-one lymph node samples, eleven bone marrow, three peripheral blood and one bowel biopsy were analysed. Tissue biopsy samples or red cell lysed peripheral blood or bone marrow cells in lithium heparin were fixed in paraformaldehyde and permeabilised with saponin before incubation with the mouse anti-human Bcl-2-100 monoclonal antibody which was detected with a goat anti-mouse FITC secondary. Samples were analysed on a Beckman Coulter EPICS-XL analyser using the Coulter Expo software program. A mouse leukemia cell line stably overexpressing the human Bcl-2 served as the positive control.

Results

Histological diagnosis	Number of cases	Bcl-2 overexpression	Bimodal pattern of positivity*
Follicular lymphoma	6	6	3
Other lymphoma -mantle cell (1) -marginal zone (2) -prolymphocytic leukemia (2) -peripheral T cell lymphoma (2) -Hodgkin's lymphoma (2) -CLL/SLL (4) -Burkitt's lymphoma (1) -Large cell lymphoma (2)	16	4	1
Non-malignant	14	2	0

* On the bcl-2 histogram, a bimodal expression pattern was observed when two distinct bcl-2 positive peaks occurred with >20% of the cells having a relatively higher intensity of bcl-2 staining.

Bcl-2 overexpression was found in a wide range of haematological malignancies as well as in healthy patients. All follicular lymphoma patients were positive for Bcl-2 and 50% of these samples had a bimodal pattern of Bcl-2 positivity.

Conclusions
Our results show that we can readily determine Bcl-2 protein expression by indirect immunofluorescence and flow cytometric analysis of peripheral blood, bone marrow and lymph node samples. In this pilot study, overexpression of Bcl-2 alone does not appear to distinguish between different histological subtypes of lymphoma or even from reactive/non-malignant states. The significance of bimodal positivity in follicular lymphoma is unclear in view of the small numbers studied. In conjunction with other disease markers, it may be possible to exploit the ease of intracytoplasmic staining for oncoproteins as part of the diagnostic work-up for patients with lymphomas and leukaemias.

B097

Unrelated Cord Blood Transplantation in Children with Acute Lymphoblastic Leukemia – Risk Factors for Relapse

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GVHD is reduced after unrelated cord blood transplantation (UCBT). Whether this fact increases relapse is unknown. To study risk factors for relapse we analysed 195 children transplanted with UCB for ALL who were reported to EUROCORD and transplanted between July, 1994 and December, 2001. At diagnosis the median age was 4 years and WBC was $35x10^9$/l (0.6-1326). The karyotype was normal in 34%, abnormal in 45% (55% high risk) and not available in 21%. 17% had T-ALL. At CBT median age was 7 yrs (0.4-16) and weight was 25kg (5-86Kg). 120 children were good risk (CR1 n = 35, CR2 n = 85)) and 75 had more advanced disease. 16 patients had a previous BMT. Median follow-up after CBT was 31 mths (0.6-78mths). Conditioning consisted of TBI containing regimen in 70%. GVHD prophylaxis was single agent (Csa or FK506) in

17, MTX containing regimen in 39 and Csa and steroids in 129. Median nucleated cells (NC) infused was $3.8x10^7$/kg (0.52-21.3). The CB donor was 6/6 HLA matched in 11%, 5/6 in 43%, 4/6 in 42% and 3 or 2/6 in 4%. Neutrophil recovery to $0.5x10^9$/l was achieved by day 60 in 84% of children in a median of 29 days. Patients receiving $>3.7x10^7$/kg NC had a 95% probability of neutrophil recovery compared to 81% in the remainder (p>0.001). At day 100, TRM was 34%. Probability of aGVHD II-IV was 37% and cGVHD was 34%. Estimated 2-year EFS was 38% for children transplanted in CR1, 36%, in CR2 and 29% for others (p=0.41). Overall relapse risk was 28%; 43% in CR1, 35% in CR2 and 32% in advanced phase (p=0.69). Estimated relapse risk at 2 years was 47% with abnormal karyotype and 25% with normal karyotype (p=0.02). Relapse occurred in 47% for patients receiving MTX for GVHD prophylaxis versus 21% for others (p=0.015) and in 19% for patients with aGVHD $\geq$ II versus 33% for others (p=0.02). Shorter time from diagnosis to CBT (p=0.09) and relapse on therapy in patients transplanted $\geq$ CR2 (p=0.02) was associated with increased risk of relapse. In summary, in multivariate analysis, the most important factors in increasing relapse were karyotype abnormalities at diagnosis (p=0.03), use of MTX for GVHD prophylaxis (p=0.007), time from diagnosis to CBT (<25 mths; p=0.05) and absence of aGVHD (p=0.01).

B098

Scedosporium prolificans – A Case Series of Disseminated Infection in Clinical Haematology and Stem Cell Transplant (SCT) Patients

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Background
Scedosporium prolificans is a multi-drug resistant fungus first described as a human pathogen in 1984. It is probably a soil saprophyte and has been isolated from potted plants and air samples from hospitals in Spain and Australia, some of which were undergoing building refurbishment. *S. prolificans* has been associated with localised as well as disseminated infection. Disseminated infection most frequently occurs in the immunocompromised patient, particularly those with neutropenia as a result of chemotherapy or SCT. Effective treatment is lacking and most disseminated infections are fatal.

Methods
Six clinical haematology and SCT patients with disseminated *S. prolificans* infection diagnosed within a 19-month period at a single institution were identified using hospital charts, radiological and bacteriological reports.

Results
Patient details are summarised below.

Age/ Sex	Diagnosis	Therapy	Clinical manifestations	Neut $x10^9/L^{\dagger}$	Source of culture (n)	Outcome
19/M	ALL	Allogeneic SCT	Septic arthritis, fever	3.45	Synovium/ fluid (7), blood (1), wound (2)	Death
54/M	MDS	Allogeneic SCT	Fever, pulmonary infiltrates, multi-organ failure	2.95	BAL‡ (1), sputum(4), blood (1), catheter tip(1)	Death
25/F	MDS	Nil	Pulmonary infiltrates, sinusitis	10.6	Sputum (5), nasal tissue (2), blood (1)	Death
23/M	AML	Allogeneic SCT	Fever, pulmonary infiltrates, rash	0.01	Blood (5), skin (1), sputum (1), BAL (1)	Death
55/F	Multiple myeloma	Allogeneic SCT	Fever, back pain, sinusitis	7.35	Sinus tissue (1), bone (3), aneurysm (1)	Alive
29/M	NHL	Unrelated donor SCT	Fever, multi-organ failure	0.01	Blood (3)	Death

†at time of first positive culture ‡BAL=bronchoalveolar lavage

S. prolificans was isolated from ≥2 specimens in 5 patients and from blood alone in a single patient. Positive cultures were most commonly obtained from blood (n=5), followed by respiratory tract specimens (n=4). All 6 patients received antifungal therapy; one received the combination of voriconazole and terbinafine. This anti-fungal combination has demonstrated in vitro synergy and may improve therapeutic response. Five patients died, at a median time of 25 days (range 2 - 509) after the

first positive culture for *S. prolificans*. The survival of the remaining patient was associated with graft rejection and autologous recovery. Building construction near an outpatient haematology treatment facility pre-dated all infections. This construction may have increased patient exposure to *Scedosporium prolificans*.

Conclusions
These cases of disseminated *S. prolificans* infection stress the importance of this emerging fungal pathogen in patients with haematological malignancies, particularly during hospital rebuilding work. The resistance to standard antifungal therapy and associated high mortality of this rare but clinically important fungus is highlighted.

B099

Type 2 Dendritic Cell Progenitor Numbers in Donor Peripheral Blood Stem Cell (PBSC) Grafts Correlate with Increased Chronic Graft versus Host Disease (GVHD) and Survival following Transplantation

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Type 1 and type 2 dendritic cells play an important role in immune regulation following allogeneic transplantation. Type 2 dendritic cell progenitors are recognised by a CD8-/CD3-/$CD4^{bright}$/low side-scatter ($CD4^{bright}$) phenotype. Higher $CD4^{bright}$ numbers in donor bone marrow (BM) are reported to have adverse effects on survival and are associated with increased relapse and decreased chronic GVHD. This study assesses the relationship between clinical outcomes (death, relapse, GVHD) and donor graft constituents, particularly $CD4^{bright}$ numbers in PBSC rather than BM.

80 consecutive patients between January 1999 and February 2002 with haematological malignancies underwent PBSC transplantation. Complete data on transplanted lymphoid subset numbers were available for 55 patients. $CD3^{+}$, $CD34^{+}$ and $CD4^{bright}$ cells were enumerated by four-colour flow cytometry and results expressed per kilogram recipient body weight. Analyses were performed as of 10 June 2002. Overall survival was defined as time from transplantation to death or last contact. Event free survival (EFS) was defined as survival without evidence of relapse. Standard conditioning regimens and GVHD prophylaxis and management protocols were followed. Acute (aGVHD) and chronic GVHD (cGVHD) were defined using IBMTR criteria. Survival curves were produced using Kaplan-Meier methods and statistics performed using the Pearson chi-square test.

The median patient age was 48 years (range 17 – 61); 58% were male. Transplants were performed for ALL-7, AML-17, CML-5, NHL-11, CLL-1, PLL-1, HD-2, MDS-4, AA-1, MM-4, MF-2. 40% of patients survived to the reference date with a median follow-up of 470.5 days (range 129 – 1216). EFS was 68%, 40% and 35% at 100 days, one and 3 years respectively. A similar pattern for overall survival was observed. The median $CD4^{bright}$ number was 4.26 $x10^{6}$/kilogram (range 0.17 – 22.05). Higher numbers were associated with improved survival after transplantation (p=0.036). Relapse (30%) was the commonest primary cause of death at a median of 180 days (range 70 – 350) post-transplantation. The relapse rate for patients receiving higher and lower numbers of $CD4^{bright}$ cells was 14% and 30% respectively (p=0.168). aGVHD developed in 79% of patients (grade I-II in 44%; III-IV in 35%). No association between $CD4^{bright}$ numbers and the incidence of aGVHD was demonstrated. 39 patients survived to day 100; 21 (54%) developed cGVHD. Higher $CD4^{bright}$ numbers were associated with an increased risk for cGVHD (p=0.041).

The number of $CD4^{bright}$ cells in PBSC is higher than that previously reported in BM. The content of $CD4^{bright}$ cells in donor PBSC grafts is associated with increased cGVHD and survival following transplantation.

B100 **P40**

Rapid and Complete Chimerism Occurs after Non-Myeloablative (Mini-) Allogeneic PBSC Transplantation (PBSCT) using Melphalan and Fludarabine as Conditioning Chemotherapy

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Allogeneic transplantation offers the possibility of cure for some haematological malignancies. An increased risk of transplant-related mortality precludes the use of myeloablative conditioning in older patients and those with significant co-morbidities. Mini-PBSCT uses lower doses of conditioning chemotherapy and/or radiotherapy to improve the safety of allogeneic PBSCT and relies on the graft-versus-tumor effect to cure patients. The clinical significance of the persistence of recipient haemopoietic cells (mixed chimerism) is not well understood but has been suggested to be a prognostic indicator following mini-PBSCT.

We have measured haemopoietic chimerism in 12 mini-PBSCT recipients (7 male and 5 female, median age 51.5 (36-63) years) not eligible for standard PBSCT. There were 4 patients with AML, 2 with CLL and 2 with CML, as well as single cases of MM, HD, Waldenstrom's macroglobulinaemia and myelofibrosis. All patients received fludarabine (25 mg/m^2 x5), melphalan (140 mg/m^2 x1) and ATGAM 15 mg/ m^2 from day -4 to day +5 prior to transplantation. GVHD prophylaxis was cyclosporin 3 mg/kg/day IVI for 3-6 months and mycophenolate mofetil 15 mg/m^2 BD from day 0 to day +27.

Chimerism was measured using polymorphic minisatellite (VNTR) markers in granulocytes (pellet), monocytes (CD14$^+$/CD15$^+$), T-cells (CD3$^+$) and NK cells (CD2$^+$, CD3$^-$) purified sequentially from peripheral blood by immunomagnetic separation. Informative VNTR markers were amplified by PCR using 100 ng of genomic DNA as a template and digoxygenin-labeled deoxynucleotide triphosphate precursors. Following agarose gel electrophoresis and Southern blot, PCR products were detected by enhanced chemiluminescence and the chimerism evaluated by comparison with serially diluted recipient cells in donor cells. The sensitivity of this procedure was between 1 and 5%. Chimerism was assessed monthly for 6 months and then at 9 and 12 months post transplant. More frequent measurements were made when mixed chimerism was evident. Monitoring of monocyte and NK cells was discontinued in recent patients as it gave the same results as granulocytes and CD3$^+$ cells, respectively.

No graft rejection occurred. 11 of 12 patients achieved >90-95% donor chimerism by 1 month post mini-PBSCT. Donor granulocyte engraftment either preceded or was concurrent with that of donor CD3$^+$ cells engraftment. Complete and stable donor chimerism was achieved in 10 patients within 2 months. Transient appearance of recipient granulocytes and CD3$^+$ cells (5%) was observed in one CML patient 5 months after transplant. Substantial mixed chimerism at 1 month was evident in the myelofibrosis patient (<50% donor) which was managed by ceasing the mycophenolate mofetil; >90% donor chimerism quickly developed accompanied by the onset of severe acute GVHD.

In summary, rapid, complete and sustained complete donor chimerism was achieved with the use of melphalan, fludarabine and ATGAM as conditioning chemotherapy prior to mini-PBSCT.

B101 **P18**

Stopping Interferon-α For 10 days prior to Bone Marrow Examination (BMAT) for Patients with Chronic Myeloid Leukaemia (CML) does not increase the Chances of a Successful Chromosomal Analysis

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Chromosomal analysis of bone marrow metaphases is the most important predictor of response to interferon and of long-term survival of patients with CML. Unfortunately, chromosomal analysis frequently fails presumably due to the antiproliferative effect of interferon. It is common practice to stop interferon for 7-10 days prior to BMAT in the belief that this will improve the chances of a successful chromosomal analysis. This study assessed, with a combination of retrospective and prospective data, whether or not this manoeuvre does improve the chances of a successful result.

Thirteen patients receiving interferon underwent 125 BMATs (median 12; range 1-19) over a median of 3 (range 0.25-7.5) years. For the past 2.5 yrs, patients have alternately continued the interferon prior to BMAT or stopped the drug for 10 days prior to BMAT. Prior to that time the continuation of interferon was random or not recorded. Twelve of 13 patients were male; median age was 50 (21-63) yrs. Median Sokal score was 0.75 (0.46-2.77) and median Hasford score was 994 (158-1988).

A successful chromosomal analysis was achieved in 77 of 125 (62%) BMATs. This was true for 19 of 37 (51%) of those BMATs where the interferon was stopped for 10 days prior; 22 of 30 (73%) of those BMATs where the interferon was continued (p=0.07). In addition 4 of 8 (50%) of those BMATs where the interferon was stopped for >10 days for toxicity reasons (median 58 days; range 15-157 days) and 32 of 50 (64%) of those where it was unknown whether or not the interferon was ceased had a successful analysis. 12 of 13 (92%) of patients had a successful chromosomal analysis prior to commencing interferon.

Assessing all BMATs (n=125): the chances of a successful chromosomal analysis decreased during the first 2 yrs on interferon and then remained steady: prior to interferon - 92% successful; during first 6 mths of interferon - 95% successful; during second 6 months - 75% successful; during 2nd year - 57% successful; during 3rd year - 42% successful; during 4th year - 58% successful; during 5th year - successful 58%; and for 6th year onwards - 59% successful.

The next successful chromosomal result after a failed result was always (35 of 35) a major cytogenetic response (1-34% Ph+ metaphases; n=13) or a complete cytogenetic response (no Ph+ metaphases; n=22).

In summary, the chances of a successful chromosomal analysis did not depend on whether or not the interferon was stopped for 10 days prior to BMAT but decreased with time on interferon and with greater chromosomal responses.

B102

Tailored Volume Leucapheresis (LP) Minimises the Number Of Leucaphereses Required To Achieve Adequate $CD34^+$ Stem Cell Numbers For Autologous Peripheral Blood Stem Cell (PBSC) Transplantation

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The optimal method for PBSC collection is uncertain. The aim of this pilot study was to minimise the number of LP collections required to achieve a predetermined stem cell number in the final product. LP volumes were tailored for each patient based on peripheral blood $CD34^+$ cell count and the postulated leucapheresis collection efficiency (CE) of 40% using the Baxter-CS3000+ cell separator.

Twenty patients were prospectively assessed. The tailored LP volume was calculated using the formula:

$$\frac{\text{Target } CD34^+ \text{ cell number per kg x patient weight (kg)}}{\text{Machine efficiency x } CD34^+ \text{ cell number per litre of peripheral blood}}.$$

A minimum of 5 and a maximum of 14.5 litres of peripheral blood were processed per collection. The CE was calculated for each LP.

Patient characteristics:17 males, 3 females; median age 54 (range 22-66) years; diseases NHL (7), MM (8), ALL (2), germ cell tumour (1) and HD (2). All patients received prior chemotherapy and one had additional radiotherapy. Patients were mobilised with G-CSF alone (10-20ug/kg/day; n=8), or chemotherapy plus G-CSF (10-20ug/kg/day; n=12).

Twenty patients underwent 40 LP; a median of 2 LP (range 1-3) per patient. The pre-LP peripheral blood $CD34^+$ concentration correlated with the harvested $CD34^+$ cells, r^2=0.75. The median CE was 37% but the range was wide (19–73)%. Importantly, for patients with an initial CE <37% the CE remained consistent with subsequent LP. Predictors of CE <37% appear to be a low blood volume and/or a diagnosis of NHL. In 16/20 patients (80%) the PBSC ($CD34^+$) target was reached. Median platelet pre LP was 146 (range 45-637) $x10^9$/L; and post LP was 89 (range 37-441) $x10^9$/L. In comparison with an estimated number of standard LPs (7L) that would have been required to collect the equivalent number of $CD34^+$ cells, there was a saving of 1-2 LP per patient. A total of 24 LP were estimated to have been saved.

We have demonstrated the benefit of tailoring LP volume to reduce the number of LP per patient based on a CE of 40%. We predict that by varying the CE for each patient (a) prior to LP based on disease and blood volume or (b) after the first LP, based on the calculated CE, we may further reduce the number of LP required for autologous PBSC transplantation. This may form the basis of a randomised trial comparing PBSC collection of tailored leucapheresis volumes with the conventional leucapheresis volume of 7 litres.

