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HANDBOOK VOLUME I:
FINAL PROGRAMME & ORAL ABSTRACTS

30 October - 2 November 2011 Sydney Convention & Exhibition Centre, Sydney, Australia

A joint scientific meeting of the:

Haematology Society of Australia and New Zealand
Australian and New Zealand Society of Blood Transfusion
Australasian Society of Thrombosis and Haemostasis
with the
XIIth Congress of the International Society of Hematology, Asia-Pacific Division
16th Congress of Asia-Pacific Blood and Marrow Transplantation
International Society for Cellular Therapy: Australia

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Sunday 30 October **0830-1000**
HSANZ Symposium 1: Haemoglobinopathies ***Auditorium B***

Overview of Sickle Cell Disease

John Porter
Department of Haematology, University College London, UK

Sickle cell disease (SCD) was the first genetic disease for which the molecular mutation was characterised. A single β-globin amino acid substitution results in reversible polymerisartion of Hb molecules, under hypoxic conditions causing haemolytic anaemia, NO scavenging, and intermittent vaso-occlusion, the latter causing severe pain from bone marrow necrosis ('crises'). Clinical presentation is diverse, both between patients and within an individual's lifetime. Genetically-determined disease modifiers include persistence of HbF and the co-inheritance of *alpha*-thalassaemia. Recognised acquired precipitants include inflammation, infection, dehydration, and hypoxia. SCD is a multisystem disorder; in childhood, life-threatening anaemia may be caused by acute splenic sequestration or by parvovirus B19 infection. Functional hyposplenism, results in a high risk of *pneumococcal* septicaemia. Stroke affects about one child in 10 and this risk is predicted by performing yearly trans-cranial Doppler ultrasonography, stroke being more likely in children with increased flow rates. Chest syndrome causes significant mortality; here, red cells sequester in the lung tissue causing life threatening hypoxia, which can be effectively treated by timely exchange transfusion. This intervention is also indicated for acute stroke or in preparation for major surgery. Chronic organ damage becomes more common with advancing years, affecting: the bones (avascular necrosis); the eye (proliferative retinopathy), kidney (renal failure), heart (pulmonary hypertension), skin (leg ulceration). Repeated blood transfusions cause iron overload, which is difficult to assess using serum ferritin alone, non-invasive measurement of LIC by MRI being the most reliable approach. Iron overloading rates can be decreased with exchange rather than top-up transfusions but chronic overload can be safely treated with oral deferasirox. Hydroxyurea is an effective prophylactic treatment, decreasing haemolysis, vaso-occlusion, pain rate, hospital admissions and chest syndrome. Bone marrow transplantation from matched sibling donors has a procedure related mortality of about 5% and patient selection requires a detailed understanding of prognosis with non-curative treatments.

Sunday 30 October **0900-1030**
HSANZ Symposium 1: Haemoglobinpathies ***Auditorium B***

Iron Chelation in the Haemoglobinopathies – Australian Guidelines and Clinical Studies

P Joy Ho
Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

The transfusion-dependent haemoglobinopathies are an important problem in Australia, where significant numbers of patients with thalassaemia and sickle cell disease are treated. Iron overload is one of the most critical issues, and iron chelation is therefore crucial in the prevention and management of complications such as cardiomyopathy, cirrhosis and endocrinopathies. The development of new oral iron chelators such as deferasirox and deferiprone has widened treatment options and improved adherence, an important factor in iron control. Magnetic resonance imaging (MRI) techniques for the measurement of cardiac and liver iron load can be used to individualise therapy according to organ iron load, although availability is currently restricted and should be improved. These innovations in management and the diversity of settings in which Australian patients are managed have highlighted the benefit of compiling guidelines on iron chelation. This symposium provides a forum to discuss Australian practice and the recently published guidelines.

Iron-overload cardiomyopathy is the leading cause of mortality in thalassaemia major, and the development of cardiac MRI has been a significant advance. An Australian multi-centre study of MRI assessment of cardiac and liver iron was set up to evaluate the effect of deferasirox. An interim analysis at a planned sample size reassessment was completed recently, including 42 patients, of whom 32 had thalassaemia major and 3 sickle cell disease. In the haemoglobinopathy cohort (n=35), a reduction in cardiac iron was observed with a significant increase in cardiac T2* from 16.1 to 18.4 ms (p=0.002). Further analysis was performed based on the T2* baseline values: 5-10 ms, no change detected (n=6; 8.5 to 8.0 ms; p=0.7; non-significant); 10-20 ms, improvement of 26% (n=8; 14.8 to 18.7 ms; p=0.002); >20 ms, increase of 12% (n=21; 31.7 to 35.5 ms; p=0.04). The largest increase in LVEF was observed in moderate cardiac siderosis (baseline T2* 10-20 ms; increase of 5.7%, 55.9% to 61.6%, p=0.046). These interim results indicate for the first time that deferasirox induced a simultaneous and statistically significant increase in both cardiac T2* and cardiac function in patients with moderate cardiac siderosis. Descriptive analyses have shown a reduction in liver iron over one year of treatment. It is anticipated that Australian units will continue to participate in clinical studies investigating new protocols and compounds for iron chelation.

Sunday 30 October **0900-1030**
HSANZ Symposium 1: Haemoglobinpathies ***Auditorium B***

Update on Thalassemia and Hemoglobinopathies: From Bench to Bed

Vip Viprakasit

Abstract not received at time of going to print

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Sunday 30 October **0900-1030**
ANZSBT Symposium 1: Platelet Issues ***Auditorium A***

Prophylactic Platelet Transfusion

Sherrill J Slichter
Puget Sound Blood Center and University of Washington School of Medicine; Seattle, Washington, USA

Introduction
There are two aspects of prophylactic platelet (plt) transfusions that can be controlled by the patient's physician: 1) whether to provide plt transfusions prophylactically to prevent bleeding or only therapeutically for significant bleeding [i.e., World Health Orgaization (WHO) ≥ Grade 2 bleeding]; and 2) what should be the prophylactic plt transfusion trigger.

Therapeutic (T) *Versus* Prophylactic (P) Plt Transfusions
In 106 consecutive autologous stem cell transplant (SCT) patients who received only T transfusions, 19% of patients had WHO Grade 2 bleeding. In another study, 171 consecutive patients receiving autologous SCT for hematologic malignancies were randomized to receive either T or P plt transfusions. Plt transfusions were reduced by 27% in the T arm, and 46% received no transfusions. WHO ≥ Grade 2 bleeding was 28.7% T *versus* 9.5% P, and one T patient had an intracerebral bleed without sequelae. In contrast, in another randomized T *versus* P plt transfusion trial in AML patients receiving chemotherapy, 7 T patients had intracerebral bleeds with 2 fatalities *versus* none in the P arm.

Transfusion Trigger
In two small studies in thrombocytopenic patients not being supported by plt transfusions, bleeding risk was not substantially increased until the plt count was ≤5,000/µl. In a recent study involving over 1,272 patients receiving chemotherapy for acute leukemia, bleeding was assessed on 24,309 days. At morning plt counts of ≤5,000/µl, WHO ≥ Grade 2 bleeding occurred on 25% of the days *versus* 17% at plt counts between 6,000/µl and 80,000/µl ($p<0.001$). Several randomized clinical trials have compared prophylactic plt transfusion triggers of 10,000/µl *versus* the previously-accepted standard of 20,000/µl. All showed no increase in bleeding risk at the lower trigger, and cost savings were 22% to 33% less because of fewer plt transfusions.

Conclusion
Therapeutic-only plt transfusions may not be safe for all thrombocytopenic patients, and a prophylactic plt transfusion trigger of 10,000/µl is safe and cost-effective.

Sunday 30 October **0900-1030**
ANZSBT Symposium 1: Platelet Issues ***Auditorium A***

Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic Uremic Syndrome (HUS): A Community Perspective

James N George
Departments of Medicine and Biostatistics & Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

The Oklahoma TTP-HUS Registry is a population-based inception cohort of 415 consecutive patients over 22 years for whom plasma exchange treatment was requested for a diagnosis of TTP or HUS. Although only 68 (23%) of the 299 patients with ADAMTS13 measurements had severe deficiency (<10% activity), quite different from reports of selected patients, these patients are often described as having “typical” TTP. Patients with severe ADAMTS13 deficiency usually respond quickly to plasma exchange and steroids; patients with complicated courses or relapses may be effectively treated by the addition of rituximab; they have a 41% risk for relapse over 7.5 years while patients without severe ADAMTS13 deficiency rarely relapse. Patients with severe ADAMTS13 deficiency may not be severely ill: 34% had no neurologic abnormalities, not even confusion or headache; 49% had normal serum creatinine values; 78% had no fever. Only 3 (4%) of the 68 patients had the complete “pentad” of clinical features: microangiopathic hemolysis, thrombocytopenia, neurologic and renal abnormalities and fever. Two of these patients were subsequently discovered to have sepsis, not TTP, as the cause of their illness; one had systemic lupus erythematosus and TTP. These observations confirm the validity of the current nonspecific diagnostic criteria: only microangiopathic hemolytic anemia and thrombocytopenia without an apparent alternative etiology. The classic “pentad” of diagnostic features must be forgotten. Multiple disorders, such as systemic infections and malignancies, can mimic the presenting clinical features of TTP. Disorders described as TTP or HUS can have multiple etiologies other than severe ADAMTS13 deficiency and some may respond to plasma exchange. Clinicians face the dilemma of a disorder with 10% survival if untreated, with no explicit diagnostic criteria, and an urgent need for plasma exchange treatment that has increased survival to 80% but which has a high risk of critical complications.