B105 **P36**

Generation of Specific Leukaemia Cytotoxic Cells using Dendritic-Like Cells derived from Acute Lymphoblastic Leukaemia

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Acute lymphoblastic leukaemia (ALL) is the least immunogenic leukaemia as shown by the low level of graft versus leukaemia (GvL) effect in allogeneic bone marrow transplant patients.

We have previously reported a novel *in vitro* technique of inducing bone marrow (BM) cells from pre-B leukaemic patient cells to develop into functional dendritic-like (DC-like) cells. In this study, the effectiveness of the DC-like ALL cells at producing a specific cytotoxic immune response in naïve T cells was investigated.

ALL BM cells were cultured in a mixture of cytokines with CD40L for 5 days to generate DC-like cells. Differentiation was confirmed by morphology and phenotype and the ALL origin established by gene rearrangement studies. Cord blood T lymphocytes were incubated with DC-like cells in a mixed cell reaction to induce the generation of cytotoxic T lymphocytes (CTLs).

The cytotoxic activity of the T cells was assayed against several targets: the *de novo* ALL cells, NALM-6, and THP1. Target cells were stained with membrane dye PKH26 and incubated with CTLs for 6 hours. Target cell viability was determined by

7AAD exclusion using flow cytometry. Perforin and IFNγ production by CTLs was also determined by intracellular staining and flow cytometry.

T cells generated from mixed cell reactions from 5/7 patients showed significant cytotoxicity against the leukaemic cells from which the DC-like cells were derived (range 6.5 to 34.3 for 1:25 ALL to T cell ratio). Three of these patients also showed a dose dependent cytotoxic response to varying ratios of CTLs (1:5, 1:10 and 1:25).

Cytotoxicity against NALM-6, a pre-B ALL cell line, was observed suggesting a
shared common antigen between patient ALL cells and NALM-6. Lack of cytotoxic activity against THP1, a monoblastic cell line, indicated specificity of cytotoxicity.

After overnight incubation, IFNγ production increased from 1% to 5.0% for $CD4^+$ and 1% to 5.9% for $CD8^+$ T lymphocytes (mean of 5 experiments). Perforin production increased by 2.9% in $CD8^+$ CTLs (mean of 5 experiments).

It is possible to produce specific cytotoxicity against ALL using ALL derived DC-like cells as the stimulus. This study demonstrates the potential for immunotherapy against ALL without requiring definition of tumour antigens.

B106 **P24**

Thalidomide Alters Myeloma Cell Surface Receptor Expression In Vitro

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Thalidomide is an effective treatment for approximately one third of patients with multiple myeloma (MM). Initially trialed on the basis of its anti-angiogenic properties, the actual mode of action in MM remains unclear. Nor is it understood why some patients fail to respond to the drug. In vitro studies have shown that thalidomide on its own is not a potent inducer of MM cell death. However, it is known to cause marked changes in receptor expression on the surface of normal lymphocytes and endothelial cells both in vivo and in vitro. Because the binding of MM cells to the bone marrow (BM) stroma is pivotal to the pathological features of MM, we hypothesised that thalidomide may interfere with this attachment by altering receptor expression on the surface of either the MM or BM stromal cells. To test this theory, we treated 13 MM patient plasma cell samples and 4 MM patient derived long term BM stromal cultures (LTBMC) with 10 μM thalidomide or DMSO as a control for 72 h in vitro, then examined surface antigen expression by flow cytometry. For each receptor, the percentage of positive cells was determined by plotting cell counts vs. fluorescence on a log scale, and for each sample the percentages of positive cells after thalidomide or control treatment was compared. None of the LTBMC showed any changes in expression of any of the markers tested (CD38, CD138, CD95, CD49, CD56, CD117, CD10). However, analysis of isolated CD138+ plasma cells from MM patient BM aspirates at the time of diagnosis (n=10) or restaging (n=3) revealed frequent and consistent changes in levels of expression of CD40 (>50% decrease in 4 samples, >50% increase in 2), CD49d (15-40% increase in 5 patients), CD44 (decreased by 30-60% in 3) and CD29 (25-75% increase in 3 samples). In addition, the majority of samples tested were found to express CD54 (ICAM-1) (8/8) and the integrin beta 7 (7/11), but expression of either of these molecules became only slightly altered in a few samples (2/8 and 3/7 respectively) in response to thalidomide treatment. When the patient plasma cells were plated onto LTBMC monolayers, no difference in adhesion was observed between treated and non treated cultures. We conclude that thalidomide influences receptor expression on the surface of MM cells, but this does not interfere with the adhesion of MM cells to the BM stroma.

B107

Methionine Synthase Genetic Polymorphism MS A2756G Alters Susceptibility to Non-Hodgkin's Lymphoma but not Multiple Myeloma

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Chromosomal translocations are believed to play an important role in the development of Non Hodgkin's lymphoma (NHL) and multiple myeloma (MM). Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS) are two enzymes involved in DNA synthesis and methylation. These enzymes are known to have polymorphisms that reduce their activity and may alter susceptibility to carcinogenesis. Although recent population studies have shown that polymorphisms which confer

high MTHFR activity increase susceptibility to colon cancer and acute lymphocytic leukemia, little work has been done to investigate this association in NHL and MM. We conducted restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) analysis for MTHFR C677T, MTHFR A1298C, and MS A2756G polymorphisms in Caucasian patients with NHL (n=151) or MM (n=90) and compared the frequencies with those of healthy controls (n=299). The frequencies of these polymorphisms in our control group were similar to those reported for other Caucasian control populations in the UK and Australia. The MM group showed no significant difference in the incidence of any of the three polymorphisms when compared to the controls. The NHL population showed a significantly higher incidence of the normal MS 2756 AA genotype (73.8% for NHL vs 62.8% for controls, p=0.02),but no difference from control frequencies for MTHFR 677 CC, CT and TT (49.3%, 39.2%, 11.5% in NHL vs. 48.5%, 44.5%, 7.0%, respectively) and MTHFR 1298 AA, AC and CC (44.1%, 46.9%, 8.9% in NHL vs. 42.2%, 47.2%, 10.5% in controls, respectively). When broken down by histologic subtype, it became apparent that these frequencies were only reflected by the Follicular Lymphoma (FL) group (n=48), in which the MS 2756 AA genotype conferred a 2.4 fold higher risk of disease (CI: 1.14 – 5.25, p=0.019). In contrast, the Diffuse large B-cell lymphoma (DLCL) population (n=74) had frequencies similar to those of the controls for all three polymorphisms. This is the first comprehensive analysis of the incidence of these polymorphisms in sufferers of MM, and our results indicate that neither of these metabolic pathways is an important contributor to the disease. In contrast, the methionine pathway may play a role in the development of NHL, whereby the normal AA genotype confers a higher susceptibility to FL but not DLCL. This finding emphasises the importance of DNA methylation in promoting particular forms of malignancy, and stresses the need for further investigation of this poorly understood mechanism.

B110 **P23**

In Vitro Analysis of Growth Inhibition and Apoptosis Induction in Human Myeloma Cell Lines by the 3'hydroxy-3methylglutaryl Coenzyme A (HMGCoA) Reductase Inhibitor Fluvastatin

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A large body of evidence indicates that the mevalonate pathway plays an important role in cell growth and survival. Mevalonate is synthesized intracellularly from 3'hydroxy-3methylglutaryl coenzyme A (HMGCoA) in a process catalysed by HMGCoA reductase, the rate-limiting enzyme in this pathway. Mevalonate metabolism yields a series of isoprenoid compounds which are incorporated into cholesterol, isopentenyl adenine, prenylated proteins and other end products essential for cell growth. As competitive inhibitors of HMGCoA reductase, statins have been shown to not only block synthesis of mevalonate but to inhibit the growth and proliferation of both normal and tumour cells. Furthermore, inhibition of HMGCoA reductase has been shown to induce cell death by apoptosis in some systems.

For this reason we have investigated the cytotoxic effect of the HMGCoA reductase inhibitor fluvastatin on human multiple myeloma cell lines LP-1, OPM-2, U266, NCI-H929 and RPMI-8226 in vitro using a tetrazolium reduction assay. After 3 days culture in the presence of 0 to 50μM fluvastatin, the Promega MTS assay reagent was used to determine the level of inhibition of cell proliferation and/or cell death. Fluvastatin concentrations as low as 2.5μM significantly inhibited proliferation of all cell lines except RPMI-8226 ($p<0.05$ by paired student's t-test). Concentrations of 25μM and 50μM significantly inhibited proliferation in all cell lines ($p<0.05$ by paired student's t-test), with inhibition at 50μM ranging from 45 to >90% for U266 to OPM-2.

Using the same assay we investigated whether the activity of fluvastatin against multiple myeloma in vitro could be enhanced by the addition of the bisphosphonate Zometa which also inhibits the mevalonate pathway. Using 80% cell inhibition as an end point, isobolograms were constructed to visualize the interaction between fluvastatin and Zometa. Isobologram analysis indicated that fluvastatin and Zometa act synergistically to induce cell death in human myeloma cell lines. To illustrate this point, >50μM fluvastatin or >100μM Zometa alone was required to induce 80% cell death in the myeloma cell line LP-1 but the combination of 25μM fluvastatin and 0.21μM Zometa has the same effect.

Our initial data indicates that fluvastatin is a potential therapeutic agent for multiple myeloma that warrants further investigation both as a single agent and in combination with other inhibitors of the mevalonate pathway.

B111

Synergistic Induction of Apoptosis by the Bisphosphonate Zometa and other Potential Therapeutic Agents in Human Myeloma Cell Lines

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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 previously reported that the BP Zometa induces varying degrees of apoptosis in human MM cell lines NCI-H929, RPMI-8226, LP-1, OPM-2 and U266. The aim of this study was to determine whether other potential therapeutic agents could enhance the apoptosis-inducing potential of Zometa. The agents chosen were dexamethasone, tamoxifen and tumour necrosis factor related apoptosis inducing ligand (TRAIL). Dose-response curves were constructed for each agent alone and in combination with Zometa using results obtained from MTS assays following 72hr treatment of myeloma cell lines. Using data from the dose-response curves, isobolograms were plotted in order to visualize the interaction between Zometa and each of the agents.

Isobologram analysis indicated that Zometa and dexamethasone acted synergistically to induce cell death in human MM cell lines. As an example, in the myeloma cell line NCI-H929 >100μM Zometa or 2μM dexamethasone alone is required to induce 50% cell death however the combination of 1μM Zometa and 1.2μM dexamethasone has the same effect. In contrast, the interaction between Zometa and TRAIL was antagonistic with even low concentrations of Zometa increasing the concentration of TRAIL required to induce the same level of cell death. In the absence of Zometa, 117.34ng/ml of TRAIL was able to induce 50% cell death in NCI-H929 cells, however in combination with 0.1μM Zometa the concentration of TRAIL required for the same effect was 211.24ng/ml. Tamoxifen showed little effect on human MM cell lines alone or in combination with zometa.

In summary, the MTS assay in a 96 well format proved a convenient method for screening the cytotoxic activity of individual agents and the interaction of agents in vitro. Isobologram analysis of data generated by MTS assays provided a simple visual interpretation indicating whether combinations resulted in synergistic (ie Zometa and dexamethasone) or antagonistic (Zometa and TRAIL) interactions. Our data provides a rationale for the treatment of MM with Zometa in combination with the conventional therapeutic dexamethasone, however the combination of Zometa and the novel therapeutic agent TRAIL is contra-indicated although both agents have shown promising results as single agents in vitro.

B112 **P48**

Temporary Triple Lumen Central Lines as an Alternative to Hickman's Catheters for Patients undergoing Intensive Dose Chemotherapy Regimens

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Central venous lines (CVL) are an integral part of managing patients receiving intensive dose chemotherapy. In Australia the commonest CVLs in this setting are Hickman's Catheters (HC). HCs require surgical or interventional radiological placement and leave a tunnel with a foreign body as a potential site of infection. Since 1997 our institution has primarily used triple lumen central lines (TLCL) instead of HCs because of easier placement.

This study looks at outcomes of these CVLs at our institution. Data collection is prospective and sequential. Data relevant to each CVL inserted is entered recording the line type, the insertion complications and the reason for removal.

From July 1997 to May 2002, 314 patients underwent a total of 620 CVL placements. 274 patients had malignancies requiring intensive dose chemotherapy regimens either with or without autologous blood or bone marrow transplant (BMT) and 40 had chronic bone marrow failure states – eg myelodysplasia. 76 placements with vascaths for PBSC harvests, 47 implanted venous access devices and 77 PICCs are not included in this analysis.

The average duration for TLCLs and HCs was 28 days (1-147 ± 2 SD) and 69 days (1-149; P<.0001) respectively.

The total number of insertion complications was 81 (21%) for TLCLs and 8 (20%) for HCs, although the number and types of varied between the two catheter types. Early removal occurred in 100 (26%) cases in TLCL and 12 (30%) in HC. The major reason for early removal was suspected or proven line sepsis and occurred in 67 (18%) of TLCL and 27 (23%) of HC.

Despite the significant difference in the average dwell-time between the two types of CVL, our data demonstrates that TLCLs are able to safely remain in situ long-term in some patients. The number and types of insertion complications varied, but there was no overall statistical difference between the two. Similarly, reasons for early removal varied, but the overall rate between the two catheter types was not statistically different. The number of HCs in our study is low limiting conclusions. Studies with comparable numbers of HCs report similar rates and reasons for early removal, although infection rates of as high as 40% occur.

We believe that TLCLs provide a safe, convenient alternative to HCs in the management of patients requiring intensive dose chemotherapy regimens and supportive care, thus negating the need for surgical or radiological placement.

B113

CD34 Selected Allo-Transplants with DLI in Older Patients

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To reduce early transplant related mortality in older patients due to acute graft versus host disease, a study was undertaken to allotransplant 40 to 60 year old patients with haemopoietic malignancies, using Filgrastim-mobilised HLA-identical donor blood cells which were CD34+ selected using Isolex devices. To counteract a possible increase in relapse rate due to the T cell depletion donor leucocyte infusions were planned.

Donors were leukapheresed to achieve a target CD34+ cell collection of 5 million/kg recipient body weight after selection. The median number of CD34+ cells collected was 9.9 million/kg recipient body weight (range 1.9 to 22.9) and a median of 5.2 million/kg (range 1 to 11.5) were recovered after selection. The median number of CD3+ cells infused was 0.24 million/kg (range 0.02 to 0.83), a 3 log depletion of T cells. Conditioning therapy was TBI 12 Gy and Cyclophosphamide 120mg/kg. GVHD prophylaxis was Cyclosporin only. Engraftment was rapid in all cases and graft failure occurred in only 1 of 17 patients. Transplant-related mortality of patients who received selected CD34 cells was 6% (1/17) at 3 months and 20% (3/17) at 6 months. Acute GVHD of grade 2 or more occurred in 2 patients (12.5%). Donor leucocyte infusions on day 60 or 90 were able to be given to six patients with resolved grade 1 or no acute GVHD in an attempt to reduce relapse. DLI was unable to be given to more patients because they had active or resolving acute GVHD or were still on therapy for previous GVHD. Relapse occurred in 8 of the 17 including 3 of the 6 who had DLI. Extensive chronic GVHD has developed in 6 of 12 evaluable patients receiving selected CD34+ cells; 2 of these had received DLI. Seven of the 17 (41%) patients are alive at median follow-up of 56 months. This approach even in older patients led to a low early transplant mortality because of rapid haemopoietic reconstitution and low acute GVHD. Relapse remains a significant problem and the impact of DLI on the relapse rate is limited by the difficulty in giving DLI because of low grade GVHD.

B114 **P01**

Multiparameter Flow Cytometry for the Detection of MRD in Precursor B ALL

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During the last two decades major progress has been made in the technology of flow cytometry and in the availability of a large range of monoclonal antibodies against surface membrane and cytoplasmic antigens. Flow cytometric immunophenotyping has become a diagnostic tool for the analysis of normal and malignant leukocytes and it has proven to be a reliable approach for the investigation of minimal residual disease in leukaemic patients during and after treatment.

In this study we used four-colour flow cytometry to investigate the B cell compartment of normal bone marrow in order to generate a frame of reference for the identification of leukaemia-associated phenotypes in precursor B ALL. 30 normal bone marrow samples were analysed whose age ranged from 2 to 50 years with 2 different four-colour combinations of antibodies CD19 APC/CD45 PerCP/CD10 FITC/CD20 PE and CD19 APC/CD45 PerCP/CD34 FITC/CD9 PE. A series of dual parameter displays were created in which normal B cell precursors occupied predictable regions. We then tested these antibody combinations on a series of 25 precursor B ALL and found that 95% of cases showed an abnormality. When blast cells from a leukaemia cell line (NALM 6) were serially diluted into normal bone marrow we could reliably detect 1 blast in 10^4 normal cells.

Because the pattern of antigen expression in normal B cells is reproducible it is possible to generate templates to define the normal B cell differentiation pathways which allows the discrimination of malignant precursor B cells and can therefore be used for MRD detection.

B115

Rituximab Therapy for Patients with Recurrent Rheumatoid Arthritis (RA) Post Haemopoietic Stem Cell Transplantation (HSCT)

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HSCT for patients with severe RA has been recently investigated in an attempt to provide disease remissions in patients who have failed all available therapy. Case series and an Australian randomised trial have confirmed that significant responses can

occur in these patients but that disease recurrence still occurs usually in the 6-12 month period, although sustained remissions can occur up to 2 years post HSCT. Thus a major objective of future work in this area is the ability to maintain responses. Immune reconstitution data reveal that 75% of rheumatoid factor (RF) positive patients in our trial had a rise in RF titre associated with recurrence. In many cases this corresponded with reconstitution of B cells suggesting a major role for B cells and their secretion of RF in disease pathogenesis. Recently a trial using cyclophosphamide and rituximab in resistant RA patients showed significant responses prompting our group to hypothesize that the antibody may be of benefit to patients who had previously received high dose cyclophosphamide and a HSCT. We have conducted a phase 1/2 trial involving 10 patients in which they have been treated with 1g rituximab 2 weeks apart along with 250mg methylprednisolone. Follow up on these patients reveals major responses in 8/10 patients – 5 ACR 70 (70% improvement in disease parameters), 3 ACR 50. The responses were associated with a reduction in RF titre, CRP, joint counts and loss of B cells in the peripheral blood confirming that B cells may be an important target for therapy in RA. 4 patients have subsequently had recurrence of disease associated with a rise in RF titre and in most cases B cell reconstitution. These patients will be re-treated with the same regimen to confirm the initial findings. This pilot study provides important data for the design of future trials in RA and confirms the pivotal role for B cells in the pathogenesis of RA.

B117

Effect of Imatinib on CML Progenitor Adhesion

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Aim
To characterise further the defect in CML progenitor cell adhesion to the bone marrow microenvironment, and to identify the effect of the ABL tyrosine kinase inhibitor, imatinib, on correcting this deficit.

Methods
The expression of certain cell adhesion molecules was assayed on CML cells, normal cells and on mobilised PBSC by standardised semi-quantitative flow cytometry. Functional assays of adhesion to fibronectin (fn) were performed using flow sorted CD34+ cells from normal donors and from chronic phase CML in the presence of a range of individual cytokines and cytokine combinations. These tests were repeated after incubation of cells in 3uM imatinib for 16 hours.

Results
CAM expression of CD11a, CD18 and CD58 was reduced in the CML CD34+ population as compared to normal CD34+. The CML pattern of CAM expression was similar to that observed in therapeutically mobilised PBSC. Using the Philadelphia chromosome positive cell line MEG-01, we have been unable to effect a change in the expression of these molecules in response to imatinib. Adhesion of normal CD34+ cells to fn was augmented by SCF, IL3 and GM-CSF, however CML CD34+ cells showed increased adhesion to this substrate only with SCF. Imatinib treatment neither increased the adhesion of these cells to fn, nor corrected their response to IL-3 and GM-CSF.

Conclusions
The adhesive defects of primary CML cells are not reversed by 16 hour treatment with imatinib. We suggest therefore that these defects are not related to the immediate effects of the BCR-ABL chimeric protein. They may arise from its down stream signalling, may result from pre-leukaemic changes, or may be related to the type of cell that is transformed in CML. The lack of effect of imatinib on the adhesive function of CML cells is likely to indicate that an important mechanism of action of this therapeutic agent is not through alterations of the interaction of CML progenitors with their microenvironment.

B118

Genetic Status of Chronic Myeloid Leukaemia Patients and the Clinical Course of Disease

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Aim
To determine the effect of karyotype, BCR-ABL transcript, ABL-BCR transcript and D-FISH deletion on presentation characteristics, duration of chronic phase and overall survival of CML patients.

Methods
55 CML patients were investigated by conventional cytogenetic analysis, RT-PCR and D-FISH. Case notes were reviewed for the patient's clinical data at presentation, response to therapy, duration of chronic phase and overall survival. Median follow up was 115 months (range 86 - 228 months). Median estimated duration of chronic phase and survival were 71.4 months and

97.5 months respectively. An additional 8 cases were analysed by D-FISH because they had a complex Philadelphia rearrangement (Ph) (bringing the total number in this group to 13) and 9 cases because they had additional abnormalities at presentation (bringing the total number in this group to 14).

Results
The 55 patients in the original study group all expressed BCR-ABL; 22 had b2a2, 29 had b3a2 and 4 had both transcripts. 30/55 also expressed ABL-BCR. With regard to the parameters studied there was no significant difference between these groups. All patients with ABL-BCR expression lacked D-FISH deletions. Of the 25 ABL-BCR negative patients, 7 had deletions detected by D-FISH. 0/14 with additional cytogenetic abnormalities at presentation and 5/13 patients with a complex Philadelphia rearrangement had D-FISH deletions. Kaplan-Meier plots showed significantly shorter duration of chronic phase in cases of complex Ph (p=0.0481), although overall survival was not significantly different.

Discussion
Type of BCR-ABL fusion transcript and presence of ABL-BCR fusion transcript did not affect clinical parameters at presentation of CML, nor did they identify patients with differing response to therapy or survival times. Literature reports show that D-FISH deletions are a marker of poor outcome for CML patients treated with alpha interferon and conventional chemotherapy. Our data does not support this finding, perhaps due to the small number of patients who had deletions. Deletions are not more common in the patients with additional abnormalities at presentation, although they were seen in 38.5% of patients who had a complex Ph. The shorter duration of chronic phase for this latter group may suggest that many harbour smaller deletions than can be identified by D-FISH.

B119 **P05**

When the t(9;22) and the t(15;17) Lack Clinical Significance

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The international system for human cytogenetic nomenclature (ISCN), 1995 states that 2 cells must bear the same translocation for it to be considered to be representative of a clonal change. A number of publications have explored the significance of single cell cytogenetic abnormalities (SCA), but they have focussed on numerical changes. Structural changes are known to occur as SCA, but they are more common following chemotherapy and radiotherapy and do not usually take the form of recognised, non-random leukaemia-associated changes. We now report 2 diagnostic dilemmas. The first concerned a patient presenting with acute lymphoblastic leukaemia and 1 of 40 cells with t(9;22)(q34;q11.2), the remaining 39 cells showed an apparently normal female karyotype. The second was a patient with M2 AML on morphology who had +8 in 29 cells and 1 cell with +8,t(15;17)(q22;q21).

Confirmation of the clonal nature of these translocations would have a profound impact on the treatment and the counselling given to these patients. Using the techniques available to us (FISH, RT-PCR and immunocytochemistry) clonality of the observed SCA could not be proven. Even if SCA appear identical to recognised, disease-related cytogenetic abnormalities, they must be investigated fully before a decision can be reached regarding their significance.

It is uncertain whether these SCA are random events that simply mimic the Philadelphia chromosome and the t(15;17) of M3 AML, and whether they arose in vivo or in vitro. Bose et al demonstrated by RT-PCR that normal individuals can express BCR-ABL at very low levels – we may have identified the rare cytogenetic equivalent of this finding.

B122

Molecular Monitoring of Acute Promyelocytic Leukemia (APL) Using DzyNA RT-PCR

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Aims
To assess the quantification of *PML/RARα* fusion transcripts by DzyNA RT-PCR as a method for diagnosing APL and monitoring disease levels in response to therapy.

Method
The catalytic properties of dnazymes were exploited in the development of a single step real-time quantitative RT-PCR assay (DzyNA RT-PCR), which was used to quantify the levels of *PML/RARα* fusion transcripts in total RNA from the bone marrow of patients with APL. Protocols allowed amplification of both L-type and V-type fusion transcripts and levels were

normalized to an endogenous control transcript *BCR* to control for variability in the integrity of RNA from patients' specimens. Relative transcript levels were expressed as a percent ratio of disease transcripts to control transcripts (RDC %).

Results and Discussion
PML/RARα transcript levels were quantified retrospectively in patients enrolled in the APML3 clinical trial currently being conducted by the Australasian Leukaemia and Lymphoma Group (ALLG). Fusion transcripts were detected in all specimens collected at disease presentation in a cohort of 39 patients thus confirming the diagnosis of APL. Patients expressed a wide range of transcript levels at diagnosis (average RDC 185 %, range 16 – 2342%) and there appeared to be higher levels in patients with V-type, as opposed to L-type, transcripts. Relative fusion transcript levels were also quantified in serial samples collected at regular intervals during and following treatment, and these correlated well with the clinical histories of individual patients. DzyNA RT-PCR consistently detected rises in fusion transcript levels three to six months prior to morphological and cytogenetic evidence of relapse.

Conclusions
Molecular analysis by DzyNA RT-PCR has utility as a tool for diagnosing and monitoring APL and allows early and reliable prediction of relapse. The ability to quantify leukaemia-specific transcript levels may assist clinicians in tailoring therapy for individual patients. Further, it provides a powerful tool for rapid and accurate monitoring of the efficacy of therapeutic regimens being assessed in clinical trials.

B123

Hereditary Hyperferritinaemia-Cataract Syndrome and Unexplained Familial Hyperferritinaemia in Australasia

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Hereditary hyperferritinaemia-cataract syndrome (HHCS) is an autosomal dominant condition presenting clinically as high serum ferritin levels (600 to >3000µg/L) with normal iron saturation, and a distinctive cataract morphology due to accumulation of ferritin deposits in the lens. This is the result of point mutations or deletions in the iron responsive element (IRE) at the 5' end of the ferritin light chain gene. Normally, 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. HHCS can be readily misdiagnosed as haemochromatosis leading to inappropriate venesection. We have previously reported three Australian families with this condition. We now report a further two families, one from Australia and one from New Zealand.

PCR amplification of genomic DNA and sequencing analysis was used to identify patients containing a mutation in the L-ferritin IRE. Two out of three members of the Australian family had a mutation at position +36 (C-A, London 2). Of the New Zealand cohort, three of five family members investigated revealed a new mutation at position +32 (G-C). Mutations at this position have been previously reported, however they are from G-A and G-T.

In addition to our HHCS cohorts, we have identified several families with inherited hyperferritinaemia, both with and without cataracts, who lack mutations in their L-ferritin IRE. In order to further elucidate potential mechanisms of hyperferritinaemia in these families, we have developed methods to measure the H-ferritin to L-ferritin ratios at both the messenger RNA (using Real Time PCR) and protein levels (using ELISA) in monocytes from normal individuals and from patients with haemochromatosis, acute phase hyperferritinaemia, HHCS, and unexplained hyperferritinaemia.

Preliminary results suggest that the absolute levels, and the ratios, of H and L ferritin mRNA and protein are characteristic in these groups, and may provide insight into novel mechanisms of familial hyperferritinaemia.

To date, only 25 families world-wide have been identified with HHCS. However, the discovery of 5 families with HHCS in Australasia indicates that this syndrome and other forms of inherited hyperferritinaemia, are potentially more common than first thought, and may be significantly under diagnosed.

B124

Generation of $CD4^+$ or $CD8^+$ CMV-Specific T Cells Using Dendritic Cells Pulsed with Different CMV Antigen Types

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Aims

Adoptive transfer of donor-derived cytomegalovirus (CMV)-specific cytotoxic T cell (CTL) clones can restore immunity in allogeneic stem cell transplant recipients, providing protection against CMV disease. However, both $CD4^+$ and $CD8^+$ CMV-specific T cells may be required to provide immediate and long-term restoration of immunity after adoptive transfer of T cells. Therefore we have investigated the use of different antigen types for the generation of $CD4^+$ and $CD8^+$ CMV-specific T cells using dendritic cells incubated with A) CMVpp65 (495-503) nonamer peptide, B) CMV cell lysate preparation, or C) adenovirus encoding CMVpp65 (Ad5pp65). METHODS: Monocyte-derived DC were generated from CMV seropositive donors and pulsed with pp65 peptide, CMV lysate, or transduced with Ad5pp65 and co-cultured with autologous PBMC. Cultures underwent two stimulations in the presence of 10 U/ml IL-2 during the first two weeks. After the second stimulation, cells were cultured in 50 U/ml IL-2 for 14 days and allowed to expand. CMV-specific cultures were then analyzed for specificity through flow cytometric phenotyping and tetramer staining, as well as cytotoxicity assays. RESULTS: DC efficiently stimulated CMV-specific T cells to expand from CMV-seropositive donors when pulsed with a range of CMV antigens. DC pulsed with CMVpp65 peptide produced CTL cultures that were >90% $CD8^+$ $Tetramer^+$ and had > 500-fold expansion of total cell number and > 1.7 x 10^5-fold tetramer expansion after 4 weeks in culture. Similarly, DC transduced with Ad5pp65 generated a strong $CD8^+$ CMV-specific response that was focused towards the pp65 (495-503) immunodominant epitope. Cultures stimulated with transduced DC expanded at similar rates as CTL generated by peptide-pulsed DC, and the frequency of CMV-specific CTL was similar (>90% $CD8^+$ $Tetramer^+$). DC incubated with CMV lysate (generated from CMV infected fibroblasts) produced a T cell culture, which was primarily $CD4^+$, but contained some CMV-specific $CD8^+$ (~ 10-30%). All cultures showed specific lysis of DC incubated with the appropriate antigen as well as killing target cells pulsed with pp65 (495-503) peptide. CONCLUSION: We present three different methods for stimulating CMV-specific T cells in vitro, and show that the presentation and processing of the antigen type by DC affects the type of cells generated in culture. The use of different CMV antigen preparations may be considered for generating either $CD4^+$ or $CD8^+$ CMV-specific T cells for adoptive immunotherapy.

B125

Optimising a Blood DC Preparation for Immunotherapy

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Objective
To establish a blood dendritic cell (BDC) isolation strategy for cancer immunotherapy. Large-scale production of BDC is now feasible using magnetic immunoselection based on the monoclonal antibody (mAb) CMRF-56.

Methods
Healthy adults underwent an apheresis procedure without mobilisation. Apheresis products were used for large-scale immunoselection with the mAb CMRF-56 and the CliniMACS® (Miltenyi Biotec, Germany) automatic closed system. Buffy coats were used for small-scale optimisation of the isolation conditions.

Results
We have demonstrated that small scale immunoselection of BDC with the mAb CMRF-56 produced a positive fraction of up to 86% $CMRF\text{-}56^+$ cells including up to 65% $CMRF\text{-}56^+$ $CD14^-$ $CD19^-$ DC, *i.e.* a 100 fold enrichment from the 0.5% starting DC population. The yield of $CMRF\text{-}56^+$ DC varied with individuals (n=20) with a mean of 66% (range 21%-91%) of the available BDC numbers (calculations based on total blood volume and percentage of DC prior to apheresis). The total number of cells after isolation in the positive fraction was an average of 1.2% of the starting PBMC population. The proportion of BDC ($CD14^-$ $CD19^-$ $CMRF\text{-}56^+$) in the positive fraction averaged 27% (range 6% - 65%). DC isolated were shown to be fully functional. We applied CliniMACS® technology to the immunoselection procedure, which allows the direct use of apheresis products. The procedure generated $CMRF\text{-}56^+$ preparations of similar quality to those observed in the smaller scale experiments, with a total product of up to 88 x 10^6 cells. In the process, we identified additional variables requiring optimisation for a large-scale preparation, and they include: overnight culture of PBMC at higher densities (optimal 50-80 x 10^6 cells/mL), protein additives (human serum albumin, HSA from the Australian Red Cross at a concentration of 0.75%), culture containers (various gas-permeable Teflon bags are being assessed) and the need for shaking during the incubation. We will present further the data regarding this clinically suited isolation procedure.

Conclusions
We describe a clinically applicable large-scale procedure for obtaining blood DC for cancer immunotherapy.

B126 **P34**

Autologous Stem Cell Transplantation at Liverpool Hospital

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Background
Peripheral Blood Stem Cell Transplantation has been shown to be of benefit in a number of specific malignant haematological conditions such as relapsed NHL, AML, MM. It may or may not be of benefit in other haematological, and solid organ tumours. A full Autologous PBSCT service has been provided at Liverpool hospital since 1995. We reviewed the outcomes of all patients transplanted at Liverpool Hospital between the initial transplant in 1995 and December 2001.

Methods
We performed a retrospective analysis of 102 patients having a total of 114 transplants (including 1 syngeneic, 8 patients having 2 transplants each and 2 patients having 3 transplants each) in regard to mortality and morbidity, engraftment time, blood product usage and utilisation of TPN. We also assessed different patient diagnostic groups and their relative outcomes.

Results
During the period under analysis, of the 114 transplants, there were 98 transplants in 93 patients with haematological conditions (the majority being NHL and MM) and 16 transplants in 9 patients having solid tumours. There was one death related to the procedure (<1%). Of the 89 patients in which follow up was available, at a median follow up of 16 months (mean 21.8 months, range 0-74 months), median long-term survival was 53%. Of the major subgroups survival was 70.7% amongst haematological malignancies and 50% amongst solid tumours. Time to engraftment defined as PMN >0.5 x 10^6, was related to number of CD34+ cells infused with <2 x 10^6 CD34 cells/kg infused taking a mean of 20 days and requiring a mean of 132 units of platelets and 13.6 units of packed cells each; 2-4 x 10^6 CD34 cells/kg infused taking a mean of 10.36 days and requiring a mean of 18.1 units of platelets and 3.4 units of packed cells each; >4 x 10^6 CD34 cells/kg infused taking a mean of 9.7 days and requiring a mean of 11.7 units of platelets and 3.1 units of packed cells each. During 2000 and 2001, 14 of 46 transplants required the use of TPN for a mean of 4.9 days.

Conclusions
In the group of patients treated with autologous PBSCT at Liverpool hospital between 1995 and 2001, the mortality of the procedure was negligible with good overall results. Long-term survival was comparable to national results.

B127 **P45**

Cefotetan Associated Haemolysis

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Prophylactic antibiotics are commonly used prior to surgery for the prevention of infection with contaminating organisms that may be present. Although generally safe, severe and life threatening complications may occur following this practice.

Cefotetan is a second-generation cephalosporin in wide spread use in general and obstetric surgery for prophylaxis because of its good broad-spectrum anti-microbial cover. It has been previously associated with autoimmune haemolysis. We report 4 cases of severe life threatening haemolysis that presented to our unit following administration of a single dose of intravenous cefotetan. Three of these were associated with obstetric surgery and the other was a general surgical case.

All cases presented with fever and symptoms of anaemia around day ten and showed evidence of autoimmune haemolysis with profound anaemia. The mean haemoglobin at presentation was 45g/L (range 41g/l-45g/l). The blood films showed the presence of spherocytes and marked polychromasia. The bilirubin and lactic dehydrogenase (LDH) were markedly elevated in each case and the haptoglobins were undetectable. The direct antiglobulin test was positive with both IgG and C3d. Using Diamed-ID / Coomb's microtube and saline tube anti-human globulin techniques, the mechanisms of antibody formation were shown to be antibodies to cefotetan in the patient's plasma and drug-anti-drug complexes in the patient's plasma.

In each case there was a delay in diagnosis. This was due to a search for an alternative explanation for the anaemia and cause of the fever (eg. Sepsis, blood loss). All patients recovered rapidly following treatment with intravenous steroids and blood transfusion in each case. Bilirubin and LDH levels returned to normal by day 8.

This report demonstrates that cefotetan-induced haemolytic anaemia is a severe life threatening condition. Physicians and surgeons should be aware of this serious complication of cefotetan to avoid misdiagnosis, withdraw the offending drug and to institute appropriate therapy. If the diagnosis is not considered there is the potential to readminister the offending drug with dire consequences including severe intravascular haemolysis. The possibility of cross reactivity with other cephalosporin must also be considered.

Patients should be informed of the nature of their allergy. A personal medical alert bracelet, hospital headsheet, and hospital computer record should be kept detailing the serious adverse event in order to prevent further drug exposure and subsequent haemolytic episodes. The occurrence of 4 cases in a relatively short time-period may be due to the increased use of cefotetan for surgical prophylaxis or a change in the composition of the drug.