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Sunday 30 October **0900-1030**
ASTH Symposium 1: Coagulation and Innate Immunity ***Bayside 204***

Platelets in Innate Immunity

Dermot Cox
Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland

While the role of platelets in thrombosis and haemostasis is well established their role in the innate immune system is less well recognised. As the first responders to any injury platelets play an important role in orchestrating the immune response to pathogens. Once activated by contact with bacteria at a site of injury they release the contents of their granules. Secreted anti-microbial peptides are bactericidal to many bacteria. Platelets also secrete cytokines such as CD40L and RANTES that attract other immune cells to the site of injury. These immune functions are mediated by distinct receptors that are also found on other immune cells. Pattern-recognition receptors such as Toll-like receptors facilitate platelet activation by lipopolysaccharide while FcγRIIa responds to antibody-bound bacteria. However, over-stimulation of platelets during infection plays a role in pathogenesis. Thus, platelet activation by bacteria during sepsis can lead to disseminated intravascular coagulation which is associated with thrombocytopenia and localised formation of thrombi in the microvasculature. Equally infective endocarditis occurs as a result of platelet activation on the surface of an infected heart valve. These immune receptors on platelets may be suitable drug targets to prevent some of the complications of infection.

Sunday 30 October **0900-1030**
ASTH Symposium 1: Coagulation and Innate Immunity ***Bayside 204***

A Role for Platelets in the Innate Immune Response Against the Malaria Parasite

Simon Foote
Menzies Research Institute Tasmania, Hobart, Tasmania, Australia

There is a competition between the parasite and the host. If the malarial parasite can reproduce sufficiently rapidly, it can reach a parasitaemia that is lethal to the host. However, if its rate of growth is slowed, the adaptive immune response of the host can kill the parasites before the critical parasitaemia that spells lethality for the host. The host response that controls the growth of malarial parasites is the innate immune response. We have recently used knockout animals and ENU mutagenesis to identify novel innate immune mechanisms that control the growth of parasites in the blood of mice. One of these mechanisms involves platelets. Platelets are able to specifically bind to infected red cells and kill the parasite within. We have identified some of the molecules involved in binding of platelet to infected red cell and the platelet effector molecule.

Sunday 30 October **0900-1030**
Nurses Symposium 1: Blood in Practice ***Bayside Terrace***

Leading Practice and Quality in Transfusion

Elizabeth Pirie
Scottish National Blood Transfusion Service, Edinburgh, Scotland

Blood transfusion nursing covers many aspects of donor and patient care including whole blood collection, donor and therapeutic apheresis, tissue banking and in the last decade hospital based transfusion practice. The UK Serious Hazards of Transfusion (SHOT) scheme however has demonstrated that the main cause of morbidity and mortality in patients receiving a transfusion is caused by errors in practice. Nurses are integral to the transfusion process; they are involved in every stage of the transfusion process from, informing the patient, assisting in the decision to transfuse, taking the blood sample for pre-transfusion testing, collecting the blood component from the Blood Bank or satellite refrigerator, undertaking the pre-administration checks, administering the component, monitoring the patient during the transfusion episode to managing any suspected transfusion reaction or event.

In the UK there has been increasing emphasis on the necessity to deliver services around the needs of patients, and nursing roles are seen as key to the delivery of this objective. High quality person-centred care however, should be based on sound evidence and not custom or ritual. In transfusion, surprisingly there is a paucity of research evidence and as a result practice is often based on clinical guidelines and expertise.

From our experience in Scotland there is no single intervention or magic bullet that will reduce or minimize the risk to patients. This talk will focus on the merits and challenges of establishing a national education and audit programme and the facilitation of national guidelines and clinical standards. By engaging with clinical and blood service colleagues we have made a positive impact on the standard of transfusion care patients receive.

Sunday 30 October **0900-1030**
Nurses Symposium 1: Blood in Practice ***Bayside Terrace***

Prescribing of Blood in Australia by Nurses: Where Are We Up To?

Beverleigh Quested
Australian Red Cross Blood Service, Blood Safe, Adelaide, South Australia

This paper discusses some of the issues related to the prescription of blood by nurses in Australia. Some blood products in Australia are scheduled as drugs (eg RhD immunoglobulin) with similar legislative requirements for prescription to other drugs. Fresh blood components such as red cells, platelets, plasma and cryoprecipitate are exempt from scheduling with no legislative prescribing requirements though guidelines in development refer to the prescription of blood by nurses. Blood transfusion can save lives but is not without risk.

Nurses and nurse practitioners in some areas like Haematology are keen to undertake a wider role in transfusion. Not all nurses will need to prescribe blood transfusions and the knowledge required to do so relates to specialised practice settings, with the resulting scope of practice discussions.

The prescribing of blood components products by the health professionals needs to acknowledge the following:

- Awareness of current Patient Blood Management Guidelines, clinical and research evidence to ensure the transfusion is appropriate with expected benefits outweighing the potential hazards
- Transfusion product knowledge including patient groups who may need special transfusion requirements or alternative treatments that may be appropriate
- Ordering and interpretation of the prerequisite pathology testing of blood groups and antibody presence needed
- Discussing the risks and benefits of transfusion to obtain consent as well as appropriate actions needed if the patient refuses blood
- Appropriate risk assessment, legal implications and indemnity insurance and documentation

Currently scope of practice for nurse prescription of blood occurs at the individual clinical units or institutions that consider the nurses expertise and determine the suitability of an individual nurse to prescribe. Such variability does not support the development of current or future nursing practice or the development of an education framework.

Sunday 30 October **0900-1030**
Nurses Symposium 1: Blood in Practice ***Bayside Terrace***

Giving and Taking: Developments in Nurse Led Red Cell Exchange

Claire Dowsing
Apheresis Service, Department of Clinical Oncology, The Royal Melbourne Hospital, Melbourne, Victoria, Australia

Sickle cell disease is one of a broad spectrum of haemoglobinopathies characterised by haemolytic anaemia and microvasculature occlusion. The long-term effects of these disorders can result in organ damage, severe painful crises, emergent hospital admissions and potentially shortened life expectancy. Current treatment modalities include hydroxyurea therapy and / or transfusion programs.

At the Royal Melbourne Hospital a chronic red cell exchange (RCE) program has been in operation for the past ten years treating patients with homozygous sickle cell anaemia and sickle cell beta thalassaemia. Patients with a history of recurrent veno-occlusive crises, silent cortical ischaemia on MRI, pulmonary hypertension, acute chest syndrome, multi-organ crises, pregnancy or poor tolerance of hydroxyurea are enrolled. Currently 16 patients attend the program on a 4 – 6 weekly schedule. The principle outcome measures are HbS <30% post exchange, Hct 30% ± 3% in keeping with the American Society for Apheresis (ASFA) guidelines.

The program is nurse-led by a dedicated apheresis nurse coordinator who manages patient schedules and follow-up treatment, and includes patient assessment and referral to medical staff as required. A minimum of 2 annual visits incorporating a thorough assessment of organ function is also scheduled with the treating Haematologist. Despite the necessity for regular apheresis treatment, this program has compliance for attendance near 100%. 62% of patients have not experienced an acute crisis requiring hospital admission since commencing the program and no patient has discontinued since enrolment. Patients report positive benefits to their lives as a result; including the ability to participate in full-time work or study (70% of current patient cohort), resumption of activities such as swimming and reported increased energy levels. This apheresis based red cell exchange program for patients with sickle cell disease, while resource intensive, has produced qualitative and quantitative improvement in the lives of these patients.

Sunday 30 October **0900-1030**
APBMT/BMTSANZ Symposium 1: GVHD **Bayside Gallery B**

Segregating GVHD from GVL Responses in Humans

Stanley R Riddell, Seitaro Terakura, Tori N Yamamoto, Cameron J Turtle, Rebecca A Gardner, Michael Hudecek, Michael C Jensen, Marie Bleakley, Edus H Warren
Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Despite the potency of the graft versus leukemia effect (GVL) after allogeneic hematopoietic stem cell transplant (HCT), leukemia relapse remains a major obstacle to a successful outcome, and strategies to augment GVL are often associated with graft versus host disease (GVHD). One appealing strategy that has yet to be fully realized is to separate GVL from GVHD based on the specificity of donor T cells for distinct minor histocompatibility antigens that are expressed selectively on recipient hematopoietic cells, including the leukemia. The limitations of this approach include the difficulty isolating T cells that are specific for the few minor H antigens that are known to be selectively expressed on hematopoietic cells, and the requirement that donor and recipient be appropriately disparate in antigen expression. An alternative strategy that we are pursuing is to engineer donor T cells to express chimeric antigen receptors that consist of an scFV fragment of an antibody specific for a tumor associated cell surface molecule linked to T cell receptor (TCR) signaling and costimulatory domains. Initial studies with autologous T cells transduced to express first generation CARs that lacked costimulatory domains gave disappointing results, but more recent work in animal models and in pilot clinical trials with a CD19 specific CAR that targets B cell lineage malignancies demonstrate potent and durable antitumor activity. The application of this approach to allogeneic HCT requires the selection of donor T cells that lack alloreactivity for T cell transduction, which can be accomplished by transducing virus-specific T cells. Advances in cell selection and transduction methods allow the rapid production of bi-specific T cell products that have potent antitumor activity in animal models and can now be tested in clinical trials in HCT recipients to evoke a GVL effect without GVHD.

Sunday 30 October **0900-1030**
APBMT/BMTSANZ Symposium 1: GVHD **Bayside Gallery B**

Antigen Presentation in Transplantation: Where and When?