B128 **P12**

Effects of Inhibitors of the Chemokine Receptor CXCR4 on Acute Lymphoblastic Leukemia Cells *In Vitro*

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Objective
SDF-1 is a key regulator of the behaviour of normal and leukemic pre-B cells. It is possible that inhibiting SDF-1 driven processes in pre-B acute lymphoblastic leukemia (ALL) may have therapeutic implications. A number of SDF-1 inhibitors, including SDF-1 derived peptides, peptides from polyphemusin II and bicyclams, have been developed in order to inhibit HIV infection of T cells. In this study we examined the ability of two of the most potent SDF-1 inhibitors, the bicyclam AMD3100 and the polyphemusin II derived peptide T140, to modulate pre-B ALL cell responses to SDF-1 *in vitro*.

Methods
The ability of AMD3100 and T140 to block SDF-1 specific receptor CXCR4 was examined using flow cytometry. Double chamber chemotaxis assay, and migration into bone marrow stromal layers were used to examine the effects of these inhibitors on chemotactic and migratory responses of pre-B ALL to SDF-1.

Results
The polyphemusin II derived inhibitor T140 and the bicyclam AMD3100 effectively inhibited binding of the anti-CXCR4 monoclonal antibody 12G5 on the pre-B ALL cell line NALM6, with IC_{50}s of 2 and 10 nM, respectively. Similar results were obtained with 8 ALL samples. Chemotaxis of pre-B ALL cells to SDF-1 was completely blocked in the presence of 10 μM AMD3100 and 1 μM T140. AMD3100 and T140 at 1μM were able to attenuate the migration of pre-B ALL cells into bone marrow stromal layers.

Conclusion
The ability of CXCR4 inhibitors to modulate these biologically important functions of leukemic cells warrants further investigation.

B131

MRD Detection in CBF Positive AML

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CBF positive AML is recognised as a good prognosis subset of AML. Routine allogeniec transplant in first CR has not been beneficial, since many patients are cured with chemotherapy alone. However 40 - 50% of patients with CBF AML still relapse. It has not been possible to predict relapse using qualitative PCR for the CBF fusion transcripts, however, quantitative PCR (QPCR) monitoring shows promise as a predictive assay. The aim of this study was to establish QPCR for both AML1-ETO and Type A CBFB-MYH11 fusion transcripts and monitor patients through treatment and remission. Using Taqman technology, transcript numbers are normalised against a housekeeping gene, (PGK) which is in turn normalised against an external calibrator (transcript from positive cell lines) to ensure that intra-run variation is minimised. Results are then expressed as an absolute copy number. Sensitivity these assays is one in 1 million cells (CBFB-MYH11) and 1 in ten million cells (AML1-ETO). Using these techniques we have studied 11 AML patients with AML1-ETO fusion transcripts, and 9 with CBFB-MYH11 type A transcripts. Median follow-up for the AML1-ETO patients is 13 months, (range 3-50) and 2 patients have relapsed. Median follow-up for CBFB- MYH11 patients is 11 months, (range 2-33) and 3 patients have relapsed.

In 4 of the 5 relapses, rising transcript levels were observed in the 2 – 3 months prior to clinical relapse. No samples were received from the 5th patient prior to relapse. Patients in ongoing remission have been characterised by transcript levels below 10^{-5} for AML-ETO and 10^{-4} for CBFB-MYH11 in our small series. Further data will be required to verify this and to identify threshold levels of transcripts predictive of relapse. Once such levels have been established, these assays may allow early clinical intervention with autologous or allogeneic transplant in patients destined to relapse.

B134 **P25**

Fludarabine Induced Immune Thrombocytopenic Purpura in Low Grade B and T cell Lymphoproliferative Disorders: A Report of Three Cases and Review of Pathogenesis

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Three cases of fludarabine associated autoimmune thrombocytopenia are presented, and the literature is reviewed.

Our cases include one patient with T cell Prolymphocytic Leukaemia and two with B cell Chronic Lymphocytic Leukaemia. To our knowledge, this is the first case reported of an autoimmune complication following fludarabine in T-cell lymphoproliferative disease.

The induction of autoimmune phenomena with fludarabine appears primarily to involve haemopoietic cells, in particular red cells and platelets, with few reports of non-haematological autoimmune complications. The same spectrum of autoimmune cytopenias are associated with untreated low-grade B-cell lymphoproliferative disease, suggesting that purine analogues may act synergistically to enhance a pre-existent underlying process in this setting.

However, the occurrence of Fludarabine-induced thrombocytopenia in a patient with T-cell lymphoproliferative disease suggests the possibility of a common pathway which is disease-independent, perhaps involving dysregulation of T suppressor cells allowing abnormal expansion of autoreactive B cell clones. Fludarabine has been shown to be selectively more toxic to T-cells than B-cells, with production of a profound, rapid and often persistent drop in CD4+ cells. Disruption of T-cell networks may therefore facilitate emergence of autoreactive clones.

B135

Severe Myelosuppression with Low-Dose Methotrexate

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Aim
To determine causes for severe myelosuppression in patients treated with low dose methotrexate.

Methods
Cases of methotrexate toxicity were identified at our institution from Pathology and Adverse Drug Reaction databases. Case reports were identified from Medline. Only cases with Grade 3 to 4 haematological toxicity were included in the analysis. The reasons for toxicity were determined where possible, along with cause of death. Age, methotrexate dose, renal function, concurrent treatments and comorbidities were determined and compared with nadir cell counts and death to determine risk factors using two tailed t-tests.

Results
Seven cases were identified at our institution between 1999 and 2001. A further 53 case reports, with adequate information to enable analysis, were identified. The majority of patients were treated for rheumatoid arthritis or psoriasis. Cases had a mean age of 58 years (range 44-91) and had been treated for a mean of 13.8 months (1 dose to 144 months). 18 (30%) patients died, all due to infectious complications of neutropenia. A single patient was subsequently diagnosed with multiple myeloma and 3 had folate deficiency, but no other predisposing primary haematological disorders were found. Drug interactions featured prominently as causes for toxicity, including co-trimoxazole, non-steroidal anti-inflammatory agents and leflunamide in two cases at our centre. Acute and chronic renal failure were also commonly cited causes of toxicity. In 10 cases methotrexate was accidentally taken in excess of the prescribed dose and in 6 cases no clear cause could be identified. Twenty cases (33%) occurred within 2 months of commencing methotrexate. Only the nadir neutrophil and leukocyte counts were significantly associated with death ($p=0.046$ and 0.004, respectively). There was a trend to lower neutrophil counts with lower creatinine clearances ($p=0.06$). Age ($p=0.223$) or dose ($p=0.12$) were not associated with death or nadir neutrophil counts in affected patients.

Conclusion
Life threatening and fatal pancytopenia due to low dose methotrexate therapy continues to occur in patients with known risk factors for methotrexate toxicity. In the majority of cases, risk factors for toxicity may be identified before or during treatment, providing the opportunity for early intervention to prevent this severe complication. Unexplained pancytopenia occurs in a small proportion of cases, suggesting a potential role for polymorphisms of methylenetetrahydrofolate reductase, thymidylate synthase or other enzymes involved with folate metabolism or purine synthesis. There does not appear to be any clear method to pre-select patients most at risk of septic death during pancytopenia.

B136 **P44**

Bone Marrow Aplasia Following Campath-IH Treatment in T-cell Prolymphocytic Leukaemia

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The use of the humanised monoclonal antibody Campath-IH, directed against the pan-lymphocyte antigen CD52, has been reported to be effective in lymphoproliferative disorders. We report a patient with T-cell prolymphocytic leukaemia (T-PLL) treated with Campath-1H following failure of fludarabine, who developed prolonged and ultimately fatal bone marrow aplasia. Although marrow aplasia has been previously reported in T-PLL following treatment with Campath-1H, the mechanism of marrow injury is unknown. Major haemopoietic toxicity has also been reported following Campath-1H therapy in B-cell chronic lymphocytic leukaemia, complicated by fatal opportunistic infection, but the incidence of marrow aplasia in this setting is not specified in the literature reports.

CD52 is a small glycosylphosphatidylinositol (GPI) linked glycoprotein, present on lymphocytes, monocytes and dendritic cells, but is not expressed on stem cells. Successful peripheral blood stem cell mobilisation and transplantation has been reported following Campath-1H, indicating preservation of stem cells during treatment.

Possible mechanisms of bone marrow injury producing aplasia, following Campath-1H therapy may include:

1. Suppression of regulatory T lymphocytes resulting in dysregulation of autoreactive lymphocyte clones, leading to autoimmune aplastic anaemia.
2. Acquisition of CD52 by stem cells, by transfer from T-PLL cells, leading to aberrant CD52 expression. Such transfer of the CD52 antigen has been documented in spermatogenesis. Non-specific incorporation of other GPI linked proteins into cell membranes has been documented in vivo, and could potentially occur in disorders with high levels of soluble CD52 liberated by complement-mediated cell lysis.
3. Decreased expression of other GPI linked proteins leading to a PNH phenotype. Although cells with a PNH phenotype have been reported following Campath-1H administration, the mechanism whereby this may be a cause for aplasia is uncertain.

Bone marrow aplasia has been consistently reported following use of Campath-1H in T-PLL, and may represent a unique but very serious adverse event confined to this subgroup of patients. Further investigation of the underlying mechanism/s will result in a greater understanding of the role of CD52, and the effects of Campath-1H on the haematopoietic system. Furthermore, susceptible individuals may be identified and preventative strategies designed to counteract this serious adverse effect.

B138

Lineage-Specific Variation in Responses of Philadelphia Chromosome Positive (Ph+) Cell Lines to Arsenic Trioxide (ATO) and Imatinib Mesylate (IMB) Alone and in Combination

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Aim
To evaluate responses of different Ph+ myeloid and lymphoid leukaemic cell lines to therapeutically attainable levels of ATO and IMB, and determine the impact of lineage on the combined action of these agents in vitro.

Methods
Myeloid CML blast crisis cell lines (K562, AR230), and Ph+ ALL cell lines (ALL1, TOM1), were maintained in culture for 3 days in the presence of ATO, IMB and ATO plus IMB. Cell counts were determined electronically, and viability, cell cycle status and degree of apoptosis by flow cytometry. Isobolograms were plotted to determine whether these agents acted in a synergistic, additive, or antagonistic manner in inducing growth inhibition.

Results
ATO and IMB exerted varying degrees of growth inhibition on all cell lines. The nature of the response of K562 cells to ATO was concentration dependent, starting from accumulation in S phase at lower levels, through G2/M arrest, up to apoptosis at much higher levels. The predominant effect of IMB at the usual therapeutic levels was induction of apoptosis, with a trend to synergistic action of these agents, in combination, in inhibition of proliferation.

AR230 cells displayed a similar response to ATO, and were more sensitive to IMB, where the dominant response was apoptosis. There was, in contrast, a trend for ATO and IMB to be antagonistic in inhibiting growth.

ALL1 cells were extremely sensitive to both agents, but displayed a different response pattern, with little change in cell cycle profile or apoptosis. In these cells the combination was antagonistic.

TOM1 cells underwent apoptosis with ATO, but less so with IMB. ATO acted in a synergistic manner with IMB in these cells.

Conclusion
Substantial lineage variation was found in the nature and degree of response of Ph+ acute leukaemia cell lines to ATO and IMB. Factors additional to inhibition of abl tyrosine kinase, therefore modulate the potency of IMB anti-leukaemic activity in evolved Ph+ disease. ATO, in some instances, produced greater toxicity than IMB and acted in a synergistic manner with IMB, indicating a potential therapeutic role for combined treatment. Contrasting responses in other cell lines, however, indicates the likelihood of major individual variation in clinical disease response to ATO and IMB.

B139

The Effect of $CD34^+$ Stem Cell Selection on Patient Outcome in Multiple Myeloma

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The degree of tumour contamination of transplanted stem cell autografts may adversely affect clinical outcome. Studies have indicated that patients transplanted with PBSC products containing low numbers of myeloma plasma cells or with allogeneic stem cells have a better outcome than those transplanted with higher numbers. Positive stem cell selection, based on CD34 antigen expression, enables the passive depletion of contaminating tumour cells. T cells are also depleted during stem cell selection and this may result in delayed immune reconstitution. This study evaluates and compares engraftment, incidence of infection and survival outcome of myeloma patients transplanted with CD34 selected or unselected PBSC grafts.

In a non-randomised study, 22 patients (12 male, 10 female, mean age 56.2 $\pm$ 1.4 (SEM) years) received CD34 positively selected PBSC autografts following myeloablative therapy, while 18 patients (9 male, 9 female, 51.0 $\pm$ 2.5 years), treated similarly and within the same time frame, received unselected PBSC autografts. CD34 positive selection was performed using the Isolex 300i (Baxter) system. Reduction in malignant plasma cell content of selected PBSC products was monitored by flow cytometry and by PCR for the IgH rearrangement. Engraftment was recorded as days to neutrophils $\geq 1.0 \times 10^9$/L and platelets $\geq 50 \times 10^{12}$/L. All infections were noted up to 2 years post transplant. Patient survival was analysed using the Kaplan-Meier method.

Patients received selected grafts containing $2.4 \pm 0.2 \times 10^6$ $CD34^+$ cells/kg or unselected grafts of $3.3 \pm 0.7 \times 10^6$ $CD34^+$ cells/kg. CD34 selection resulted in a 2-3 log reduction of plasma cells, however 75% of products remained positive by PCR for the malignant clone. Neutrophil and platelet engraftment was not different for patients receiving selected (15.7 $\pm$ 0.5 days, 22.6 $\pm$ 2.2 days respectively) or unselected grafts (14.1 $\pm$ 1.0 days, 18.4 $\pm$ 1.6 days). However, patients receiving selected grafts had a higher incidence of infection (86%) than those receiving unselected grafts (61%). No difference in overall survival ($p=0.45$) was seen, although median survival has not been reached for either group. The Kaplan-Meier mean survival for patients receiving selected grafts is 48 months and 43 months for the unselected group.

CD34 selection of PBSC with the Isolex 300i does not result in a significantly prolonged time to engraftment post transplantation but is associated with a higher incidence of infection. Despite a significant reduction in the number of malignant plasma cells in selected grafts, no difference in overall survival was observed between recipients of selected or unselected grafts.

B140

Vinorelbine and Gemcitabine with Filgrastim (VGF) Support Shows Significant Activity Against Advanced Lymphoma

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Both vinorelbine and gemcitabine have single agent activity in patients with advanced lymphoma. We have previously reported preliminary data from an outpatient pilot study of vinorelbine and gemcitabine with filgrastim (VGF) support in advanced lymphoma and now expand on that initial experience.

Forty patients with relapsed or refractory non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD) were accrued between February 2001 and April 2002. 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/m^2 and gemcitabine 1000 mg/m^2 on days 1 and 8 with filgrastim at 5 μ/kg/day days 2 to 7 and day 10 until neutrophil recovery or day from 9 until neutrophil recovery.

Median age was 58 years (range, 23-74). Diagnoses included follicular B-cell NHL (n = 13), DLC B-cell NHL (n = 7), peripheral T-cell NHL (n = 10) and HD (n = 8) with a median of 3 prior treatment regimens (range, 1-11). Twelve patients had undergone prior ASCT and 15 had never achieved a prior CR. At the time of treatment, disease status was primary refractory (n = 3), refractory relapse (n = 1), first or second relapse (n = 26) or third or subsequent relapse (n = 13). To date 114 VGF cycles have been delivered with 32 patients having received 2 or more cycles and 38 patients are evaluable for response on an intention-to-treat basis. Treatment was generally well tolerated with unplanned hospital admission required during 27 (24%) of treatment cycles. Grade 4 haematological toxicities were uncommon (neutropenia 13% and thrombocytopenia 15%). The commonest non-haematological toxicity was phlebitis with 10 (25%) patients experiencing 1 or more episodes. There were 2 infectious deaths (invasive Aspergillosis; Legionella pneumonia) and 2 other non-lymphomatous deaths (AMI; pneumonitis) all occurring despite haematological recovery. Twelve and 4 patients proceeded to ASCT or alloSCT, respectively. Overall response rate with VGF was 55% (n = 21) (5CR + 4Cru + 12PR) including 7 patients (58%) with a prior ASCT. A further 7 patients (18%) had stable disease (<50% response). With a median follow-up of 9 months the actuarial probability of overall and progression free survival at 12 months is 65% and 59%, respectively. We conclude that VGF shows significant activity against advanced lymphoma and can be administered in an outpatient setting.

B141

Both Mitochondrial (Type 2) and Non-Mitochondrial (Type 1) Apoptotic Pathways are Activated in Authentic Multiple Myeloma Cells by Apo2L/TRAIL

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TNF-alpha related apoptosis inducing ligand (TRAIL/Apo2L) is a member of the TNF family and has unique potential as an anti-tumour agent. We have demonstrated previously that TRAIL induces apoptosis of primary multiple myeloma (MM) tumour cells from MM patients but does not appear to be harmful to normal haemopoietic cells. Furthermore, we have demonstrated that one or both of the effector receptors for TRAIL (R1 and R2) is present on 90% of primary MM tumour populations and that TRAIL resistance is not due to expression of TRAIL decoy receptors (R3 and R4). To develop a rational approach to overcoming TRAIL resistance and the development of synergistic drug combinations with anti-MM potential we have endeavoured to define the mechanism(s) by which TRAIL induces apoptosis. Five authentic MM cell lines expressing both R1 and R2 were used as targets for TRAIL evaluation. At 1 hour post-TRAIL treatment 3 lines OPM-2, RPMI 8226 and LP-1 demonstrated sensitivity to TRAIL with 39%, 37% and 34% apoptosis, respectively, whereas NCI H929 and U266 were resistant with only 8% and 5% apoptosis, respectively. The 3 sensitive lines all demonstrated efficient cleavage of Pro-caspase 8 within 15 minutes of TRAIL treatment but this was delayed and reduced in the resistant lines. All 5 lines exhibited similar cytosolic accumulation of both Cytochrome C and SMAC confirming activation of the Type 2 pathway. To clarify the relative contributions of the Type 2 pathway it was inhibited by pre-incubation with a Caspase 9 inhibitor and the effect on TRAIL-induced apoptosis determined. This revealed a negative correlation between uninhibited TRAIL-induced apoptosis and the effect of Type 2 pathway inhibition with a reduction in apoptosis of 7.1%, 20.3%, 48.8% and 64.3% for the lines RPMI8226, LP-1, NCI H929 and U266, respectively. The latter is consistent with a greater reliance on the Type 2 pathway in MM cells with low TRAIL-induced Procaspase 8 activation efficiency. In contrast, there is significant redundancy in cells with efficient TRAIL-induced Procaspase 8 activation. Based on this we hypothesised that enhancing Type 2 pathway activation in cells relatively resistant to TRAIL may enhance TRAIL-induced apoptosis. Preliminary experiments with NCI H929 have demonstrated significant synergy between TRAIL and dexamethasone consistent with this hypothesis. We conclude that both mitochondrial and non-mitochondrial apoptotic pathways are activated in MM cells by TRAIL and this observation provides a basis for a rational approach for the evaluation of synergistic anti-MM drug combinations with TRAIL.

B142

Donor Pretreatment with G-CSF Prevents Chronic Graft-Versus-Host Disease Apfter Allogeneic Stem Cell Transplantation

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The incidence of extensive chronic GVHD (cGVHD) after allogeneic stem cell transplantation with G-CSF mobilised peripheral blood stem cell (PBSC) products is increased compared to that after bone marrow transplantation and necessitates prolonged, expensive and poorly effective immunosuppressive therapy. G-CSF is known to alter T cell function and PBSC products contain 10-20 times the number of T cells relative to bone marrow grafts, both of which may increase cGVHD. We have studied this in two well-described models of cGVHD manifested by immune complex glomerulonephritis (DBA/2 → B6D2F1) and sclerodermatous hepatic and cutaneous fibrosis (B10.D2 → Balb/c). The former model induces GVHD to major and minor HA and the latter to minor HA only. Donor mice were treated with G-CSF or diluent for 6 days and transplanted with unseparated splenocytes that contained equal numbers of T cells. In the immune complex model, 80% of SCT recipients that received grafts from control treated donors develop nephrotic syndrome compared to 25% in the recipients of G-CSF treated grafts ($P<0.01$) and mortality is decreased from 45% to 15% ($P<0.05$). Although donor engraftment and B cell activation are equivalent in both groups, the reduction in cGVHD following donor G-CSF pretreatment occurs in the setting of donor Th2 differentiation manifested by reduced interferon-γ and increased IL-4 production with concomitant increases in IgG1 and reductions in IgG2α auto-antibody generation. In the scleroderma model, GVHD mortality is significantly reduced by donor pretreatment with G-CSF (8% v 24%, $P<0.05$) and GVHD severity in surviving animals is reduced by 50% as determined by GVHD clinical score ($P<0.01$). However, if the donor G-CSF T cell dose is escalated 5-fold (G-CSF+T), cGVHD mortality and is increased from 8% to 60% ($P<0.01$). The severity of cutaneous cGVHD (maximum score = 4) in recipients of control, G-CSF, G-CSF+T and syngeneic grafts is 1.1 ± 0.1, 0.8 ± 0.1, 2.1 ± 0.3 and 0.0 ± 0.0 ($P<0.01$, G-CSF v G-CSF+T) and is characterised histologically by epidermal thickening, fibrosis and procollagen deposition. The severity of histological hepatic cGVHD after SCT in the same groups is 9.1 ± 0.8, 5.1 ± 1.2, 7.8 ± 1.1 and 1.2 ± 0.3 as determined by semi-quantitative histology ($P<0.05$, G-CSF v G-CSF+T). These data suggest that donor pretreatment with G-CSF induces donor Th2 differentiation which is associated with an impaired ability to induce cGVHD. Thus, the limitation of T cell doses transferred during PBSCT should reduce both acute and chronic GVHD compared to BMT, whilst maintaining the beneficial effects on graft quality.

B143

Donor Pretreatment with Progenipoietin-1 is Superior to G-CSF for the Prevention of Acute Graft-Versus-Host Disease after Allogeneic Stem Cell Transplantation

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The chimeric G-CSF and FLT-3L receptor agonist Progenipoietin-1 (ProGP-1) has potent effects on dendritic cell (DC) expansion and may be superior to G-CSF for the mobilisation of stem cells for allogeneic stem cell transplantation (SCT). We studied the ability of stem cell grafts mobilised with this agent to induce acute graft-versus-host disease (GVHD) to minor and major histocompatibility antigens in the well described B6 → B6D2F1 SCT model. ProGP-1, G-CSF or control diluent was administered to donor B6 mice. ProGP-1 expanded all cell lineages in the spleen and blood with dramatic expansion of DC and granulocyte lineages (2-log increase). Unseparated splenocytes from these animals produced large amounts of IL-10 and TGFβ (3 and >10 fold increase respectively over control and G-CSF) while the expression of T cell adhesion molecules (L-selectin, CD44) were diminished several logs. Transplant survival was 0%, 50% and 90% in recipients of control, G-CSF and ProGP-1 treated allogeneic donor splenocytes respectively ($P<0.0001$). Donor pretreatment with ProGP-1 allowed a four-fold escalation in T cell dose over that possible with G-CSF. Donor CD4 T cells from allogeneic SCT recipients of ProGP-1 splenocytes demonstrated an anergic response to host antigen and cytokine production (IFNγ, IL-4 and IL-10) was also reduced while CD8 T cell cytotoxicity to host antigens remained intact. Neither $CD11c^{hi}$ DC nor "plasmacytoid" $CD11c^{dim}/B220^{hi}$ DC from ProGP-1 treated donor animals conferred protection from GVHD. Conversely, when equal numbers of purified T cells from control, G-CSF or ProGP-1 treated allogeneic donors were added to allogeneic T-cell depleted control spleen, survival at day 60 was 0%, 15% and 90% respectively ($P<0.0001$). The improved survival in recipients of ProGP-1 T cells was associated with reductions in systemic TNFα generation (Control v ProGP-1: 227±82 v 35±14 pg/ml, $P<0.01$) and GVHD of the GI tract (semi-quantitative GVHD score: 19.5±2.1 v 9.5±0.4, $P<0.01$). These data suggest that in addition to increasing stem cell yields, donor pretreatment with ProGP-1 will also be superior to G-CSF for the prevention of GVHD after allogeneic SCT, primarily due to incremental affects on T cell phenotype and function.

B144

Autologous Platelet Cryopreservation and Reinfusion – The Liverpool Hospital Experience

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Cryopreservation and reinfusion of autologous platelets have been performed by several groups including Schiffer CA et al., 1982, Funke I et al., 1995, Bentley M et al., 2001 and Vadhan-Raj et al., 2001. The procedure was safely used by these groups to support alloimmunized patients with acute leukemia undergoing high dose chemotherapy, those undergoing autologous stem cell transplantation and those with carboplatin-associated severe thrombocytopenia, respectively. Platelets were generally mobilised by utilising rhTPO. At Liverpool Hospital two patients undergoing curative chemotherapy for acute myeloid leukemia, who were refractory to Red Cross Blood Bank donor platelet transfusions, underwent apheresis collection of their platelets, whilst in remission. Platelets were collected by apheresis following chemotherapy, cryopreserved in 5% DMSO and stored in liquid nitrogen. Following consolidation chemotherapy the platelets were transfused back into the patients. Several series of platelet transfusions were performed on both patients with one and 24 hour post-transfusion platelet counts evaluable on all series.

Total number of transfusions (two patients)	13
Median autologous platelet dose	2.4×10^{11} (range 1.8 to 3.8×10^{11}).
Median increment at one hour post transfusion	9 (range 3-24)
Median increment at 24 hour post transfusion	7 (range 0-14)

This form of treatment allows support for alloimmunized patients without the need for TPO, has no toxicity and is convenient (eliminates the difficulty of locating matched platelets from Red Cross Blood Bank and allows the storage of platelets that could be transfused as clinically needed).

B147

Quantification of Feto-Maternal Haemorrhage (FMH) by Flow Cytometry: Anti-HbF Labeling Potentially Underestimates Massive FMH Compared to Labeling with Anti-D

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Background
Many centers now routinely use flow cytometry for the quantification of feto-maternal haemorrhage (FMH), typically using either anti-D or anti-HbF to label D-positive or HbF containing fetal cells present in the maternal circulation. Which flow cytometric method is the most accurate in quantifying FMH is currently unknown.

Aim
To compare anti-D and anti-HbF labeling for the detection of D-positive fetal red cells in FMH.

Methods
Both directly conjugated FITC-monoclonal anti-D (Fluro-D, CSL, Victoria) and PE-monoclonal anti-HbF (MHFH04, Caltag Laboratories, CA) are available for the quantification of FMH by flow cytometry at our institution**.** An audit of clinical results in which both methods had been performed was initially carried out to determine if any significant discrepancies in results were present between the 2 different methods. Subsequent to this audit, 46 samples of adult D-negative blood were spiked with varying amounts of D-positive cord blood (0.05 – 10% fetal cells / sample). The number of fetal cells present in each spiked sample was then quantified by both anti-D and anti-HbF labeling to determine the relative accuracy of the two different flow cytometric methods.

Results
In total, 17 clinical samples from 14 patients with maternal blood group D-negative and fetal group D-positive had had FMH estimated by both flow cytometric methods. Although overall there was no significant difference in the size of FMH estimated by the 2 flow cytometric methods ($r=0.985$, $t=1.314$, $p=0.208$), in 2 of 3 patients with massive FMH (>25mls fetal RBC), the estimated FMH using anti-HbF was 13-32% lower than that estimated by labeling with anti-D. In the spiked samples, the percentage fetal cells detected by anti-D labeling was very similar to the percentage of fetal cells added ($r=0.994$, $t= 0.476$, $p=0.636$). However, anti-HbF labeling significantly underestimated the estimated percentage of fetal cells present ($r=0.987$, $t=4.215$, $p=0.0001$). In comparison to anti-D, the percentage of fetal cells detected by anti-HbF was also significantly lower ($r=0.995$, $t=4.761$, $p<0.0001$). On subgroup analysis, this difference in fetal cell detection between anti-D and anti-HbF labeling was only apparent in the 22 samples containing >= 1% fetal cells per sample ($r=0.992$, $t=7.873$, $p<0.000001$). In the 24 samples containing <= 0.6% fetal cells, no significant difference in detection of fetal cells between anti-D and anti-HbF labeling was observed ($r=0.892$, $t=1.670$, $p=0.11$).

Conclusions

Both our clinical and laboratory data suggest that quantification of D-positive fetal cells by flow cytometry using directly conjugated anti-HbF potentially underestimates the number of fetal cells present in samples containing >=1% fetal cells in comparison to labeling with anti-D. As such, to allow adequate immunoprophylaxis in D-negative mothers with massive FMH, we recommend that anti-D labeling should be used for the flow cytometric estimation of FMH.

B148 **P09**

Disease Persistence in ALL - Establishing Reliable Quantitative Data at the Single Cell Level

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The importance of minimal residual disease in childhood ALL as a predictor of outcome has been established by painstaking quantitation of disease markers by molecular techniques. These results have suggested that the ability of leukaemic cells to persist throughout induction chemotherapy dictates the propensity of leukaemic relapse. The genes expressed by a persistent leukaemic population are therefore of major importance. This population may represent very low cell numbers from different leukaemic sub-populations in the immediate post induction chemotherapy period (Day 35). To analyse these cells, we must sort single cells with accuracy from the background of a recovering bone marrow. Success of single cell analysis requires: (1) proof of the ability to sort single cells (2) proof that the single cell is of leukaemic origin. Hence, we have examined the ability of current technologies to reliably quantify cell number and gene expression at the single cell level using cell sorting, four-colour fluorescence detection of leukaemic populations and real-time PCR. We found RT-PCR amplification of 28S ribosomal RNA to be 100% (n = 15) efficient in amplifying cDNA from a single cell. However, the efficiency of PCR amplification of DNA representing the disease marker, the immunoglobulin heavy chain gene, was only 47.7% (SD = 9.2 n = 108). This is equivalent to a Poisson distribution derived from the detection of a single molecular event in 0.7 cells per well. This was compared with growth characteristics of the cells where sorting at 1 cell per well resulted in colony formation in 57.6% of wells (S.D. 13.1 n = 6 x 96). This last figure is consistent with growth efficiency of single sorted cells governed by a Poisson distribution. Hence, statistically we are successfully sorting at a single cell per well and determining the purity of the sort optimally for the current level of technology. We will now use this data to validate the purity of the sorted leukaemic cell population for paired diagnostic and day 35 patient samples, so that pure leukaemic clones can be analysed for the expression of drug resistance-related genes. From this the cells can be pooled and analysed by real-time PCR for the expression of selected drug resistance related genes as well as amplified for analysis by gene expression arrays.

B151

Peripheral Blood Stem Cell Viability Post Cryopreservation

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Peripheral blood stem cells (PBSC) are collected by apheresis from patients and normal donors for future transplantation. In the laboratory the cells are washed twice with normal saline, cryopreserved and stored in liquid nitrogen. At the time of cryopreservation pilot ampoules are stored for future testing.

Viability testing is performed on thawed pilot samples to determine the number of stem cells that survive processing, cryopreservation and storage. Cell viability is assessed by flow cytometry using 7AAD with analysis being performed within 30 minutes of sample thawing.

Generally harvested stem cells are washed and cryopreserved in the laboratory on the day of collection. It is sometimes necessary to hold the product at 4^{o}C overnight before processing the following day. We used the viability assay to assess the impact of cell washing and overnight storage on product viability and yield. We also examined the impact of cellular composition on product yield and viability.

We analysed data from 260 apheresis products for which estimation of viable total cells and CD34^{+} cells had been performed on the product prior to processing and on the corresponding thawed cryopreserved pilot sample.

Products that had been washed had significantly higher cell viability (Mann Whitney) as well as significantly higher post thaw viability and viable CD34^{+} yield.

There was no significant difference in product viability pre- and post-processing between cells that had been stored and those that were processed immediately. There was also no significant difference in post-thaw cell viability and viable CD34^{+} yield.

	Effect of washing			Effect of storage	
	Cells washed x 2 n=216	Cells not washed n=30		Cells processed immediately n=44	Cells stored overnight n=172
% viable cells pre-processing	98 (73-100)	98 (77-100) NS, p=0.87		98 (79-100)	97 (73-100) NS, p=0.95
% viable cells post-processing	96 (76-100)	91 (40-99) p=0.004		96 (81-99)	96 (76-100) NS, p=0.59
% viable cells post-thaw	78 (23-98)	73 (22-97) p=0.039		85 (34-97)	78 (28-98) NS, p=0.09
% $CD34^+$ recovery post-thaw	82 (13-100)	54 (21-100) p=0.004		86 (13-100)	81 (17-100) NS, p=0.25

There was no correlation (Spearman Rank) between either the number of neutrophils or the number of immature cells in the starting product and product viability or CD34 yield of the thawed cells.

We conclude that while overnight storage and the starting number of neutrophils had no effect on the $CD34^+$ yield of the thawed product, washing of the apheresis product improved both the viability and yield.

B152

Peripheral Blood Stem Cell Apheresis Collection Stored Overnight Prior to Cryopreservation

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Because of increasing laboratory workload and limited availability of equipment it may not always be possible, or practicable to process and cryopreserve Peripheral Blood Stem Cell (PBSC) harvests on the day of collection. We have analysed data from 30 patients treated at our institution who have undergone at least two apheresis collections, with one being kept overnight prior to processing and cryopreservation.

PBSC were collected using either the Baxter CS3000 or COBE Spectra. Fifty 50ml of autologous plasma was added to the harvested cells at the end of apheresis procedure. In the laboratory the stem cells were washed twice with saline to remove platelets and reduce volume. Washed cells were resuspended in 10% DMSO and 20% autologous plasma in saline at a final maximum cell concentration of 200 x 10^6/ml and frozen using Kryo 10 control rate freezer.

For samples processed on the day of collection processing commenced within an hour of collection. If samples were processed the day following collection processing commenced between fifteen and twenty hours after collection. Samples were stored overnight in a monitored blood fridge at 4^{o}C until processing commenced. White cell counts, cell viability, and volume measurements were performed on all samples prior to and on completion of washing. At the same time viable $CD34^+$ numbers were estimated by flow cytometry using 7AAD. Viable $CD34^+$ numbers and total cell viability were also estimated on pilot samples of cryopreserved cells which were thawed for analysis.

	Processed on the day of collection n=24	Processed on the day after collection n=35
% Cell viability pre-processing	98 (89-99)	97 (79-99)
% Cell viability post-wash	95 (84-99)	96 (82-99)
% Cell yield post-wash	94 (55-100)	93 (79-100)
%Viable $CD34^+$ yield post-wash	93 (49-100)	91 (59-100)
% Cell viability post-thaw	84 (42-98)	73 (34-97)
% $CD34^+$ recovery post-thaw	82 (42-100)	73 (13-100)

Using the Mann Whitney Test there was no significant difference between samples processed on the day of collection with those processed on the day following collection for cell yield and viable $CD34^+$ cell yield post-washing, or $CD34^+$ yield post-thaw. There was also no significant difference between the two groups for cell viability pre- and post-washing and post-thaw.

We conclude that storing PBSC product overnight, at 4^{o}C diluted in autologous plasma does not have a detrimental effect on the washed product or the final cryopreserved product.

B155 **P03**

MRP1 Analysis of Leukaemic Cell Lines - A Tighter Correlation of Expression and Function

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Multidrug resistance is a ubiquitous problem in the management of cancer. The role of specific drug resistance proteins in this process has been difficult to assess and the correlation between mRNA, protein expression and function of particular genes has not always correlated. We describe a flow cytometric approach to determine the contribution of MRP1 to the multidrug resistance phenotype, which results in a tighter correlation of MRP1 protein expression and function. Cell lines representing B, T and myeloid lineage leukaemia, were investigated for their expression of MRP1 and other drug-transporter superfamily members by quantitative RT PCR. Flow cytometry was used to assess MRP1 protein expression in these cell lines and to measure the activity of MRP1 using the MRP specific dye, CFDA. MK571 was used as a known specific inhibitor of MRP1. The excretion of the dye follows a second order polynomial curve which is approximately linear for the first 30 minutes. The slope of this section of the curve represents the ability of MRP1 to export the dye from the cell and is dependent on the absolute amount of MRP1 present on the surface membrane of the cell. This correlates well with the relative protein expression for each cell line as measured by flow cytometry using monoclonal antibodies specific for MRP1, but does not correlate as tightly with MRP1 mRNA as measured by real time PCR. The alteration in the slope of the curve following MK571 represents inhibition of MRP1 related excretion, which is important when comparing the transport of non-MRP1 specific substrates such as daunorubicin between cell lines. Using this approach and flow cytometric analysis of anti-apoptotic proteins, a multidrug resistance screening of leukaemic cells can be performed and analysed in a working day. This is important in the ability to implement drug resistance protocols for patients as a primary treatment strategy and to stratify the role of the various ABC transporters in the drug resistance phenotype.