Geoffrey Hill
Department of Immunology, QIMR, Brisbane, QLD, Australia

The presentation pathways for allogeneic peptides to induce graft-versus-host disease (GVHD) are unclear. We developed a bone marrow transplant (BMT) system whereby presentation of a processed recipient peptide within MHC class-II can be spatially and temporally quantified. While donor antigen presenting cells (APC) could induce lethal acute GVHD via MHC class-II, recipient APC were 100-1000 times more potent. Following myeloablative irradiation, T cell activation, initial proliferation and memory differentiation occurred in lymphoid organs independently of alloantigen. Surprisingly, professional hematopoietic-derived recipient APC within lymphoid organs had only a limited capacity to induce GVHD. In particular, dendritic cells were not required and their deletion accelerated GVHD. In contrast, non-hematopoietic recipient APC within target organs induced universal GVHD mortality, promoting high levels of alloreactive donor T cell expansion within the gastrointestinal tract and inflammatory cytokine generation. The targeting of dendritic cells to inhibit alloreactivity is likely to be counter-productive early after BMT and alternative strategies will be needed, targeting antigen presentation itself.

Sunday 30 October **0900-1030**
APBMT/BMTSANZ Symposium 1: GVHD **Bayside Gallery B**

Intestinal Homeostasis and Graft-versus-host Disease

Takanori Teshima
Center for Cellular and Molecular Medicine, Kyushu University, Fukuoka, Japan

Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (SCT), and damage to the gastrointestinal tract plays a critical role in amplifying systemic disease. Intestinal stem cells (ISCs) play a pivotal role not only in physiologic tissue renewal but also in regeneration of the intestinal epithelium after injury. Paneth cells located near ISCs within the crypt regulate intestinal microbial ecology by secreting antimicrobial peptides such as α-defensins. However, the dynamic process of damage and repopulation of ISCs and Paneth cells after SCT and its consequence are not well understood. Using mouse models, we have discovered that ISCs were injured by conditioning but rapidly recovered and restored normal intestine. ISCs are targets of GVHD and this process of ISC recovery was suppressed in GVHD. We also found that Wnt agonist R-spondin1 (R-Spo1) is a growth factor of ISCs. Injection of R-Spo1 protected against ISC damage, enhanced restoration of injured intestinal epithelium, and ameliorated systemic GVHD following allogeneic SCT by a mechanism dependent upon repair of conditioning-induced gut injury. Paneth cells were also injured in GVHD and their secretion of antimicrobial peptides was severely suppressed. The intestine of the healthy mammals is colonized with commensal microbiota, while the gram-negative proteobacteria make up a small proportion. We molecularly profiled gut flora changes in the course of experimental GVHD. Homeostasis of intestinal microbial communities was maintained in the absence of GVHD, whereas mice with GVHD showed a shift of gut flora towards an aberrant outgrowth of gram-negative rods in association with loss of Paneth cells and their expression of antimicrobial peptides. These results demonstrate that GVHD targets ISCs and Paneth cells and uncover the previously unrecognized crosstalk between hosts and the intestinal microbial ecology in the pathogenesis of GVHD and infection after allogeneic SCT

Sunday 30 October **1100-1200**
HSANZ Symposium 2: Myeloproliferative Disorders ***Auditorium B***

What's New in Myeloproliferative Neoplasms (MPNs)

Ruben A Mesa
Division of Hematology & Medical Oncology, Mayo Clinic, Scottsdale, AZ , USA

The discovery of the JAK2 V617F mutation in 2005 led everyone in the field of Myeloproliferative Neoplasms (MPNs) to believe that we were entering a new era in which the key pathogenetic molecular mutation had been discovered; indeed that we had entered an area that would be similar to what had been experienced in chronic myeloid leukemia. In the subsequent years that have followed, we have identified many additional mutations which have complicated our pathogenetic understanding to a much greater degree. We have identified several additional mutations, including mutations in MPL, the JAK2 EXON12, TET2, ASXL1, IDH1, IDH2, CBL, IKZF1, LNK, and EZH2. More questions have been raised than answered as this science of MPN pathogenesis has exploded. We have come to recognize that there is a greater familiar predisposition for the development of MPNs than had been thought previously. We have come to recognize that the JAK2 V617F is an important middle step in the pathogenesis of MPNs, but certainly is not the initiating mutation. We have come to recognize that issues of progression are central in the management of MPNs and trying to avoid progression, yet the mechanism of progression elude us. We have recognized through SNP array analysis that there are individuals who are more predisposed to development of MPNs than others. Diagnostically, we have come to an era of greater certainty in diagnostic criteria that have been witnessed by the WHO revision in 2009. Therapeutically, we have entered the era of JAK2 inhibition and have recognized these agents are a helpful, additional tool in our battle against myeloproliferative neoplasms, but do not yet represent the panacea that has long been desired. The assessment and appropriate use of these agents is currently undergoing much reflection.

Sunday 30 October **1100-1200**
HSANZ Symposium 2: Myeloproliferative Disorders ***Auditorium B***

Current Controversies in the Treatment of CML

Timothy Hughes
University of Adelaide and SA Pathology, Adelaide, Australia

Optimal first-line therapy
In Australia we may soon be in the fortunate position of having three excellent choices of therapy for patients with newly diagnosed chronic phase CML. Imatinib is a remarkably safe long-term therapy that can lead to deep molecular responses and extremely low risk of progression in the majority of patients. When you also consider the patients who can be successfully salvaged with second-line therapy after imatinib failure, around 80% will achieve excellent long term disease control if their first-line therapy is imatinib. The more potent second generation tyrosine kinase inhibitors (TKIs) dasatinib and nilotinib both achieve deeper overall molecular responses and may reduce the risk of progression to acute phase disease in the first 2 years compared to imatinib but have not yet demonstrated improved overall survival. The superior molecular responses observed were in the context of a conservative control arm where imatinib was given at 400 mg/day and patients who were intolerant or resistant came off study and were regarded as failures in terms of achieving molecular endpoints. Many of these patients would have gone on to achieve good molecular responses on second-line therapy. This is where the TIDEL II study may give us a more realistic picture of what can be achieved with an optimised imatinib approach, where a more potent TKI is substituted early in patients who don't achieve excellent molecular responses or are intolerant. So far this approach looks highly effective for patients who switch to nilotinib for intolerance but less clearly so for those switching for resistance. It also needs to be considered that the potential advantages of upfront dasatinib or nilotinib may be counterbalanced by a slightly higher risk of morbidity and mortality with these agents than is seen with imatinib. The recently identified but poorly understood risk of peripheral vascular disease with nilotinib and pulmonary arterial hypertension with dasatinib may further complicate these decisions depending on their frequency and severity.

Should TKI therapy be stopped in any patients in a complete molecular response (CMR) outside the trial setting
There is great interest in the French and Australian studies showing that 40% of patients in stable CMR can stop imatinib therapy without losing CMR. These studies are reassuring because they suggest that (1) many patients can safely stop without relapsing, and that (2) even for patients who have rapid molecular relapse upon stopping imatinib there do not appear to be long-term adverse effects – they virtually all achieve a stable CMR again after restarting imatinib. There have been no reported cases of progression or imatinib resistance in either study (over 100 patients in total). However it may be premature to recommend ceasing therapy in patients with stable CMR. Reasons for caution here are the relatively short follow up of these studies. It remains possible that late relapses will occur and that these patients may not be as responsive to restarting imatinib therapy as we have observed with early molecular relapses. Further studies of cessation are needed.

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Sunday 30 October **1100-1200**
ANZSBT Symposium 2: Complications of Transfusion: Respiratory Issues ***Auditorium A***

Overview of Transfusion Associated Circulatory Overload (TACO)/ Systemic Inflammatory Response Syndrome (SIRS) and Transfusion Related Acute Lung Injury (TRALI)

Hitoshi Okazaki
Japanese Red Cross, Tokyo, Japan

Respiratory complications of blood transfusion have been gaining much attention lately. Constriction of the airway and/or edema of the larynx are the common allergic respiratory complications of transfusion. However, it was only a decade ago that we realized that a substantial proportion of cases of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) might be related to the infusion of blood products. It was well known that ARDS could be triggered by blood transfusion. However, the etiology of blood-transfusion-induced ARDS remained unclarified, probably because blood transfusion was considered to be very rare cause of ARDS. Initial studies have revealed that anti-leukocyte antibodies such as antibodies against human leukocyte antigen or human neutrophil antibody reacting with cognate antigens activate leukocytes, which is followed by pulmonary microvasculature injury and noncardiogenic pulmonary edema. Recent studies using sensitive detection methods have shed light on the high HLA antibody prevalence in parous females, moreover, some studies of critically ill patients have shown a higher frequency of TRALI than that derived from hemovigilance data. Animal models of TRALI can be easily made by prestimulation of animals with lipopolysaccharide. Taken together, sepsis or other critical conditions, sometimes referred to systemic inflammatory response syndrome (SIRS), may be prerequisite for TRALI manifestation.

As the awareness of TRALI among clinicians grows, the number of reports of circulatory overload after transfusion increases. Overtransfusion results in cardiogenic pulmonary edema, but it is sometimes very difficult for clinicians to clearly distinguish cardiogenic from noncardiogenic pulmonary edema. Thus, transfusion-associated circulatory overload (TACO) has drawn attention recently as a differential diagnosis of TRALI.

Sunday 30 October **1130-1200**
ANZSBT Symposium 2: Complications of Transfusion: Respiratory Issues ***Auditorium A***

Laboratory Advances in Testing for HLA and HNA

Mary Diviney[1] & Rhonda Holdsworth[2]
Victorian Transplantation and Immunogenetics Service[1], Transplantation Services[2], Australian Red Cross Blood Service

Over the last ten years the technology for detection of antibodies to human leukocyte antigens (HLA) has evolved from the cell based complement dependent lymphocytotoxicity (CDC) test to solid phase assays using ELISA, Flow cytometry and more recently Luminex. Antibodies directed against HLA have traditionally been detected by the complement dependent lymphocytotoxicity (CDC) test using a panel of HLA typed cells. The percentage of cells in the panel to which the individual is sensitized is referred to as panel reactive antibody percentage or PRA. Antibody specificity can be determined using a panel of HLA typed cells but is difficult to assign when multiple antibodies, rare antibodies or HLA Class 2 antibodies are present.

The introduction of solid phase assays in particular those that use Luminex technology has increased the sensitivity of antibody detection and allows for precise definition of both HLA Class I and 2 antibody specificity. Luminex technology is a multi-analyte system consisting of an array of microspheres each coded with a specific flurochrome that can be detected using an advanced flow cytometer. Different formats of the Luminex HLA antibody test system are available; the most sensitive is the Single Antigen Bead test. Recombinant proteins are affinity purified from an immortalised cell line that expresses a particular HLA antigen and then conjugated to a microsphere, up to 100 of these beads can be combined in a single tube. Patient serum is added and after incubation and washing, a 2nd labelled antibody is added and detected in the Luminex system.

Similarly the detection of antibodies to human neutrophil antigens (HNA) has evolved from cell based assays to solid phase Flow cytometry and Luminex based assays.

This technology provides for many advantages over previous technologies, particularly increased sensitivity. The challenge is now to define cut-offs for clinical relevance.

Sunday 30 October **1100-1200**
ASTH Symposium 2: Challenges in Haemostasis ***Bayside 204***

Novel Pathogenesis-oriented Approaches for the Management of Refractory Immune Thrombocytopenia

Ming Hou
Department of Hematology, Qilu Hospital, Shandong University, 107 West Wenhua Road, Jinan, PR China

Primary immune thrombocytopenia (ITP) is a complex autoimmune disorder in which the patient's immune system reacts with platelet autoantigens resulting in immune-mediated platelet destruction and/or suppression of platelet production. ITP is a very heterogeneous condition and it is unlikely that a single pathogenetic mechanism underlies all cases. Refractory chronic ITP, which fails to respond to splenectomy and results in low platelet counts, often occurs in a small proportion of ITP patients. Treatment of refractory ITP should always be tailored to the individual patients, and novel pathogenesis-oriented approaches demonstrated certain potential for the management of refractory ITP. B-cell depletion therapy with rituximab has increasingly been used as a second line treatment option for ITP, and dexamethasone plus rituximab demonstrated a higher response rate than dexamethasone alone. Preliminary data from a multicenter study shown that rituximab plus rh-TPO yields rapid and sustained response in adult patients with refractory immune thrombocytopenia. In addition, rituximab could be effective in post-splenectomy ITP patients. FcγR blocking treatment with IVIg has been used for a long time. Our group recently found that the efficacy of IVIg or dexamethasone was associated with autoantibody species, and anti-GPIbα positive ITP patients were demonstrated to be less responsive to IVIg or dexamethasone therapy comparing with GPIbα negative patients. Agents functioned by inhibition of Fc receptor dependent signaling and blockade of B cell survival signal are under development. Immune mediated suppression of megakaryocyte and platelet development is a recently identified mechanism in ITP, in patients of this type, the TPO-receptor agonists, eltrombopag, romiplostim, and rh-TPO launched in China, have been shown to be effective, with comparatively high response rates and low toxicity. The potential mechanisms of ITP are multiple, and novel pathogenesis-oriented approaches could act as potential therapeutics for the management of refractory chronic ITP.

Sunday 30 October **1100-1200**
ASTH Symposium 2: Challenges in Haemostasis ***Bayside 204***

Bleeding Disorders of Childhood

Julie Curtin
The Children's Hospital at Westmead, Sydney, NSW, Australia

Congenital and acquired bleeding disorders may present in childhood and often these patients present challenges to the treating clinician in both diagnosis and management. An understanding of the physiology of the haemostatic system in children, which is different to that in adults, is essential in order to make an accurate diagnosis. Reference ranges for components of the haemostatic system are age dependent and physiological values may overlap with values considered deficient in an adult patient. Furthermore, practical considerations such as the need for relatively large volumes of blood for some tests (eg platelet aggregations) can make testing and subsequent diagnosis difficult. The management of the paediatric patient with a bleeding disorder is often different to that of the adult and the treating clinician needs to appreciate the natural history of the underlying bleeding disorder as well as the unique challenges that managing paediatric patients present.

The current concepts in diagnosing and managing children with common inherited bleeding disorders such as haemophilia A and B and von Willebrand Disease will be discussed. Management of these disorders in childhood with reference to prophylaxis, products available and inhibitor development will be covered. In addition, presentations of some of the rarer but important inherited bleeding disorders and the diagnostic challenges that clinicians face will be discussed.

Some acquired bleeding disorders, eg neonatal alloimmune thrombocytopenia are unique to paediatrics, others such as immune thrombocytopenic purpura are seen across the entire spectrum of ages. However, even these acquired disorders when presenting in children often have a substantially different course as compared to adults and it is essential that the treating clinical can recognise this and manage accordingly. The diagnosis and management strategies for these common acquired conditions will be discussed.

Sunday 30 October **1100-1200**
Nurses Symposium 2: The Counts of Monte Christo ***Bayside Terrace***

Complex Case Management of a Young Adult with SAA

Wendy Londal
Sydney Children's Hospital, Randwick, NSW, Australia

The diagnosis of a life threatening illness such as Severe Aplastic Anaemia (SAA) in a child is a frightening concept for any parent or child to deal with. Aplastic Anaemia (AA) is an illness characterised by failure of the bone marrow to undergo effective and sustainable haematopoesis. It can be acquired or inherited however, in many cases, causes in children unknown.
AA can be defined as moderate, severe and very severe. In the severe and very severe cases, first line treatment includes a bone marrow transplant, preferably from a matched sibling. In the absence of this, immunosuppressive therapy (IST) is given. Without treatment, death is almost inevitable. When no donor is found and IST fails, treatment options move from one of cure, to one of palliation - with the aim of managing or treating the clinical signs and symptoms and making life as comfortable as possible when the risk of fever, infection and bleeding is so high. In most cases, death is usually associated with overwhelming infection or a significant bleed (ie: intracranial haemorrhage).
This case study will address the specific challenges faced by a young person when two rounds of IST failed and no donor match for a bone marrow transplant were ever found. In the latter stages of his illness, this young man's care and management was compounded by co-morbidities including the diagnosis of Type 1 IDDM and other back–to-back life threatening health issues. Added to this, was his dire psychosocial situation that necessitated care in a home with medical support.
This facilitated interactive session reflects on the multidisciplinary approach to his complex case, spanning two different health centres and involving a variety of medical specialities as well as highlighting the challenges this young person faced in the context of his illness.

Sunday 30 October **1100-1200**
APBMT/BMTSANZ Symposium 2: Preventable/Early Transplant Complications
Bayside Gallery B

Treatment and Prevention of Hepatic VOD following SCT: Current Status and Future Directions, With a Focus on Defibrotide

Paul Richardson
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Hepatic veno-occlusive disease (VOD) is an important conditioning-regimen-related toxicity associated with stem cell transplantation (SCT). Severe VOD is associated with a 100-day post-SCT mortality rate of >80%, and develops in up to 70% of cases.[1] There is no approved treatment for VOD; current standard therapy comprises best supportive care.
Defibrotide (DF) a first-in-class polydeoxyribonucletide, is the most promising therapeutic agent for VOD to date. Specifically, Phase II and III trials and investigational new drug protocol studies of DF in the treatment of severe VOD post-SCT demonstrated complete response (CR) in 35–46% of patients by Day+100, with encouraging Day+100 survival and favorable tolerability[2–4]. A phase III historically controlled trial in patients with severe VOD with multi-organ failure (MOF) demonstrated CR of 24% in DF-treated patients, versus 9% in the matched historical control cohort (HC); p=0.015 (protocol 2005-01[5]). Furthermore, CR strongly correlated with Day+100 survival in this study (p<0.0001). DF has also demonstrated efficacy in VOD prevention. In a phase III prospective randomized trial of prophylactic DF in children at high risk of VOD post-SCT, VOD incidence was reduced by 40% in patients receiving DF compared with untreated control (p=0.049)[6]. Pooled analysis of phase II/III trials comparing DF-treated patients who met 2005-01 protocol criteria (n=201) with the HC (n=32) demonstrated a significantly increased Day+100 CR rate of 30% for DF-treated patients versus 9% for HC (p=0.0015). Moreover, Day+100 survival was 40% for patients receiving DF versus 25% for HC (p=0.0294). Although used in a critically ill population, DF was generally well tolerated; rates of hemorrhage did not increase with DF treatment compared with control (47% and 72%, respectively in protocol 2005-01).
Future directions include continued investigation of prophylactic DF and further elucidating the role of DF treatment in high-risk groups such as sirolimus- or mylotarg-exposed patients, and mismatched allogeneic SCT patients. Earlier intervention with DF, prior to the development of advanced MOF, and combination approaches with other agents targeting endothelial injury and hepatocellular dysfunction may also warrant additional investigation. Additional studies should also help identify those patients most likely to benefit and refine prevention and treatment strategies to further improve outcomes.

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Sunday 30 October **1100-1200**
APBMT/BMTSANZ Symposium 2: Preventable/Early Transplant Complications
Bayside Gallery B

Managing Viral Infections Beyond CMV in Haematopoietic Stem Cell Transplantation (HSCT)

Nicole Gilroy
Blood & Marrow Transplant Network, NSW, Australia

The management of CMV in HSCT has employed a range of strategies including pre-transplant screening, primary prevention of CMV acquisition in CMV seronegative transplant recipients; prophylaxis aimed at maintaining viral latency; preemptive therapy implemented before CMV disease develops using markers of viral reactivation such as pp65 and PCR, and the use of CMV active antiviral drugs and immunotherapies to manage disease if it develops.