B157 **P21**

Filgrastim Can Mobilise CD34+ Cells in Patients with Chronic Myeloid Leukaemia Patients Who Achieve Complete Cytogenetic Response (CCR) on Imatinib Mesylate Without Affecting Blood Levels of BCR-ABL

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Aims
CML patients who achieve CCR whilst on Imatinib (Glivec, formerly as STI571) have a low risk of progression in the first 2 years but no data exists on long-term stability. Autologous stem cells (PBSC) collection when the leukaemic burden is lowest is rational and several strategies are being assessed. We have studied mobilisation with G-CSF (Filgrastim, Amgen, USA) while continuing imatinib therapy to assess the subsequent level of leukaemia and the adequacy of cell yields.

Methods
10 CML patients with confirmed CCR by conventional cytogenetics were enrolled in the prospective study. Filgrastim 10 μg/kg/day s.c. was given with ongoing imatinib at the previously established dose. Harvest was commenced on day 4. Cell yields were measured by the mononuclear cells (MNC) and CD34+ cells and correlated with the duration of CML and imatinib treatment. Serial blood specimens before and after mobilisation were collected for *bcr-abl* transcript levels using quantitative RT-PCR as previously described.

Results
(a) After an average 3.2 runs of apheresis (range 2-5), the median CD34+ cell yield was 2.27 x 10^6/kg body weight (bw) (range 0.64-4.35) with a median CD34+ cell yield per apheresis 0.67 x 10^6/kg bw (range 0.32–2.18). Two patients (20%) had inadequate collection (<1.0 x 10^6/kg) while 2 had a borderline collection (1.7-2.0 x 10^6/kg). No significant adverse effects were noted.
(b) The duration of imatinib therapy did not correlate with either the CD34+ yield (r = 0.14) or the MNC yield (r = -0.57) per apheresis. Similarly, the duration of CML did not correlate with either parameter (r = -0.08 and 0.41 respectively).
(c) Serial blood *bcr-abl* levels remained below 0.5% in all patients: at a median 0.06% pre- and during mobilisation (range 0.0-0.42%) compared to a median 0.03% post-mobilisation (0.03; range 0.0-0.28%) after a median follow-up of 23 weeks.

Conclusions
Filgrastim at standard dose allows an adequate autologous PBSC collection in the majority of imatinib treated CML patients in CCR

Neither the duration of CML, nor the duration of imatinib administration appear to affect the PBSC yields.

The process of Filgrastim-mobilisation does not appear to have any significant effect on the leukaemia level of CML as quantitated by RT-PCR of the blood *bcr-abl* transcript levels. Comparative studies of the minimal residual disease status of the apheresed products using quantitative PCR and FISH are ongoing.

B158

Monitoring Glivec Activity in Vitro and in Vivo Using Western Blotting of Crkl Phosphorylation

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The anti-CML drug Glivec (formerly STI571) blocks the binding of ATP to the kinase of BCR/ABL, preventing substrate phosphorylation. Point mutations within the ATP binding pocket of Bcr-Abl, are thought to be responsible for Glivec resistance and relapse.

Our aim is to monitor Glivec inhibition of kinase activity using phosphorylation of the adaptor molecule Crkl by BCR/ABL, both at the level of in vivo tissue levels, and to allow *in vitro* analysis of the inhibition of BCR/ABL by titration. This will enable us to determine if intracellular Glivec levels are sufficient to achieve full inhibition of wild type kinase activity, and to examine BCR/ABL mutants for resistance to Glivec.

Methods
Primary CML cells and cell lines were cultured alone or with Glivec at 0-10 micromolar for 2 hours or overnight. To assess *in vivo* levels of Glivec activity, primary CML cells from blood were taken before and after therapy commenced. In some experiments, drug treated cells were cultured in medium without Glivec to assess the kinetics of reversal of Crkl phosphorylation inhibition. Protein lysates were run on 10% PAGE gels and transferred to nitrocellulose membranes. Enhanced chemiluminescence detection of anti-Crkl antibody binding allowed quantitation of both Crkl and phosphorylated Crkl. As a model of *in vivo* Glivec levels, NOD/SCID mice were injected with primary CML cells by the peritoneal route, followed by recovery of cells from the peritoneum after different doses of orally administered Glivec to assess inhibition of Crkl phosphorylation.

Results
Primary CML cells show inhibition of Crkl phosphorylation with an IC50 of around 1 micromolar. In contrast, cells from patients who have developed BCR/ABL mutations and resistance exhibit an IC50 of >10 micromolar. Preliminary experiments revealed partial inhibition of Crkl phosphorylation in CML cells taken from patients on Glivec therapy, which may be related to the finding that removal of Glivec from cultured CML cells results in a return to baseline phosphorylation of Crkl within approximately 2 hours.

Conclusions
Our data show that primary CML cells can be used to estimate IC50's of Glivec for wildtype and mutant BCR/ABL. Preliminary results show it is possible to demonstrate inhibition of Crkl phosphorylation in primary CML cells taken from peripheral blood of patients undergoing therapy with Glivec. Generation of dose response curves which can be used to estimate effective tissue levels of Glivec, as well as detect mutated BCR/ABL less sensitive to Glivec inhibition. However, sample handling must be tightly controlled, as there is relatively rapid reversal of inhibition of Crkl phosphorylation when cells are removed from Glivec-containing environment.

B159

Evidence for the Persistence of Leukaemic Cells in Glivec Responders Achieving Complete Cytogenetic Remission

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Chronic myeloid leukaemia is a clonal stem cell disorder characterised by excess myeloid proliferation in the chronic phase. Curative treatment strategies have been limited to allogeneic transplant with chemotherapy affording little benefit long term. Relapses in allogeneic transplant recipients and long term interferon responders have been reported, indicating the presence of a long lived, possibly quiescent population of leukaemic cells.

Recently, complete haematological (CHR) and cytogenetic responses (CCR) have been reported in a high percentage of chronic phase CML patients treated with Glivec. We have analysed the G-CSF mobilised PBSC and remission BM samples from 8 Glivec treated patients who exhibited CCR, for the presence of the BCR-ABL fusion transcript. Q-PCR for BCR-ABL performed at remission or during mobilisation was below 0.5% in all cases (median 0.06; Range 0 - 0.42%). In two patients the level of transcription was below the level of detection of the assay (Sensitivity 1 K562 cell in 10^5 normal cells). Conversely in all cases BCR-ABL positive cells were detected using interphase FISH with results ranging from 1% to 16% (median 8.9%)(normal <1.0%). Hypermetaphase FISH analysis following growth factor stimulation failed to identify any BCR-ABL

positive cells from the mobilised collections. Interestingly FISH on interphase cells in these cultures demonstrated BCR-ABL positivity ranging from 3 to 16%. Thus we have identified a population of leukaemic cells which persist in Glivec responders but express low levels of BCR-ABL. Failure of these cells to give rise to metaphases in growth factor stimulated culture leads us to believe that these cells may be quiescent in nature.

We have previously demonstrated the existence of a population of quiescent progenitor cells (approximately 3% of CD34+ cells) in presentation material from CML patients. It is known that these cells have self-renewal capabilities and we have demonstrated their arrest in Go is transient. These cells when stimulated to divide are highly proliferative, however this proliferation can be abrogated by the addition of Glivec. We have been unable to demonstrate an effect of Glivec at clinically achievable concentrations on these cells whilst they remain quiescent. We postulate that quiescent leukaemic progenitors with low or no detectable levels of BCR-ABL are relatively insensitive to Glivec. Strategies to induce these cells to cycle may be necessary to eradicate this potential source of resistant cells.

B160

Stem Cell Mobilization in Healthy Donors

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Aim
Peripheral blood stem cells (PBSC's) instead of bone marrow can be used for allogeneic transplantation. Mobilization of stem cells in healthy donors is achieved with cytokines such as granulocyte colony-stimulating factor (G-CSF). Westmead Hospital has been using mobilized PBSC's from healthy donors for allografting since 1995. We observed a wide variability in the PBSC collection between different healthy donors. We were interested whether the pre mobilization (or resting) CD34+ cell count in the peripheral blood allows estimation of mobilization of CD34+ cells following G-CSF administration, and whether higher doses of G-CSF would allow better mobilization.

Methodology
Nineteen healthy donors, median age 42 (range 19-71) received either 2 x 5 μg/Kg G-CSF daily (n=10, group A) or 2 x 8 μg/Kg G-CSF (n=9, group B) for 5 days. Pre mobilization CD34+ cell counts in the peripheral blood for each donor prior to the administration of G-CSF and apheresis samples were quantitated for CD34+ cells using the ISHAGE gating strategies. Collection of PBSC's were performed on a Cobe Spectra.

Results
The mean pre mobilization CD34+ cell count was 3.7±1.2/μL in group A (donors who were to be given 10μg/Kg G-CSF) and 3.8±2.6/μL in group B (donors who were to be given 16μg/Kg G-CSF). We found a relationship between the number of resting CD34+ cells in the PB of healthy donors and the number of CD34+ cells that could be mobilized after 5 days of G-CSF. Normal donors who had less than 2 CD34/uL before mobilization had a low chance of achieving a target of 5x10^6 CD34+ cells/Kg after a single apheresis, compared to donors with more that 2 CD34/uL at baseline. An average 287.5x10^6 CD34+ cells were collected from group A and 647.2x10^6 CD34+ cells from group B. We found that an increase in the G-CSF dose from 10 to 16 μg/Kg was associated with a mean 2.3 fold higher CD34+ cell mobilization. The target of collecting >5.0 x10^6 CD34+ cells/Kg in first apheresis was achieved in 44% of donors in group A and in 67% in group B, respectively.

Conclusion
Preliminary data indicate that pre mobilization PB CD34+ counts may serve to estimate the CD34+ cell mobilization potential and that higher doses of G-CSF can mobilize more stem cells in healthy donors.

B162 **P22**

Detection of Occult Multiple Myeloma (MM): Experience with F-18 FDG PET and Tc-99m Sestamibi Imaging

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Objective
Multiple myeloma (MM) may be difficult to assess due to absent or small volume paraprotein, bone marrow biopsy sampling variation, or persisting abnormalities on skeletal surveys (SS) following therapy which may not represent active disease. In small studies, both FDG-PET (PET) and Tc-99m sestamibi scans (MIBI) have been used to identify sites of occult bony and soft tissue disease in MM. This analysis aims to compare the results of PET and MIBI scans in MM patients (pts).

Methods

14 pts had concurrent PET and MIBI scans. Medical records and scan results were reviewed to assess: (a) the ability of the scans to identify occult disease; (b) concordance between the scans; and (c) their impact on management.

Results
5 pts had scans at diagnosis or as baseline in 'difficult-to-assess' disease. 7 pts had scans for suspected progression. 2 pts had re-staging scans following therapy. In 7 pts (50%), MIBI identified additional bony disease sites not seen on routine SS. In 3 of these cases, other disease markers also suggested progression. In 1 case, MRI of the positive site on MIBI was negative. In 2 cases, sites of MIBI positive disease were confirmed with MRI and CT respectively. In 4 pts (29%), the PET identified additional disease sites to routine imaging. 2 of these pts had sites of soft-tissue plasmacytomas identified. MIBI generally detected more disease sites than PET (7 cases). In only 1 case was a site of disease not seen on MIBI identified by PET. In 7 of the 14 cases, the scans impacted on management. In 3 cases, absence of active disease was confirmed. In 2 cases, residual active disease requiring further therapy was identified. In 1 case, a diagnosis of MM was confirmed in a pt with a negative SS, borderline marrow infiltrate and low-volume serum paraprotein. In this case, PET detected a scapular plasmacytoma which was confirmed on biopsy. A diagnosis of MM was made in a further pt thought to have a solitary plasmacytoma. In this case, both PET and MIBI revealed a further plasmacytoma with an extensive soft-tissue component.

Conclusions
PET and MIBI scans may be useful additional diagnostic tools for detecting occult myeloma. MIBI appears to be more sensitive for detecting additional disease sites. However, PET scans have detected sites of soft tissue disease not seen with other imaging modalities.

B163

Age ≤ 65 is the Major Predictor of Favourable Outcome Following Thalidomide (T) Therapy for Relapsed and Refractory Multiple Myeloma (MM)

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Background and Objectives
MM patients (pts) with relapsed or refractory disease have a poor outlook. Initial studies with T demonstrated efficacy, however there has been a lack of large, prospective multicentre confirmatory studies in pts with the more typical age distribution of MM. In 1999, we initiated a Phase II trial using T ± interferon α-2B (INF) to treat MM pts. Objectives were to determine response rate (RR), toxicity, overall survival (OS) and progression-free survival (PFS); and to determine if known prognostic factors predicted RR, OS and PFS.

Methods
75 pts were treated on a prospective, multi-centre phase II trial. Pts commenced T at 200mg/d po, increasing by 200mg q14d, to 800mg/d or individual maximum tolerated dose (iMTD). After 12 weeks, pts were planned to continue their iMTD of T and start concurrent INF (1.5-3.0 MU, SC, TIW) provided blood parameters were acceptable (ANC > 1.5 x 10^9/L, plts > 75 x10^9/L, LFTs < 3X ULN). T ± INF was continued until progressive disease or intolerance.

Results
72 pts were evaluable for response using SWOG criteria (i.e. PR: ≥50% fall in 'M' component) at >4 weeks of T. Median age was 64 yrs (range 36-83). Mean iMTD of T was 370mg with 39% achieving a iMTD of 800mg/day. No unexpected toxicities of T occurred. 2 pts had non-fatal thromboembolism. Overall response rate (RR) was 28% with 1 CR and 58% stable disease (SD). RR among pts ≤ 65 v >65 was 38% v 14% (p = 0.020). At a median follow-up of 18 months (range 6-26) the median PFS and OS were 5.5 mo and 14.6 mo, respectively. Multivariate analysis demonstrated significant prognostic factors for inferior OS were age >65 (median >26 v 9.2mo; p=0.005), raised serum LDH (p=0.011), raised serum creatinine (p=0.019) and chromosome 13 deletion (p=0.003). PFS was also significantly better for younger pts with actuarial 1 yr PFS of 31% v 13% (p=0.045). Dosage administered was not affected by age. 19 pts received concurrent T ± INF, 13 for >4 weeks. Of these, 3 maintained SD, 6 maintained a PR, and 4 improved from SD to PR. The addition of INF increased levels of fatigue, headache and neutropenia. 11 pts (58%) ceased INF due to toxicity.

Conclusions

1) Our results confirm the RR to T of prior trials, in a prospective multi-centre setting in patients with a median age of 64yrs, 2) INF may improve RR in selected pts and 3) age >65 predicts a RR of only 14% compared to 38% in younger pts. This latter result will have substantial impact on planning management for pts with relapsed or refractory MM.

B164 **P31**

Clonal T Lymphocytes and Elevated IL-5 Levels in Episodic Angioedema and Eosinophilia (Gleich's Syndrome)

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Background
Abnormal T cell clones, often of T-helper (CD4+) phenotype, have been found to occur in the idiopathic hypereosinophilic syndrome (HES), which is characterised by systemic organ dysfunction/damage due to accumulation of eosinophils or their products. The cytokine IL-5 produced by T-helper lymphocytes has been implicated in the pathogenesis of this disease. Gleich's syndrome is a rare condition with the hallmarks of hypereosinophilia and episodic angioedema but no end organ damage [NEJM, 1984]. We describe this condition associated with T-cell clonality.

Clinical history
A 57-year-old man experienced episodic urticaria, oedema of the limbs and face, leg rash and weight gain over the last 13 years. Prednisolone-responsive cycles occurred every 3-5 weeks, with complete interval resolution of symptoms. Apart from non-specific lethargy there were no other systemic features. He has had no significant past history implicating an infective cause for the eosinophilia. Clinical examination was normal.

Results
Over a 9-year period the most constant feature of his disease has been an isolated peripheral blood eosinophilia up to 27.5 x 10^9/l (n<0.06 x 10^9/l) and an IgE level of 5-10 times normal. Skin biopsy revealed dermal oedema, scattered eosinophils, and perivascular lymphocytes and eosinophils. Bone marrow examination revealed increased normal eosinophils and their precursors, and small aggregates of mature T cells. No mitoses were obtained to permit cytogenetic evaluation. BCR-ABL PCR was negative. Inguinal lymph node biopsy showed only benign reactive changes. Echocardiogram, colonoscopy, abdominal ultrasound and CT chest to pelvis showed no organ involvement. No microbiological cause of the eosinophilia was identified on faecal or urine examination. Strongyloides serology was negative. T cell receptor gene rearrangement studies of marrow and peripheral blood show an identical monoclonal band on three occasions. Flow cytometric analysis of peripheral blood revealed an abnormal T cell population with the phenotype CD2+ CD3- CD4+ CD7+ CD8- CD56- CD25+ CD95+. Serum IL-5 levels were markedly elevated at 647ng/ml (assay sensitivity 39 ng/ml, normally undetectable).

Conclusions
This patient fulfils the criteria for episodic angioedema and eosinophilia, rather than HES. We demonstrate for the first time a monoclonal T-helper cell clone in this disease which we believe is involved in the pathogenesis, most likely by the secretion of IL-5, a cytokine that controls the production, activation and localization of eosinophils.

B166

Central Nervous System Prophylaxis can be Improved by the Addition of Systemic High-Dose Methotrexate (HD-MTX) to Intrathecal Chemotherapy in Patients with Intermediate-Grade NHL

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CNS relapse is a devastating and usually fatal complication of NHL, despite optimal management. In intermediate grade NHL (IG-NHL), patients considered at high risk receive intrathecal (IT) prophylaxis, but studies suggest that this approach may be inadequate in this group of patients (ASH 2000, abstr. 3147). We therefore assessed the efficacy of adding systemic HD-MTX to IT prophylaxis, on the basis of its known penetration into CSF and brain parenchyma.

Methods
From 1/91 to 12/01 42 newly diagnosed patients with IG-NHL by the Working Formulation and negative baseline CSF cytology received IT prophylaxis. Patients were classified into 3 groups (1) CHOP-like chemotherapy & IT (N=23), (2) systemic chemotherapy with HD-MTX (≥1g/m^2 eg Hyper-CVAD) & IT (n=12) and (3) CHOP-like chemotherapy & IT followed by 2 doses of MTX 3g/m^2 24 hour CIVI q14d (n=7) (as per Ann Oncol 11:685,2000).