CMV has provided a useful template for the management of other viral diseases in transplantation. Beyond CMV there are other viruses such as Hepatitis B, Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV) for which antiviral prophylaxis has been the mainstay of disease prevention in those already exposed. For these viruses, the requirement for prolonged or repeated courses of antiviral drug therapy may fail to control disease when compliance is suboptimal and drug resistance emergent. Adenovirus and Epstein Barr Virus (EBV) are implicated in severe disease sequelae; hence pre-emptive algorithms for their management are favoured, although the lack of standardised molecular testing and a baseline or dynamic threshold for pharmacological intervention are areas of ongoing clinical research. The management of polyomaviruses (BK, JC), and the Human Herpes Viruses HHV6, HHV7 and HHV8 rely on clinical recognition and the availability of laboratory diagnostics to guide appropriate therapy.

In addition to the above viruses, all transplant recipients are at risk of severe and often life-threatening complications from exogenous viral pathogens, in particular the ubiquitous community respiratory viruses. Therapeutic options for these viruses are limited, and therefore influenza vaccination of transplant recipients and their close contacts, the avoidance of sick contacts, and the screening and isolation of symptomatic patients in hospital is essential in preventing nosocomial outbreaks.

Sunday 30 October **1100-1200**
BMTSAA Symposium ***Bayside 102***

Microbial Screening of HPC - What should we be doing?

Pamela Clark[1], Annette Trickett[2]
[1] *Sydney Cord Blood Bank, Randwick, NSW, Australia*
[2] *The NSW Agency for Clinical Innovation BMT Network, NSW, Australia*

Introduction
Collection and processing of haematopoietic progenitor cells (HPC) is associated with significant risk of microbial contamination and hence relevant standards mandate microbial screening of the final product. There are limitations with currently used methods and hence this study was undertaken to determine the most optimal screening method for HPC.

Materials and Methods
Retrospective data on 537 contaminated cord blood units (CBU) were analysed for the detection ability of different types of bottles used (aerobic, anaerobic or paediatric). Additionally, 22 CBU were collected and processed using a closed system and deliberately spiked with one of eleven QC organisms obtained from BioMeriuex (BioBall Multishot 550) at varying concentrations. Samples from different product fractions were tested for contamination using BacT/Alert (bioMérieux) and BACTEC (Becton Dickinson) blood culture bottles incubated for a minimum of 7 days at 35°C.

Results
Retrospective data showed detection rates for obligate anaerobic organisms of 98% in the adult anaerobic bottle compared to 8% in the paediatric bottle and 2% in the adult aerobic bottle. Detection rates for aerobic organisms were 70% in the aerobic bottle compared to 47% in the anaerobic and 41% in the paediatric.

After spiking CBU with QC organisms at 10 CFU/mL, detection rates were 56% in aerobic and anaerobic bottles inoculated with 10mL plasma each, 89% in aerobic and anaerobic bottles each inoculated with 3mL RBC fraction and 67% in paediatric bottles inoculated with 1mL final product. Three organisms were detected in the plasma or RBC fractions but not detected in the final product.

Conclusions
This study demonstrates that optimal microbial screening methodology for HPC includes a combination of testing a discard fraction during processing in addition to testing the final product using either the BacT/Alert or BACTEC automated detection system. Testing only a small sample of the final product is suboptimal for sensitive detection of contaminating microbes, particularly obligate anaerobes.

Sunday 30 October **1100-1200**
BMTSAA Symposium ***Bayside 102***

Centralised Provision of Quality Management Service for NSW BMT Units

Annette Trickett
The NSW Agency for Clinical Innovation (ACI) Blood & Marrow Transplant Network, Sydney, Australia

In 2002, the Blood & Marrow Transplant (BMT) Network was established to create a forum for collaboration between the 15 government-funded BMT units in New South Wales. The goals of the Network include education, review and improvement of clinical practice, utilisation of group expertise/resources, and development of common policies, standard operating procedures (SOPs) and forms. The Network is centrally managed through an executive committee of elected representatives and centralised secretariat that includes a Quality Manager and 2 FTE Quality Officers.

The Network has developed a comprehensive quality system that is managed via Q-Pulse commercial software, which is accessible by all key BMT personnel. The system is used for electronic management of document approval and control, non-conformance reporting and corrective/preventative actions, audits, supplier qualification, and equipment scheduling. Automated email notification ensures that personnel are informed of all pending actions. SOPs are generated as either generic documents to be released to all relevant facilities (eg. donor screening) or, if necessary, as site-specific documents for release to a single facility.

The Network Quality Manager has a planned program to implement this system into all collection, laboratory and clinical units. Currently, 125 BMT personnel from 6 laboratories and 11 HPC collection/transplant units have Q-Pulse accounts; over 850 documents are active and 95 suppliers have undergone qualification. Multicentre validations are being performed, and the audit, non-conformance and equipment functions of Q-Pulse are also being utilised. Five BMT laboratories and 3 apheresis units have successfully undergone NATA accreditation using the Network quality management system and 1 BMT program has attained FACT international accreditation.

In addition to being extremely cost-effective, the BMT Network quality management system is driving clinical improvement and adoption of common protocols across clinical, apheresis and laboratory facilities.

Sunday 30 October **1100-1200**
BMTSAA Symposium ***Bayside 102***

Reporting Post-thaw Viable CD34 Counts on Frozen Grafts Does It Really Matter?

Vicki Antonenas
Sydney Cellular Therapies Laboratory, Westmead Hospital, Westmead, NSW, Australia

The number of CD34+ cells infused into patients at the time of autologous or allogeneic transplantation is a clinically important variable, but the viability of these cells has not been extensively documented and correlated with haematological recovery. In a retrospective study, we analysed the recovery of viable CD34+ cells before and after cryopreservation on 145 autologous stem cell products collected from adult patients with haematological malignancies at Westmead Hospital (2005 to June 2011), using a single platform viable CD34 assay. This assay is suitable for counting viable CD34+ cells for fresh and post thaw products. For the 145 harvest samples, the median viable CD34+ cell count was 5.8x10^6/kg (range 1.6-32 x10^6/kg) before freezing and 3.5x10^6 CD34/kg (range 0.5-28.3 x10^6/kg) after thawing. The median recovery was 67.5% (range 18-95%). Further examination of the correlation between the kinetics of haematological recovery and the number of viable progenitor cells infused, particularly at the lower end of the accepted dose range (<2x10^6 CD34/kg) will be presented.

Sunday 30 October **1100-1200**
BMTSAA Symposium ***Bayside 102***

Adoptive Transfer of Antigen-Specific T Cells for Treatment of Opportunistic Infection After BMT

Leighton Clancy
Sydney Cellular Therapies Laboratory, Westmead Hospital, Westmead, NSW, Australia

Allogeneic haematopoietic stem cell transplantation (HSCT) has the potential to cure haematological malignancies but has a high rate of procedure related morbidity and mortality. Infections are the most common cause of non relapse mortality in these patients accounting for almost 20% of all deaths in the first and second year post-transplant. In healthy people, viral infections are controlled by a specific cytotoxic T lymphocyte (CTL) response. We and others have demonstrated that the adoptive transfer of virus specific CTL grown in the laboratory from the blood of their healthy donors is highly effective therapy for the treatment or prevention of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections in allogeneic HSCT recipients.

In clinical trials at Westmead Hosptial 41 allogeneic HSCT recipients have now received prophylactic infusions of CMV specific CTL. We have recently changed the manufacturing process to generate CTL lines directly from G-CSF mobilised haematopoietic progenitor cell collections using only 2-4mls of a typical collection (1-3%). This has the benefit of utilising established transplant centre procedures for donor assessment, infectious disease testing, product collection, labelling and transport. In addition the number of opportunistic pathogens targeted by each CTL product has been broadened to include CMV, EBV, Adenovirus and Varicella-Zoster virus. One problem with CTL therapy is the time needed for preparation of a therapeutic product which can take several weeks. To overcome this delay, recent studies have investigated the use of partially HLA matched, pre-prepared virus specific CTL from third party donors to treat patients with viral illness. These studies have shown that minimally HLA matched CTL can exert potent antiviral effects *in vivo*. Complete clearance of viral diseases resistant to standard therapy have been observed in patients treated with third party T cells matched at only one or two HLA antigens with the recipient. This less stringent requirement for HLA matching suggests a small bank of virus specific T cells derived from approximately 30 donors would provide most patients in Australia in urgent need of therapy with access to a partially HLA-matched T cell product. Ultimately, such a bank could be used as a national resource for the treatment of immunocompromised patients with potentially fatal viral infections.

Sunday 30 October **1200-1300**
ASTH: Barry Firkin Oration ***Auditorium B***

HIT and Mist: The FOG Has Lifted on Platelets

Beng H Chong
Haematology Department, St George Hospital & Department of Medicine, SGCS, UNSW, Kogarah, NSW, Australia

Heparin-induced thrombocytopenia (HIT) and other thrombocytopenias have been my research interests since 1979. Not surprisingly, I also became interested in the regulation of platelet production. FOG-1, GATA-1, Fli-1 and NFE2 are major transcription factors (TFs) involved in this regulation. An accident in 1990s took my research to FOG-2 and GATA-4, key TFs in cardiac development. In this presentation, I shall discuss my research in these three areas. I saw a patient with HIT in 1979 when I was a haematology registrar. He was probably the first case of HIT diagnosed in Australia. Several cases of HIT had already been reported in USA but none in UK and continental Europe. At that time, the pathogenesis of HIT was unknown and there were no clinical diagnostic criteria, no reliable lab test and no effective treatment. Over the next 10-15 years, we made key contributions to the elucidation of the disease mechanisms, proposed clinical diagnostic criteria, established reliable laboratory tests and an effective treatment for HIT. We confirmed that HIT was caused by an IgG antibody that induced strong platelet activation via platelet Fc gamma RIIA receptors and also via its Fab domain. In the 1980s, we carried out the only randomized control trial in the treatment of HIT by comparing danaparoid with dextran 70. Until then, there were many “hits and misses” in our attempts to find an effective HIT treatment. With advent of new anticoagulants in 1990s, I mistakenly thought that HIT would no longer occur after the “mist has lifted”. I deliberately changed my research direction to the regulation of platelet production. We discovered a previously unrecognised negative feed-back mechanism that regulates platelet production, mediated by bone marrow stroma cell-derived thrombopoietin (TPO). In addition, platelet production is also regulated by a network of transcription factors such as FOG-1, GATA-1, Fli-1 and NFE2. We discovered these TFs interact with each other in their regulation of genes involved in megakaryopoiesis. When we over-expressed GATA-1 and NFE2 in megakaryocytes, platelet production was increased; this allows us to make enough platelets *in vitro* for platelet function studies. This is a proof in concept experiment that may lead in future to large scale *in vitro* platelet production for patient treatment. In an attempt to clone human FOG-1, we accidentally cloned human FOG-2 (not yet cloned then) and this led us to study the nuclear import mechanisms and SUMOylation of GATA-4 and FOG-2 and their roles in cardiac development and cardiac hypertrophy. In conclusion: HIT, MISS and FOG are a major part of the landscape in my research journey in Australia.

Sunday 30 October **1400-1530**
HSANZ Symposium 3: Myeloma ***Auditorium B***

Current Status of the Treatment of Multiple Myeloma: The Emerging Role of Novel Therapies

Paul Richardson
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

The last decade has seen remarkable progress in the therapy of multiple myeloma with the development of combination regimens that incorporate the proteasome inhibitor Bortezomib and the immunomodulatory agents Thalidomide and Lenalidomide. Advances in clinical practice have been predicated on our greater understanding of the biology of myeloma and critically its interaction with the bone marrow microenvironment as well as cortical bone. Most importantly, patients diagnosed today are living significantly longer than those diagnosed in the pre-novel therapy era. The integration of novel treatments around stem cell transplantation and the emerging treatment paradigm of induction, intensification if appropriate, consolidation and maintenance applied across both transplant eligible and ineligible patients has emerged as a key treatment algorithm in modern myeloma management. However, in spite of these advances, nearly all patients relapse, as reflected by the lack of any plateau in survival curves stemming from clinical trials evaluating currently available treatment options. Substantial challenges thus remain.

In this context, the emergence of novel combination therapies has become central to improving patient outcome. These are classically rationally designed and informed by bench research utilizing models that are increasingly informative. Second generation novel agents and derived targeted small molecules are under development as part of combination strategies, which build upon the therapeutic backbone of immunomodulatory therapy and proteasome inhibition. Monoclonal antibodies are now finding their place in the therapeutic armamentarium. Further refinement of autologous stem cell transplantation (ASCT) and its utilization in selected patients, versus sparing this in others and keeping ASCT in reserve is currently under study. In the same context, allogeneic stem cell transplant, which remains investigational, will also hopefully benefit from the impact of integration with novel treatments, making it a modality associated with less toxicity and better outcome.

Furthermore, improvements in emerging technologies to help understand heterogeneity in myeloma, and the complexities of its biology through genomics and proteomics should enhance the ability to pursue more tailored treatments. This, together with continued new drug development, offers the real hope of yet further clinical benefit and the increased probability of making this a chronic illness in the majority of patients.

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Sunday 30 October **1400-1530**
HSANZ Symposium 3: Myeloma ***Auditorium B***

Current Knowledge of the Molecular Pathogenesis of Myeloma

Wee-Joo Chng
Department of Haematology-Oncology, National University Cancer Institute of Singapore, National University Health System, Singapore

Over the last two decades, the application of genomics technologies that allow the interrogation of interphase cells, as well as the advent of molecular and sequencing technologies have greatly increase our understanding of the genetic abnormalities that underlie the development of myeloma. Through careful dissection of large cohorts of patients, we have now developed a framework for the molecular pathogenesis of myeloma: from the initial events that establish the malignant clone, to transforming events, to later events, these step-wise accumulations of genetic abnormalities that cover different oncogenic properties also reflect potential areas for therapeutic targeting and even rationale drug combinations. Further, there appear to be two dichotomous genetic pathways that are quite distinctive based on the ploidy of the tumor. Adding to this complexity is the revelation of clonal heterogeneity at different stages of disease and treatment by recent studies using highly sensitive and high-resolution sequencing techniques. Understanding the clinical and biological significance of this heterogeneity will be a major challenge in the coming years.

Sunday 30 October **1400-1530**
HSANZ Symposium 3: Myeloma ***Auditorium B***

Current Issues in Immunotherapy of Myeloma

DE Joshua, R Brown, PJ Ho, J Gibson
Institute of Haematology, Royal Prince Alfred Hospital, Bosch Institute, University of Sydney

There is considerable circumstantial evidence for immune mediated control of disease in myeloma. For example stable levels of paraprotein during plateau phase disease and the impact of GvM in the allogeneic setting demonstrate the ability of the host's immune system to control the disease. The dramatic response to immunomodulatory agents also infer that immunological control mechanisms are present

Why then has immunotherapy proven to be only marginally, if at all, effective in myeloma? Possible explanations include the inability to select the most appropriate antigen, antigen dominance when whole cells are used, exhaustion and anergy of cytotoxic T cell clones, absence of immunodominant peptides within immunoglobulin idiotypes, and perhaps most importantly, the complex immunosuppressive interactions between the tumour and its host causing dysfunctional dendritic cells, an inbalance in the control by Treg/Th17 cells and an increased number of T-cells with acquired regulatory functions .

In solid tumour systems dysfunctional T cell regulation can lead to tumour escape. In myeloma our studies on the Treg /Th17 axis demonstrate the number of Tregs in the blood of patients is increased, Th17 cells are reduced, and Treg function is impaired compared with aged matched controls. The addition of TGF beta induces additional suppression of T-cell proliferation by Tregs, while IL12 reduces Treg function. New data on cell membrane protein transfer from plasma cells to T-cells (trogocytosis) demonstrates that myeloma cell membrane antigens can easily be passed to T-cells. Acquired expression of antigens such as HLA-G or CD86 can be transferred to T-cells and HLA-G+ T-cells become induced Tregs in patients with myeloma. HLA-G and CD86 expression is quite heterogenenous on malignant plasma cells ranging from 0-96% and carry adverse prognostic significance. Initial data shows that HLA-G is expressed in increased amounts on the SP cells in myeloma , suggesting failure to target the stem cell population is another reason for the ineffectiveness of immunotherapy.
Therefore these complex interactions make induction of successful immunotherapy problematic, not only related to the inability to choose the appropriate antigen but predominately because of the complex immunomodulatory interactions between the tumour and its host.

Sunday 30 October **1400-1530**
ANZSBT Symposium 3: Iron Metabolism ***Auditorium A***

Iron Metabolism in Transfusional Iron Overload

John Porter
Department of Haematology, University College London, UK

Cellular iron uptake is mediated by interaction plasma transferrin (Tf) with the Tf receptor, while cellular iron egress is modulated by interaction of plasma hepcidin with membrane ferroportin. Iron is redox active but rendered 'safe' by binding to plasma Tf or to cellular ferritin. With iron-overload, 'free-iron' species promote free radical generation and organelle damage. In health, storage iron rarely exceeds 2g, but repeated blood transfusions inevitably cause iron overload as each red cell unit, contains approximately 200 mg of iron. In Thalassaemia Major (TM) the mean iron loading rate is 0.4mg/kg/day (range 0.3-0.6mg/kg), accumulating initially in macrophages of spleen, liver and bone marrow, then in liver hepatocytes, finally spreading extra-hepatically with endocrine morbidities and mortality from cardiomyopathy. While some factors determining the propensity to extra-hepatic iron distribution are understood, there is considerable heterogeneity, both between different iron-overloaded conditions, and between individuals sharing the same diagnosis. Plasma iron species unbound to transferrin and are referred to as plasma non-transferrin bound iron (NTBI). It has been widely assumed that the pattern of extra-hepatic iron distribution reflects that of plasma NTBI clearance but evidence linking NTBI levels to clinical outcome is sparse. There are clear data linking control of serum ferritin, liver iron concentration (LIC) and myocardial iron (mT2*) to clinical outcome. Iron chelation therapy aims to tap two major chelateable iron pools; the first is iron derived from the catabolism red cells, and the second is from hepatocellular iron. Iron chelators generally remove 'MRI-visible' storage iron faster from the liver than from the heart. However if a patient with high myocardial iron levels develops heart failure, clinical benefit from chelation therapy is often seen before measurable changes in mT2* occur. With modern chelation therapy and monitoring, morbidity and mortality from transfusional iron overload are now fallin substantially.

HAA 2011 HSANZ ANZSBT ASTH

Sunday 30 October **1400-1530**
ANZSBT Symposium 3: Iron Metabolism ***Auditorium A***

Iron Management in Blood Donors: A Trial of Post-donation Iron Replacement

Joanna Speedy, Denese C Marks, Kathryn L Robinson, Hugh R Capper, Phillip J Mondy, Tania Brama, Anthony J Keller
The Australian Red Cross Blood Service, Melbourne VIC, Australia.

Maintaining iron balance in blood donors presents a challenge to blood services globally. Local strategies including haemoglobin screening, minimum donation intervals, nutritional advice, and ferritin testing for donors with low haemoglobin, are not sufficient to adequately manage the iron status of susceptible donors. To address this, the safety and efficacy of post-donation iron replacement in Australian female whole blood donors was investigated.

Methods

282 female whole blood donors aged 18 to 45, were randomised in a double-blinded trial to receive an eight week post-donation daily course of 45 mg carbonyl iron or placebo. The primary endpoints compared total body iron (TBI) and proportion of donors with ferritin<15 ng/mL at 12 weeks post-donation. Secondary endpoints included haemoglobin and incidence of gastrointestinal complaints.