Results
Overall, median age was 51 (15-80), 71% stage IV and 50% IPI ≥3. CNS relapse risk (Blood 91:1178, 1998) was high in 36%, and 40% had other risk factors (eg high risk sites of disease, epidural extension). In groups (1) – (3) CR was attained in

65%, 75% and 86% of patients respectively (P=0.5). CNS risk factors were similar; Median CNS risk score 1,1 and 1 (P=1.0), median IPI 3,2 and 1 (P=0.2). Median number of IT treatments were 4,6 and 5 (P=0.04). HD-MTX required a median of 4 days of inpatient care, and were not associated with any significant toxicity. CNS recurrence occurred in 6 patients, all of whom were in group (1). Sites of recurrence were spinal cord in 2, brain parenchyma in 2 and leptomeninges in 4. 3 year actuarial CNS relapse rate was 20 ± 8%. The groups receiving HD-MTX had a lower rate of CNS recurrence (0% at 3 y) compared with group (1) patients without HD-MTX (31 ± 11% at 3y) (P=0.04). Overall survival in groups (2) and (3) was more favourable. 3y actuarial survival rates were 32 ± 12% for group (1) and 80 ± 11% for groups (2) and (3) (P=0.1).

Conclusion
Although based on a small, retrospective series, these data demonstrate that IT prophylaxis alone is inadequate in IG-NHL. The addition of systemic HD-MTX, either as a component of primary therapy or administered following CHOP-like chemotherapy, reduces CNS recurrence and may improve overall survival.

B167 **P47**

Pure Red Cell Aplasia due to Parvovirus B19 in a Patient Treated with Campath-1H

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Campath-1H is a humanized monoclonal antibody directed against the CD52w antigen, which is present on normal and malignant human B-and T-lymphocytes and monocytes. Campath-1H is increasingly used for a variety of B- and T-cell diseases. Campath-1H causes a predictable, severe and prolonged depletion in CD4 and CD8 T-lymphocytes. Opportunistic infections are therefore common after Campath-1H, most commonly bacterial infections and reactivation of CMV or HSV infections (Blood. 2002;99:3554). This report illustrates an important consequence of iatrogenic immunosuppression: pure red cell aplasia (PRCA) as a result of parvovirus B19 infection following Campath-1H.

Study Design
A 56-year-old woman of Malaysian descent presented with a febrile illness and obtundation after recent Campath-1H treatment for refractory mycosis fungoides. She was anaemic despite repeated transfusions, and had a severe lymphopaenia of both CD4 and CD8 T cell subsets. There was a severe reticulocytopaenia.

Results
Full septic workup failed to find a cause for the patient's illness until a bone marrow biopsy showed findings consistent with PRCA. Testing for parvovirus B19 showed positive parvovirus B19 IgM at low titre as well as a positive blood PCR for parvovirus DNA. Treatment consisted of intravenous immunoglobulin (IVIG: 1.5g/kg over 4 days). The patient developed a reticulocytosis and became transfusion independent 12 days later. Fevers and mental obtundation settled. Repeat bone marrow biopsy one month later showed resolution of the PRCA.

Conclusions
Parvovirus B19-induced PRCA should be considered in patients who develop fever and anaemia after treatment with Campath-1H. The disorder can be successfully treated with IVIG.

B168 **P20**

Oxpentifyllin, Ciprofloxacine, Dexamethasone and 1,25-Dihydroxivitamin D3 Treatment in Patients with Myelodysplastic Syndromes (MDS). Preliminary Results of an Ongoing Pilot Study

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Myelodysplastic syndromes are clonal haematological disorders characterised by ineffective haemopoiesis. The main pathogenetic feature is the increased apoptosis which translates clinically into peripheral cytopenia despite the hypercellular bone marrow. The aim of our study was to use a combination of drugs that suppress apoptosis and promote differentiation in the myelodysplastic marrow.

Material and methods
Patients with proven MDS and IPSS > 0 were eligible into the study. After three months of observation, patients started on study drugs for a total period of 16 weeks. All drugs were administered orally. Treatment started with oxpentifyllin 400 mg tds, ciprofloxacine 500 mg b.d. and dexamethasone 4 mg daily for the first two weeks after which the dose of oxpentifyllin was increased to 800 mg tds. Vitamin D3 was introduced on week 9 of treatment in addition to the previous drugs at weekly incrementing dose, starting at 0.5 μg/day and up to 2.25 μg/day until the end of 16 weeks. During the treatment with vitamin D3 patients were educated to keep low their daily calcium intake. At the end of 16 weeks, treatment was ceased and patients were followed for another 6 months. Blood and platelet transfusion was given as needed.

Results
16 patients were referred for enrolment since April 2001. There were 13 males and 3 females. Median age was 62 years (range 32-80). The diagnosis according to FAB classification was RA in 5 cases, RAEB in 9 cases and RARS in 2 cases. 11 patients had normal cytogenetics (4 RA and 7 RAEB) and 5 patients had various cytogenetic abnormalities, including –7 in one (RAEB), -Y in one (RARS), t(2;4) in one (RA) and complex abnormalities in two (RAEB) patient. The IPSS was 0, 0.5, 1, 2.5 and 3 in 4, 4, 5, 2 and 1 patients, respectively. Of 12 patients eligible into the study, 4 completed the full 16 weeks of treatment, 1 is still under treatment, 4 stopped earlier due to progressive disease (n=2) or significant side effects (oedema and thrombocytopenia)(n=2), 1 died before starting treatment (not assessable) and 2 refused treatment. The most significant side effects among other patients were insomnia and irritability. Among those who completed the treatment, 1 patient (previous IPSS 2.5) progressed into AML and two patients showed transient improvement in neutrophil and/or platelet count. One patient with the initial diagnosis of RARS showed a reduction of ring sideroblasts from 20% to 10% and of bone marrow blast cells from 7% to 4%. He retained, however, the dysplastic features.

Conclusion
This is an interim report on an ongoing study. Preliminary results confirm a role for this type of palliation in patients with MDS. This is highly supported by patients' satisfaction.

B170

Negative Selection of Contaminating Tumour Cells from Stem Cell Autografts in Two Models of Malignancy

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Negative selection of contaminating malignant cells from haemopoietic stem cell autografts is limited by the availability of monoclonal antibodies or other ligands directed against neoplastic cells. The Thomsen-Friedenreich (TF) antigen is an important carcinoma-associated marker related to metastasis and is recognised by the lectin peanut agglutinin (PNA). The PNA binding epitope is also expressed in some haematological malignancies, including by bone marrow plasma cells in multiple myeloma. In this study the potential of PNA as an agent to remove neoplastic cells from stem cell autografts was determined in two diverse models of malignancy, breast carcinoma and multiple myeloma.

PNA reactivity of metastatic breast carcinoma cells, present in stem cell autografts or in bone marrow biopsies, was established by dual label fluorescent microscopy. PNA reactivity of plasma cells in peripheral blood stem cell (PBSC) harvests from myeloma patients was established by flow cytometry and by histochemical and fluorescent staining of flow sorted cells. Non-reactivity of peripheral blood $CD34^+$ cells with PNA was determined by flow cytometry. A negative selection procedure was developed using PBSC samples contaminated with cultured breast carcinoma cells (MCF-7), biotinylated PNA and streptavidin magnetic microbeads (MACS). Experimental PNA purging was carried out for a bone marrow autograft of a breast cancer patient and for PBSC samples from 12 myeloma patients. Breast carcinoma cells were detected by conventional immunohistochemical staining. Myeloma plasma cells were quantitated by immunophenotype and also detected by PCR for the IgH rearrangement.

In the developmental procedure, PNA purging of PBSC samples, artificially contaminated with MCF-7 cells, resulted in tumour cell reduction of >3.3 log, a recovery of $CD34^+$ stem cells of 62.7 $\pm$ 3.6% (mean $\pm$ SEM) and total nucleated cells (TNC) of 36.1 $\pm$ 3.2%. Colony forming ability was maintained. A 3.1 log depletion of tumour cells and a yield of 46.6% $CD34^+$ stem cells was obtained for a bone marrow harvest containing 0.5% metastatic breast carcinoma cells using the procedure. Following PNA purging of PBSC samples of myeloma patients, stem cell recovery was 58.5 $\pm$ 3.7% and plasma cell loads were reduced by 0.65->2.69 log, however, most remained positive for the malignant clone by PCR. PBSC samples were enriched for T cells following PNA purging from 14.0 $\pm$ 1.4% $CD3^+$ cells to 24.6 $\pm$ 4.7%.

In conclusion, PNA has potential as an agent to remove contaminating neoplastic cells from haemopoietic stem cell autografts for some malignancies, without compromising engraftment capacity or immune function.

B171

Significant Incidence of BCR-ABL Kinase Domain Mutations associated with Disease Progression in Chronic Phase CML Patients Refractory or Intolerant to Alpha-Interferon Treated with Imatinib Mesylate

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Chronic myeloid leukaemia (CML) is characterised by the activated tyrosine kinase BCR-ABL. Use of the kinase inhibitor imatinib has had a marked impact on treatment. A significant number of patients in chronic phase (CP) who are refractory or intolerant to alpha-interferon achieve major cytogenetic responses (MCR, 60%). The incidence of MCR in accelerated phase (24%) and blast crisis (16%) is lower and is not always sustained, with a high risk of resistance to imatinib. Clinical resistance has been associated with recurrent BCR-ABL activity due to the development of mutations in the kinase domain that interfere with imatinib binding. We have previously reported that BCR-ABL gene mutations developed in over 50% of resistant patients who were treated with imatinib for advanced phase CML. Although a less common event in CP, imatinib resistance has been reported in 8-15% of patients. We investigated 69 imatinib treated CP patients intolerant or refractory to interferon. We aimed to determine the incidence of mutation development and its association with clinical resistance in this group. Patients were selected from those with available RNA of adequate quality. Nine patients in complete cytogenetic remission (CCR) had BCR-ABL levels too low for mutation analysis. Thirty-two of the remaining 60 patients achieved a MCR within six months of imatinib (18 complete). Three lost the MCR and mutations were detected in all 3. Two of these developed additional chromosomal abnormalities indicating disease acceleration after achieving CCR. Both have responded to increased imatinib dose and maintain MCR. The third patient lost MCR after 12 months. Mutations were detected in two other patients who achieved MCR. 28/60 patients never achieved MCR, 9 entered acute phase and BCR-ABL mutations were detected in 7/9. The patients with mutations had received imatinib for a median of 7.5 months (3-18) at the time of mutation detection. The median time since diagnosis was 4.5 years (1.1-9.3) compared to 3.5 years (0.5-17.7) in patients with no mutations which supports the possibility of increasing genetic instability with time. Thus overall 12/69 (17%) of all CP patients and 10/12(83%) of CP patients with imatinib resistance had kinase mutations identified. This study suggests that although the incidence of imatinib resistance in CP patients is less than in the advanced phases, the frequency of mutations in resistant patients remains high.

B172 **P49**

Pure Red Cell Aplasia Caused By Anti-Recombinant Human Erythropoietin Antibodies

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We describe the first series of Australian cases of pure red cell aplasia (PRCA) associated with recombinant human erythropoietin (r-HuEPO) therapy caused by anti-erythropoietin antibodies. Four patients treated with r-HuEPO for anaemia associated with chronic renal failure developed PRCA between July 2000 and July 2001 (Table 1). Three of the cases were from the same renal unit comprising in the order of 300 patients. Bone marrow aspirate and trephine (BMAT) confirmed PRCA. Other causes of PRCA were excluded and inhibition of erythroid colonies by patient serum in vitro was demonstrated. Anti-erythropoietin antibodies were demonstrated by immunoprecipitation in all cases. Administration of r-HuEPO was by the subcutaneous route and this appears to be a possible link that has emerged with the development of anti-erythropoietin antibodies in this context worldwide.

Aplasia is prolonged despite withdrawal of the offending agent. We found only one of four patients became transfusion independent, although not all were treated with immunosuppression, similar to the cases described in recent reports.

We recommend early investigation for PRCA in cases of refractory anaemia when patients are receiving treatment with r-HuEPO. All patients, apparently refractory to r-HuEPO should have a BMAT.

Table 1 Characteristics, treatment and outcome of the 4 patients

Age and sex	**Renal Disease**	**Interval from start of therapy to refractory anaemia**	**Treatment**	**Outcome**
73 M	Focal and segmental glomerulosclerosis	26 months	Corticosteroids and cyclophosphamide	Transfusion free 6 months after diagnosis EPO maintained at 20 000U/week
59	Polycystic kidney	13 months	Transfusion support	Transfusion dependent

M	disease			
44 F	Diabetic nephropathy	8 months	Immune globulin Azathioprine Plasma exchange Cyclophosphamide Antithymocyte globulin and cyclosporin	Transfusion dependent 2-3units every 3 weeks
49 F	Diabetic nephropathy	10 months	Considering cyclophosphamide and pancreatic and renal transplant	Transfusion dependent 2-3units every 3-4weeks

B173

Massive Expansion of High Purity, CMV-specific Cytotoxic T Cells by Peptide-Pulsed Dendritic Cell Stimulation

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Aims
The adoptive transfer of antigen-specific $CD8^+$ T cell clones has proven to be an effective method to restore immunity in viral and malignant disease. However, the requirement of greater than 10^9 target-specific T cells for efficacy has proven difficult for routine clinical practice. We present a method for generating 10^9 highly pure, cytomegalovirus (CMV)-specific cytotoxic T cells (CTL) in 4 weeks for adoptive immunotherapy for CMV in allogeneic stem cell recipients using CMVpp65 peptide-pulsed dendritic cell (DC) stimulation followed by rapid expansion.

Methods
Monocyte-derived DC generated from HLA-$A2^+$, CMV-seropositive donors and pulsed with CMVpp65 peptide (495-503) were used to stimulate autologous PBMC. After one week of stimulation, cultures were stimulated a second time with fresh DC pulsed with peptide and cultured in the presence of low concentration IL-2 (10 U/ml). On day 14, CMV-specific CTL cultures were enumerated and resuspended in media containing 50 U/ml IL-2 at a cell density of $<5 \times 10^5$ cell/ml and cultured either in T-flask or in a suspension bioreactor. CTL cultures were fed every 1 to 2 days by a half volume media change and expanded for 2 more weeks. Cells were analyzed for phenotype, CMV-specificity (by MHC Class I-pp65 tetramer) and for function in cytotoxicity assays against peptide-pulsed and CMV-infected target cells.

Results
Peptide-pulsed DC produced a dramatic expansion in CMV-specific CTL ($CD8^+$ $Tetramer^+$) from CMV seropositive donors in which there was greater than a 500-fold expansion in total cell number and resulted in >90% purity (as measured by tetramer). These cells grew rapidly (doubling time ~1 day) in media supplemented with IL-2. After only two consecutive weekly DC stimulations, the purity of CMV-specific CTL increased in the absence of further specific stimulation. Cells transferred to a suspension bioreactor expanded at a similar rate to those grown in T-flasks, allowing the expansion of equivalent numbers of T cells in a single vessel with fewer manipulations. CMV-specific CTL showed specific lysis towards peptide-pulsed target cells as well as CMV-infected, HLA-$A2^+$ fibroblasts. Furthermore, the purity and function of the CMV-specific CTL remained high during the expansion phase of culture resulting in $>10^9$ CMV-specific T cells for adoptive immunotherapy.

Conclusions
The use of CMVpp65 peptide-pulsed DC stimulation has proven to be an effective and simple method in rapidly generating CMV-specific CTL in sufficient quantities for clinical evaluation. Furthermore, the use of bioreactor technologies may allow greater expansion with fewer manipulations.

B175 **P19**

Re-induction of Haematological Response with the Addition pf Hydroxyurea to STI 571 for the Treatment of Chronic Myeloid Leukemia

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Introduction
STI571 produces a durable haematologic and cytogenetic responses in the majority of patients with CML. The role of combined therapy with conventional anti-leukaemic agents and STI571 in patients who progress on STI571 alone is uncertain. It has been suggested that combination therapy improves patient survival and may also delay the appearance of drug resistance.

Methods and Results
Four patients with CML in chronic or accelerated phase were treated with the combination of hydroxyurea (HU) and STI571 after developing disease progression on STI571 alone. A partial haematological response was the best response recorded to STI571 in all patients. Patient age, gender, diagnosis, duration of response to STI monotherapy prior to disease progression, and response to combined hydroxyurea plus STI571 are detailed in the Table.

Patient	Age	Sex	CML Phase	STI571 monotherapy	Time to WCC <10 post HU	Duration of response
1	71	M	Chronic	7 months	10 days	5 months
2	61	F	Chronic	10 months	2 months	6 months
3	34	M	Chronic	6 months	1 week	4 months
4	59	M	Accelerated	13 months	2 months	9+ months

STI571 dose escalation was attempted in all patients prior to addition of hydroxyurea but was poorly tolerated. The addition of hydroxyurea resulted in normalisation of the white cell count and enabled the patients to be maintained on lower doses of STI571 with resolution of dose dependent side effects of STI571. This response occurred despite three of the four patients becoming refractory to hydroxyurea therapy earlier in the course of their disease.

Conclusions
The addition of hydroxyurea should be considered in patients with CML who develop disease progression despite STI571 alone. The mechanism by which hydroxyurea apparently overcomes resistance to STI571 remains poorly understood and may merit further study.

B176 **P10**

Adult T Lymphocytic Leukaemia: A Case Report and Review Describing Isochromosome 18q in Association with T(8;15) - A Previously Undescribed Karyotypic Abnormality

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Adult T cell Lymphocytic Leukaemia is a rare lymphoid neoplasm associated with HTLV1 infection. Isochromosomes of 18q have been infrequently reported in the literature and are most often associated with B cell lymphoma and lymphoproliferative disorders. A single case of adult T cell lymphoma/leukaemia (HTLV1-1+) has been reported with an isochromosome of 18q as the sole abnormality. The derivative 15 chromosome has not previously been reported.

The case report is of a 59-year-old female of Malay descent who presented in an obtunded and irritable state due to hypercalcaemia with a corrected Calcium of 4.97mmol/L. This occurred in the setting of a marked peripheral leukocytosis characterised by a pleomorphic lymphocytosis ranging from lymphoid blasts to medium sized lymphoid cells with mature chromatin pattern and highly indented nuclei (flower cells). Bone marrow aspirate and trephine histology confirmed the presence of a pleomorphic lymphoid infiltrate. Three colour flow cytometric analysis of the bone marrow confirmed the presence of a mature T cell neoplasm expressing CD 2,CD 3,CD 4,CD 25& CD 38 in the absence of CD 7, CD 34 and TdT. The patient was subsequently identified as being seropositive for HTLV1 antibodies.

Bone marrow cytogenetics revealed the following abnormal mosaic karyotype in seven of twenty six cells analysed; 46-48,XX,der(15)t(8;15)(q13;q24),+18,i(18) (q10)[cp7]/46,XX[19]. The abnormal clone, consisting of a composite karyotype, contained an isochromosome of 18q and a derivative 15 chromosome involved in an unbalanced translocation between chromosome 8. The presence of a composite karyotype is indicative of clonal evolution in this patient.

Following an initial response to Hyper CVAD chemotherapy, disease relapse was documented following the third cycle of treatment. The disease was refractory to salvage chemotherapy and anti-retroviral therapy (Interferon α [IFNα] plus Zidovudine [AZT] in addition to IFNα plus AZT and Lamivudine). The patient died from progressive disease six months following the initial diagnosis.

B177 **P50**

Delayed Onset Neutropenia Following Rituximab Therapy

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Aim
To define the clinical and laboratory features of delayed-onset selective grade IV neutropenia following rituximab therapy.

Methods
Records were examined for evidence of selective grade IV neutropenia developing after initially normal neutrophil counts in a cohort of patients receiving infusions of rituximab (375 mg/m^2). The study group comprised all patients treated with rituximab by five physicians in two institutions. Relationships were examined of the development of neutropenia to bone marrow morphology and presence of residual disease.

Results
Seven episodes of delayed-onset grade IV neutropenia were identified in this patient cohort to which 59 courses of 2 to 6 weekly infusions of rituximab had been administered. Rituximab was given as a single agent in 3, and in conjunction with cytotoxic agent therapy in 4 of the prior treatment episodes. Normal neutrophil counts had been documented >4 weeks after completion of treatment, and no additional therapy had been administered. Median time from completion of rituximab treatment was 122 days (61-168). Neutropenia was detected by presentation with febrile neutropenia in 3, and by routine monitoring in 4. Nadir neutrophil counts ranged from 0 to $0.5x10^9/L$. Bone marrow examination revealed "maturation arrest" in the neutrophil series, and no residual disease in the 6 instances marrow studies were performed. All patients were in CR on conventional criteria. The only concurrent abnormalities detected were lymphopenia in all episodes, and hypogammaglobulinaemia in the 5 evaluated. Recovery from neutropenia occurred in all instances with a median of 9 days, although duration varied widely from 4 to 148 days. Rapid spontaneous recovery took place in 2 episodes, and after administration of G-CSF in the remainder.

Conclusion
Relatively severe delayed-onset neutropenia was detected on 7 occasions after 59 courses of rituximab in this patient cohort. Neutropenia developed as a transient problem of variable duration after at least several months, in the absence of recognisable precipitating factors or evidence of recurrent disease. Some episodes were brief, and some were detected by routine monitoring, to raise the possibility that this phenomenon, briefly noted in post trial surveillance, may be underrecognised. Absence of other causative factors and an association with lymphopenia and hypogammaglobulinaemia raise the possibility of an underlying immune aetiology.

B179 **P17**

Bone Marrow Transplantation for Chronic Myeloid Leukemia in Chronic Phase- Toxicity May Not Be an Issue. The Royal Melbourne Experience 1992 – 2002

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Recent advances in the pharmacological management of chronic myeloid leukemia have raised uncertainty over the exact role of bone marrow transplantation in this disease. There is little doubt that in selected patients with a suitable sibling donor, bone marrow transplantation offers benefits in terms of survival and prolonged cytogenetic remission when compared with traditional pharmacological therapies such as interferon, hydroxyurea and busulphan. However, transplantation is associated with significant treatment related morbidity and mortality. In advanced chronic myeloid leukemia, the tyrosine kinase inhibitor, imatinib mesylate, has produced impressive results with minimal toxicity. This has lead to speculation that imatinib may be a safer and more effective therapeutic modality than bone marrow transplantation in patients with earlier phases of the disease. It is unlikely, however that imatinib is curative therapy.

Our primary objectives were to determine the morbidity and mortality of patients undergoing an HLA matched sibling allograft at the Royal Melbourne Hospital for chronic myeloid leukemia in chronic phase between 1992 and May 2002. Patients received a transplant of they had a suitable donor, had an adequate performance status and were aged fifty-five years or less. Patients and their characteristics were identified from a comprehensive institutional database of BMT patients. Forty-two patients were identified of whom 24 were male. 40 (95%) were alive at the time of review (range 23-4742 days, median 1614 days). There were two deaths, one from graft versus host disease and invasive aspergillosis (day 54 post BMT), and the other from pulmonary hemorrhage (day 202). Of the forty patients who are still alive, 36 are beyond day 180 post transplant. The median Karnovsky score in this group was 100 with 8 (22%) having a score of less than 90. 13 (36%) continued to take immunosuppressant medications.

We conclude that at our institution, bone marrow transplantation is a safe, effective and proven therapeutic modality for selected patients with chronic myeloid leukemia. In the absence of evidence to the contrary, transplantation should remain the treatment of choice for these patients.

B181 **P11**

A Novel Test to Screen the N-Ras Gene for Mutations, for Use as Markers in Childhood Acute Lymphoblastic Leukaemia

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N-ras plays a key role in the differentiation and proliferation of haematopoietic cells. Mutations have been linked to a lower rate of remission and higher risk of relapse but these links have not been strongly established. Previous studies of N-ras mutations in this disease focused on known activating mutations in codons 12, 13 and 61. It is now possible to re-examine genes using new more sensitive mutation detection techniques which can screen the entire gene rapidly, and identify mutations anywhere in the gene. Denaturing Gradient Gel Electrophoresis (DGGE) is one such technique, which has been successfully used to screen for mutations in genes such as CFTR (cystic fibrosis transmembrane conductance regulator) and Brca-1 (breast cancer, type 1).

The aim is to develop a test to screen all the exons of the N-ras gene for mutations. PCR is used to amplify all the exons of the gene in fragments of around 300 bp. DGGE is then used to identify single base mutations on the basis of a difference in melting temperature. The difference is detected through a change in mobility when electrophoresing the product on a gel containing a gradient of denaturing chemicals. To show that we can detect mutations we have designed site directed mutagenesis primers to insert a single base mutation into each PCR product. We confirm this base change causes a mobility change by electrophoresing the mutated product on a gel beside a normal product. To enable the study of archival bone marrow samples where very little DNA is available we used long range PCR to amplify the entire N-ras gene. Then we amplify individual segments for study. We have successfully amplified the whole gene using long range PCR from 3 normal blood and 10 archival bone marrow samples from fixed stained slides. We have found three polymorphisms in samples from normal individuals, one in the 5' untranslated end and two in the 3' untranslated end. This confirms DGGE can screen the N-ras gene for mutations and that there are mutations outside the sites usually studied in this gene. The technique may prove useful for testing whether mutations in N-ras are linked to disease progression.

B182 **P90**

Therakos Extracorporeal Photopheresis; Important Nursing Issues for Introducing this Procedure

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Background
Extracorporeal Photopheresis (ECP) utilizes photoactivated methoxsalen (8-MOP, Uvadex), a photosensitising agent, which selectively accumulates in T-cells and causes predominantly apoptotic cell death. Moreover, subsequent alterations in antigen presentation on T-cells initiate a secondary immune response. Here we describe the introduction of Therakos ECP at Peter Mac for the treatment of patients (pts) with chronic cutaneous graft versus host disease (cGVHD) and cutaneous T-cell lymphoma (CTCL).

Methods
The Therakos device was utilized for all procedures. For each treatment, several cycles of apheresis were performed to obtain a leukocyte-enriched blood fraction. 8-MOP was then injected directly into the recirculation (product) bag. Photoactivation of 8-MOP is achieved by exposing this leukocyte enriched blood fraction containing 8-MOP to a prescribed amount of UVA (which is a built in component of the machine). Once photoactivation is completed, the treated cells are reinfused to the patient. Red blood cells and plasma are returned to the patient without being treated.

Results
Treatment schedules varied according to the patient's disease type and tempo of progression. Most patients were treated initially once or twice a week and then monthly. We have achieved the safe introduction of this therapy with 63 procedures for 7 patients (5 cGVHD & 2 CTCL and Sezary Syndrome). Adverse effects have been minimal and included vasovagal (n=2; pt 3), access problems (n=4; pts 3 & 5) and device difficulties (n=3). There were no drug toxicities. A critical issue for administration of this therapy is the high cost. We will describe nursing issues arising from the introduction of this new therapy including:

- Staff training

- Use of Uvadex
- Patient monitoring and follow up
- Precautions
- Patient education
- Funding hurdles

B184 **P41**

Low Dose Valaciclovir for the Prevention of HSV Reactivation Following Transplant or Chemotherapy

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Introduction
The efficacy of valaciclovir (VAL) prophylaxis for herpes simplex virus (HSV) reactivation in patients (pts) following high-dose therapy and autologous blood cell transplantation (ABCT) or chemotherapy for haematological malignancies has not been extensively evaluated. The policy at PMCI has been to use VAL prophylaxis of 500mg PO daily, after an analysis of our previous experience showed that low dose aciclovir (200mg po bd) had a breakthrough (BT) infection rate of 29% overall BT consisting of 12% virological and 17% clinical. We considered a virologically proven BT rate of < 10% acceptable. We prospectively evaluated the incidence of BT HSV infection in pts from 4/2000 to 12/2001 treated with VAL

Methods
All pts who had positive HSV serology and who received high dose chemotherapy and ABCT or intensive induction / consolidation chemotherapy or T- cell immunosuppressive chemotherapy or had steroid use of > 6 weeks duration were included. Pts received VAL 500mg PO daily or aciclovir 250mg IV TDS if oral therapy not possible. Outcome measures were (a) HSV treatment based on clinical diagnosis of BT infections consisting of VAL (500-1000mg po tds) or oral aciclovir 200-400mg po 5x/day or aciclovir 5mg/kg IV tds if oral medication was not possible (b) a HSV BT confirmed microbiologically by PCR (c) response of BT infections to VAL treatment.

Results
98 pts were entered (M: F, 56:42). Indications for Val comprised: ABCT in 28, chemotherapy alone in 70. The median age was 51 (range 20-76). BT HSV infection was clinically suspected in 22 pts (22%), (2 of these pts experienced 2 BT episodes). Of these 22 patients, co-existent mucositis was evident in 10, 17 were neutropenic < 1.0×10^9. 21 had received chemotherapy alone (30%) and 1 high-dose chemotherapy and ABCT (4%; P =0.003). Microbiological specimens were not sent in 8 pts. 14 pts were investigated (1 had 2 BT episodes and was tested for HSV during one but not the second and 1 pt tested positive for HSV during one episode and negative during the second), 6 (43%) were confirmed HSV positive on PCR. If this rate of microbiological confirmation was applicable to the entire cohort of pts, the estimated overall incidence of definite (microbiologically-proven) infection would be 9.6%. Resistance was not clinically evident as lesions resolved in all treated patients. The median time from starting VAL to breakthrough was 9 days (range 2-79) for all pts who experienced a BT and the median time to a virological BT was 13 days (range 4-79).

Conclusion
The overall incidence of BT was 22% with an estimated virological BT rate of 9.6% which is less than the target of <10% and compares favourably to low dose aciclovir (P=0.13).

B185 **P13**

Comparison of Culture Systems to Produce Immunogenic AML Cells

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Effective tumour antigen presentation is essential to eradicate minimal residual disease after conventional treatments for acute myeloid leukaemias (AML). In order to induce immunity directed against the remaining tumour cells rather than tolerance, the antigen-presenting cell (APC) on which the tumour antigens are presented is of critical importance.

The aim of modifying poorly immunogenic tumour cells such as leukaemic blasts is to stimulate a specific anti-leukaemic response that could be therapeutically valuable. There has been considerable interest in the generation of leukaemia-derived dendritic cells and their ability to present tumour antigens to T-lymphocytes. We have demonstrated using a different culture

system that AML blast cells can be modified *in vitro* to an APC phenotype, characterised by upregulated expression of the co-stimulatory molecule B7-1 (CD 80) necessary for antigen presentation.

We have compared the ability of AML blast cells to upregulate the expression of B7-1 using the dendritic cell culture system (IL-4, TNF α, GM-CSF), with a second cytokine combination of IL-3, IL-6, GM-CSF, over a period of 9 days in serum and serum free media (RPMI/FCS and AIM V respectively). The blasts were subjected to 10 Gy irradiation to assess the effect on viability and B7-1 expression over this time period. We have also investigated the ability of the resulting modified blasts to stimulate the proliferation of allogeneic T-lymphocytes in mixed lymphocyte reactions.

The IL-3, IL-6, GM-CSF cytokine combination in medium containing serum was most effective at upregulating the expression of B7-1 on AML cells in comparison to the dendritic culture system of AIM V medium containing IL4, TNF α, GM-CSF. We are continuing to investigate this response with patient AML cells.

Irradiation of the leukaemic cells at 10 Gy increased the proportion of cells stained for B7-1 at day 9 in all of the culture systems by 5-15%, although there was a reduction (10-15%) in percentage of original cell numbers surviving.

In mixed lymphocyte reactions, blasts modified to express B7-1 in the culture system containing serum and the cytokines IL3, IL6 and GM-CSF were also the most immunogenic as determined by the resulting proliferation of allogeneic T-lymphocytes (40% increase). These cells were also capable of eliciting tumour-specific killing in cytotoxic T-lymphocyte assays. Irradiated, culture-modified blasts did not produce a significantly different mixed lymphocyte response to non-irradiated blasts.

This study highlights the possibility of using irradiated IL3, IL6, GM-CSF modified tumour cells as APC in a vaccine in the setting of minimal residual disease.

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Autosomal Dominant Familial Acute Myeloid Leukemia: Mutation Analysis of Transcription Factors (TFs) on Chromosome 16q

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Aims
Analyses of chromosomal translocation and inversion breakpoints in sporadic leukemias have identified many TFs as playing a role in malignant transformation and disease progression. Studies of famines with increased incidence of leukemia have so far identified mutations in the TF AML1, mapping to chromosome 21, as predisposing to leukemia. Restricting our analysis to TFs, we aimed to identify the gene(s) responsible for initiating the multi-step leukemogenic process by studying 2 large families with autosomal dominant acute myeloid leukemia and linkage to chromosome 16q.

Methodology
A candidate gene approach on both families has previously identified a critical region of 14.9 cM on chromosome 16q with a maximal combined LOD score of 3.63 (Θ-0) (Gao Q et al., Genes Chrom Cancer 2000, 28:164). Using the Human Genome Working Draft sequence, we established a gene map of the region. Known TFs were identified and mutation analysis was performed using denaturing high pressure liquid chromatography (DHPLC) and direct sequencing. Loss of heterozygosity (LOH) was assessed by single nucleotide polymorphism (SNP) analysis, fluorescence in situ hybridization (FISH) and Northern Blot analysis on lymphoblastoid cell lines from patients and control family members.

Results
Combining the chromosomal marker analyses of both families narrowed the critical region on chromosome 16q to 15.8 MB, containing 163 known and predicted genes. In this region, we identified 5 TFs important in haematopoiesis: the cofactor of AML1, core binding factor beta (CBFβ), E2F4, CCCTC-binding factor (CTCF), and nuclear factors of activated T cells NFATC3 and NFAT5. DHPLC and direct sequencing revealed the presence of 2 out of 22 SNPs in the CBFβ gene of one unaffected family member (1 SNP in the 5'-UTR and 1 SNP in the 3'-UTR), and 1 heterozygous silent mutation in exon 9 of NFATC3 in 2 leukemic family members. No other mutation was found. FSH analysis failed to detect a gross heterozygous deletion, and Northern analysis on cell lines showed normal gene expression (transcript size and abundance). Mutation and LOH analyses of CBFβ are complete; SNP and microsatellite analyses for detection of LOH for the remaining genes are underway.

Conclusions

There is so far no evidence that TFs located within the critical region on chromosome 16q are causative of the disease in these 2 families. Identification of the gene(s) responsible for leukemia predisposition in these families may improve our understanding of the initiating steps in leukemogenesis and direct future research for a specific therapeutic approach.

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A Highly Predictive Model for Efficient Peripheral Blood Stem Cell Collection Based on CD34 Enumeration and Variable Volume Leukapheresis

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Effective mobilisation of peripheral blood stem cells (PBSC) can be influenced by many factors including disease status, prior treatment and mobilisation regimes of patients. Monitoring of peripheral blood white cell count (WCC) and $CD34^+$ concentration during mobilisation can indicate the optimum time for PBSC collection by apheresis, thereby minimising the number of procedures performed per patient. In this retrospective study, data for PBSC harvests, carried out over an eighteen-month period, were collated and evaluated to determine the effectiveness of apheresis practice in our centre.

In this study, PBSC harvest data of 67 patients, mobilised between January 2001 and June 2002, was analysed. Commencement of PBSC harvest was determined by analysis of peripheral blood (PB) WCC, using the Cell-Dyn 2000, and $CD34^+$ stem cell concentration, performed by dual platform flow cytometry. The movement and amplitude of a patient's WCC was the indication for $CD34^+$ determination. The analysis of WCC and $CD34^+$ trends individualised the collection strategy for each patient, and apheresis was performed when both were on an upward trend. PBSC harvests were performed using a Cobe Spectra® continuous flow blood cell separator and a 1mL/min collect rate. The volume of whole blood processed, to obtain the requested number of stem cells, was estimated using the following algorithm:

$$\text{Blood Volume (L)} = \frac{\text{Recipient Weight (kg)} \times \text{Requested } CD34^+ \text{ Cells } (10^6/\text{kg})}{\text{PB } CD34^+ \text{ Concentration } (10^6/\text{L}) \times 0.4}$$

Of the 69 patients harvested, 27 were normal donors, 15 multiple myeloma, 15 lymphoma, 6 acute myeloid leukaemia and 6 other. Retrospective analysis of the data revealed a total of 82 leukapheresis procedures, with a mean of 1.2 collections per patient. The mean pre-collection PB $CD34^+$ stem cell concentration was 107 ± 134 (SD) x 10^6/L (range 6- 416). The number of $CD34^+$ stem cells requested ranged from 2– 10 x 10^6/kg and the mean volume of whole blood processed was 14.2 ± 5.3 L (range 6-30). The mean recovery of stem cells from processed peripheral blood was $42.0 \pm 15.5\%$. A single apheresis achieved the requested number of stem cells in 56/69 (81.2%) for all procedures and in 24/27 (89%) for normal donors and in 32/42 (76%) for other patients.

Close monitoring of peripheral blood WCC and $CD34^+$ concentration in mobilised patients and the accurate prediction of the blood volume to be processed optimises leukapheresis and restricts the number of procedures to a single collection in the majority of patients.