Results

Ferritin, TBI and haemoglobin in the placebo and iron groups were equivalent at baseline. At week 12 the treatment group had a significantly higher median ferritin (14.27 ng/mL compared to 8.34 ng/mL; p<0.001), median TBI (7.878 mg/kg compared to 5.275 mg/kg; p<0.001) and haemoglobin (134.7 ± 8.7 g/L compared to 130.1 ± 10.0 g/L; p<0.001). Of the donors receiving iron, 51.9% had a ferritin< 15 ng/mL compared to 80.5% in the placebo arm (p<0.001). Significantly more donors (51.4%) receiving iron had at least one gastrointestinal side-effect, compared to those receiving placebo (27.9%; p<0.001). Importantly 86.7% of donors receiving carbonyl iron indicated they were prepared to take iron supplementation on an ongoing basis.

Conclusion

An eight week course of 45 mg carbonyl iron daily was well tolerated and effective in ameliorating iron loss due to whole blood donation. Post-donation iron replacement may have a role in a broader strategy incorporating tailored donation intervals, ferritin screening and education. The long term effects of iron replacement on donor iron status, tolerability and acceptability could be the basis for future research.

Sunday 30 October **1400-1530**
ANZSBT Symposium 3: Iron Metabolism ***Auditorium A***

Management of Iron Deficiency in the Pre-operative Setting

Kathryn Robinson
Australian Red Cross Blood Service, Adelaide, South Australia, Australia
BloodSafe Program, Adelaide, South Australia, Australia
The Queen Elizabeth Hospital, Adelaide, South Australia, Australia

Identification, evaluation and management of anaemia in patients awaiting major surgery are important to reduce the risk of peri-operative transfusion which is associated with increased risk of morbidity, mortality and hospital length of stay. Anaemia is frequently present pre-operatively, with iron deficiency (IDA) a common cause.

The underlying cause of any anaemia and/or iron deficiency should be assessed. Both may be the result of significant underlying pathology and increase the risk of peri-operative transfusion. Ferritin is the most useful indicator of iron deficiency. In the elderly or among patients with inflammation/systemic illness, iron deficiency may still be present with ferritin levels up to 60-100 mcg/L.

Patients without a clear physiological cause of IDA (especially men and post-menopausal women) should be evaluated by gastroscopy/colonoscopy to exclude a source of gastrointestinal bleeding, particularly from a malignant lesion. Timing of investigations in relation to surgery needs to be considered based on the clinical setting and urgency of surgery, in consultation with a gastroenterologist. Screening for coeliac disease is recommended.

Once iron deficiency is confirmed, iron therapy should be commenced while the underlying cause is being established and managed. Oral iron, in appropriate doses and for a sufficient duration, is an effective first line strategy for many patients. IV iron may be required in patients with IDA who are intolerant of oral iron or fail to respond, or where rapid iron repletion is clinically important (e.g. short time to non-deferrable surgery).

Pre-operative iron therapy may also reduce the risk of transfusion in patients with suboptimal iron stores (ferritin <100 mcg/L, with or without anaemia) in whom substantial blood loss is anticipated.

Improvement strategies include development of patient pathways, engagement with general practice, anaemia clinics, patient and clinician tools/educational resources, clinical guidelines, academic detailing, identification of local champions and rapid access to IV iron infusions.

Sunday 30 October **1400-1530**
ASTH Symposium 3: Cancer and Thrombosis ***Bayside 204***

Cancer and Thrombosis

AK Kakkar

Abstract not received at time of going to print

Sunday 30 October **1400-1530**
ASTH Symposium 3: Cancer and Thrombosis ***Bayside 204***

Immunomodulating Drugs and Coagulopathies

Kate Burbury
Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia

Thromboembolism (TE) is an important complication of cancer with substantial clinical implications - with an incidence of up to 25%, depending on patient-, disease- and treatment-related risk factors.
Thalidomide and lenalidomide, immunomodulatory agents with potent anti-angiogenic and anti-inflammatory properties, have demonstrated improved outcomes particularly in patients with newly diagnosed and previously treated multiple myeloma (MM) as well as other low-grade lymphoproliferative disorders. However, these treatment regimens have been shown to further increase the risk of TE in MM patients, especially when combined with steroids and/or chemotherapy.
The pathophysiology of this increased risk is yet to be elucidated but likely involves inflammatory, endothelial and haemostatic dysregulation. As well as an elevated paraprotein, data have demonstrated markedly altered biomarker profile in patients with MM. In particular, increased VWF antigen, FVIIIc, vascular endothelial growth factor, inflammatory cytokines and cancer procoagulant, as well as acquired APC resistance, which may contribute to the hypercoagulable state.
Given disease and patient heterogeneity, bleeding and TE risks are different for all patients and individuals over time. With the emergence of novel antithrombotic agents, further understanding of the pathophysiology of both MM- and therapy-related haemostatic dysfunction and the identification of important biomarkers, may promote a risk-stratification process and allow a targeted therapeutic strategy. Moreover, although several different thromboprophylaxis strategies have been effective in lowering the risk of TE, these prevention strategies have not been prospectively compared head-to-head.
Recent evidence on the pathophysiology, the role of immunomodulatory therapy, thrombogenic biomarkers for risk-stratification and recommended prophylactic antithrombotic therapy will be discussed.

No conflict of interest to disclose

Sunday 30 October **1400-1530**
ASTH Symposium 3: Cancer and Thrombosis ***Bayside 204***

Anti-Angiogenic Therapies

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The concept of targeting tumour angiogenesis as a means of treating cancer was first proposed in 1971. Decades later, following the discovery of vascular endothelial growth factor (VEGF) and other angiogenic factors, a wave of drug development followed, focusing on angiogeneic growth factor targets or pathways. Using in vivo and in vitro models of angiogenesis several key targets were identified as important drivers of tumour angiogenesis. The first successful “targeted” AI was the anti-VEGF monoclonal antibody bevacizumab. It was first shown to be effective, when combined with chemotherapy, in metastatic colorectal cancer. Subsequently it was shown to have activity in a number of solid tumours including lung, breast, renal cell and ovarian cancers, and gliomas. A number of hypotheses exist as to how it exerts its effect including how it synergises with chemotherapy but the actual mechanisms are still to be fully elucidated, particularly in light of the negative adjuvant colorectal studies. Interesting “class” side effects have been observed in these studies including hypertension (HT), proteinuria, epistaxis and risk of haemorrhage (SCC lung), arteriovenous thromboses and bowel perforation. Stydies have since suggested HT may be a pharmacdynamic effect. Impairment of wound healing has not been a clinically significant problem. The other key class of agent to be explored are the small molecule tyrosine kinase inhibitors, targeting VEGF and often other angiogenic growth factors usually by inhibiting ATP binding in the intracellular domain of the receptors. The greatest success with these agents has been seen with these drugs used as monotherapy in renal cell carcinoma and recently in pancreatic neuroendocrine cancers. However, when combined with chemotherapy results have been disappointing. It is clear that AIs are an important class of agent however there is a great need to identify factors that may select the patients most likely to benefit.

Sunday 30 October **1400-1530**
Nurses Symposium 3: Haematology Nursing at the Edges ***Bayside Terrace***

Pushing Boundaries in Remote Haematology

Louise Underhill

Abstract not received at time of going to print

Sunday 30 October **1400-1530**
Nurses Symposium 3: Haematology Nursing at the Edges ***Bayside Terrace***

The Role of Oncology Nurse Practitioner

Keith Cox OAM
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The Nurse Practitioner (NP) is an advanced practice nurse whose practice is authorized by legislative authority not currently within the scope of registered nursing practice. The advance clinical practice roles for NP include: direct referral of patients to other healthcare professionals, prescribing selected medications and ordering diagnostic investigations. The scope of practice will be determined by the context in which the NP is working.

The role of the Oncology Nurse Practitioner may include: reviewing patients who attend the chemotherapy unit who are unwell, determining the relationship to side effects from their chemotherapy or Cancer related symptoms. Other areas of this role could encompass: coordination of complex treatments involving other modalities including Radiation and Surgery and seeing patients receiving weekly chemotherapy. The non-clinical role could comprise of clinical education of nurses, medical staff and university lecturing, involvement in developing clinical practice protocols, revision of policies, procedures and protocols, initiation and participation in research projects, service development, quality improvement, participation in hospital and community committees.

In conclusion there are established NP positions in cancer care across Australia. NPs are valuable contributors to patient care and the health care team, although roles vary greatly depending on the setting, the features of advanced nursing practice are the same.

Sunday 30 October **1400-1530**
Nurses Symposium 3: Haematology Nursing at the Edges ***Bayside Terrace***

Scope of Practice: The Advanced Practice Nursing Role in Central Venous Cannulation and Vascular Access

Tim Spencer
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It is commonly accepted that the more often a procedure is performed by an individual, the greater their expertise **BUT** why is there demarcation still between what is 'Doctor's' work and what is 'Nurse's' work? It has been about culture, history and marking ones professional turf!

Expanding opportunities for postgraduate specialist education, health service restructuring, and technological advances have all had a significant impact on the nature of the nurse's role and scope of practice and their influence on the health care system.

Nurses have embraced these changes and the opportunity they bring for 'extending the frontiers of practice'.

Australian advanced practice nurses/nurse practitioners are well positioned to bridge the divide of inequitable distribution of health services not only between metropolitan and rural/remote areas, but also within metropolitan areas.

Ultimately, the scope of nursing practice encompasses standards of professional nursing practice that identify the roles and responsibilities of the nurse in any health care setting.

Advanced practice nursing is emerging as an important strategy in improving patient safety and improving patient health outcomes and the insertion of a central venous catheter (CVC) by a trained specialist nurse clinician may promote efficiencies and potentially minimise adverse events.

Sunday 30 October **1400-1530**
Nurses Symposium 3: Haematology Nursing at the Edges ***Bayside Terrace***

Pushing Boundaries in Advanced Nurse Practice in Haematology-Radiotherapy

Tracey Dryden[1], Meinir Krishnasamy[1,2]
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Introduction
This paper will discuss the development of an advanced practice nurse (APN) role within the haematology-radiotherapy team at the Peter MacCallum Cancer Centre (Peter Mac) in Melbourne. The introduction of this role to the multidisciplinary team aims to deliver a coordinated and comprehensive package of care for haematology patients who attend for radiotherapy at Peter Mac, whilst receiving other treatment modalities at other hospitals.

Methods
There is currently a model of tumour stream specific link nurses within the radiotherapy unit at Peter Mac. Under this model, every haematology patient attending for radiotherapy is booked to see a haematology specialist nurse for a weekly consultation during treatment. However, due to resource constraints within this increasingly busy department, in practice the consultation is not always undertaken by the specialist nurse. The APN model proposes that all haematology reviews are undertaken on a specific day when the haematology-radiotherapy nurse is present and that a communication and referral pathway is developed for patients who need support when the haematology-radiotherapy nurse is not available. Other elements of the model include the development of standardised communication pathways with other referring hospitals to ensure a coordinated and streamlined approach to patient care, formal supportive care needs screening and a pre-treatment nursing consultation to prepare patients for treatment.

Implications
This paper will discuss the initial stages of the role development process, the challenges encountered and how it is changing current practice.

Sunday 30 October **1400-1530**
APBMT/BMTSANZ Symposium 3: Transplant for Haematological Malignancies
Bayside Gallery B

Reduced Intensity Conditioning Regimens for Allogeneic Transplantation

Mary M Horowitz
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Allogeneic hematopoietic stem cell transplantation (HCT) is an effective therapy for many hematologic malignancies. However, the median age of onset of most of these disorders is >50 years. Older patients may be at higher risk of regimen-related morbidity and mortality with standard myeloablative preparative regimens, as are patients, regardless of age, with significant co-morbidities related or unrelated to their cancer and its treatment. Reduced intensity conditioning regimens that facilitate donor cell engraftment while lowering the risk of toxicity from chemotherapy and radiation now allow many of these patients to undergo allogeneic HCT. The principle is to provide sufficient immunosuppression to facilitate donor cell engraftment and, with some regimens, cytotoxity for short-term disease control, to allow graft versus tumor effects to achieve long-term disease control and cure. Data reported to the CIBMTR confirm single center reports indicating good outcomes after HCT with reduced intensity regimens in older patients. Data supporting feasibility and short-intermediate term success are now available for leukemia, lymphoma and myelodysplastic syndrome and with use of HLA-identical sibling donor, haploidentical related donor, unrelated adult donor and cord blood grafts. However, some data suggest that, despite lower regimen-related toxicity, reduced intensity regimens may not lower transplant-related mortality rates to the extent hoped for, due to continuing risks of acute and chronic graft-versus-host disease. Additionally, these regimens may be associated with higher relapse risks especially with very low intensity regimens or with the use of in vitro T-cell depletion with antithymocyte globulin. There may also be differences in the efficacy of graft versus tumor effects in different diseases. Choice of conditioning regimen depends on patient-related factors predicting risks of regimen-related toxicity and disease-realtd factors predicting sensitivity to graft versus tumor effects.

Sunday 30 October **1400-1530**
APBMT/BMTSANZ Symposium 3: Transplant for Haematological Malignancies
Bayside Gallery B

Haploidentical Blood and Marrow Transplantation: Where Are We?

Tong Wu[1], Dao-Pei Lu[1,2]
[1]Beijing Daopei Hospital, Beijing; [2]Shanghai Daopei Hospital, Shanghai, China

Hematopoietic stem cell transplantation (HSCT) is a curative therapy for many diseases such as hematological malignancies, bone marrow failure and other disorders. Haploidentical family members are important alternative donor for patients who need HSCT for otherwise incurable disease but without suitable matched sibling or unrelated donor. Haploidentical HSCT (haplo-HSCT) was initiated in 1981 in our centre. The early results were poor mainly due to severe graft-versus-host disease (GVHD) and infections post-transplant. To reduce GVHD, T-cell depletion and “megadose” CD34+ cells were employed with some success. Reduced intensity conditioning has further decreased early transplant-related mortality (TRM), but the relapse rate was relatively high. In our unit, Haplo-HSCT with GIAC or GIAC 3 regimen has achieved comparable outcomes in terms of severe acute GVHD, chronic GVHD, relapse, TRM, disease-free survival (DFS), and overall survival (OS) with HLA-identical sibling transplant. New strategies have been applied in order to better manage complications post-HSCT. One of these strategies is the use of cord blood co-infusion which has reduced severe acute GVHD and early TRM significantly. The use of adoptive cellular therapy has also improved the outcome of the majority of patients with refractory cytomegalovirus, Epstein-Barr virus and aspergillus infections. Analysis of our large series of transplants demonstrated that haplo-HSCT in sex-matched donor-recipient pair has a survival advantage. Early disease stage before HSCT and high CD34+ cell infused but not donor age and HLA disparity have positive influence on DFS and OS. The data CIBMTR has confirmed that outcomes of haplo-HSCT in hematological malignancies are not associated with patient age. In summary, haplo-HSCT should be considered for the patients at early disease stage, when matched siblings or unrelated donors are not available and, sex-matched family donors are the preferred donors for haplo-HSCT

Sunday 30 October **1400-1530**
APBMT/BMTSANZ Symposium 3: Transplant for Haematological Malignancies
Bayside Gallery B

HSC Transplants for HIV Malignancies

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It is over 30 years since the first CDC reports of Pneumocystis pneumonia and Kaposi's sarcoma occurring in homosexual men in New York and California heralded what was to become a global pandemic of HIV infection and the Acquired Immune Deficiency Syndrome (AIDS). Soon after primary CNS lymphoma and the the aggressive systemic NHLs became AIDS diagnoses. Hodgkins lymphoma, although not AIDS defining has also remained of increased incidence in HIV infected individuals and in the mid 1990's two new subtypes of NHL, Primary Effusion and Plasmablastic Lymphomas, both of poor prognosis were first described associated with HIV infection.

Initial reports before the introduction of effective combination anti-retroviral therapy (cART) for the treatment of these lymphomas were very disappointing with high mortality rates due to increased treatment toxicity and high relapse and refractoriness to therapy rates. Similarly early attempts at transplantation, especially allogeneic lymphocyte and HSC transplants aimed at trying to control HIV infection were uniformly unsuccessful, although post-mortem case reports purported control of HIV infection.

The introduction of cART in the mid 1990's significantly reduced the increased incidence of lymphoma but also allowed effective chemotherapy protocols to be utilised without undue toxicity and markedly improved results. Consequently this led to a re-examination of more intensive therapies, mainly supported by autologous HSCT, with good results approximating those seen in non-HIV infected lymphoma patients. Subsequently allogeneic HSCT has been employed, mostly using reduced intensity conditioning (RIC) protocols with similar success.

Improved survivals with cART in aging populations has also been associated with an apparent increase in other cancers but to date there is little in the literature examining the role of HSC for such patients.

The possibility of transplantation being a means of improving HIV control has also been re-examined, using auto transplantation as a vehicle for introducing genetically altered lymphocytes and allogeneic transplantation to provide stem cells resistant to HIV infection.

The results of these developments will be presented, however in a global sense the best way to reduce the incidence of lymphoma as well as other cancers and opportunistic infections for those living with HIV/AIDS will be to improve access to cART.

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Sunday 30 October **1600-1730**
HSANZ Symposium 4: Acute Leukaemias ***Auditorium B***

Young Adults with AML and Genetic Risk Factors

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The last three decades have seen dramatic advances in deciphering cytogenetic and molecular lesions underlying the pathogenesis of acute myeloid leukaemia (AML). These findings have not only afforded greater insights into disease biology, but also provided useful information predicting the likelihood of any given patient achieving and maintaining remission following conventional chemotherapy, leading to the development of risk-stratified treatment approaches. However, it is becoming increasingly apparent that AML is highly heterogeneous at the molecular level. This presents a major ongoing challenge to define the individual genetic abnormalities or combinations of markers that provide significant independent prognostic information and establish their respective relationships to other pre-treatment characteristics known to influence outcome (e.g. age, presenting WBC, secondary disease). Gaining robust information on likely risk of relapse in each cytogenetically and molecularly defined subset of AML is critical to inform decisions regarding the role of allogeneic transplant. However, existing trial data are difficult to interpret due to significant variations in the cytogenetic classification schemes employed by different cooperative groups. To address this we have investigated the prognostic significance of a more extensive range of cytogenetic entities (>50), including abnormalities recognized by the WHO (2008) in a cohort of 5876 younger adults (16-60yrs) treated in successive Medical Research Council (MRC) AML trials, leading to development of a refined classification system. Integration of the cytogenetic and molecular genetic data in a hierarchical fashion within the MRC AML10/12 trials showed that outcome of patients with AML that lack internal tandem duplication mutations in the Fms-like tyrosine kinase gene (FLT3-ITD negative) and harbouring a mutation in nucleophosmin (NPM1) or biallelic CCAAT/enhancer binding protein (CEBPA) mutations have a relatively favourable outcome comparable to that observed in the core binding factor (CBF) leukaemias and hence are unlikely to benefit from routine use of allogeneic transplant in first remission.

Sunday 30 October **1600-1730**
HSANZ Symposium 4: Acute Leukaemias ***Auditorium B***

Treatment of AML: Challenge and the Future

Tomoki Naoe and Japan Adult Leukemia Study Group
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AML is a morphologically and genetically heterogeneous malignancy characterized by expansion of myeloid blasts. In a JALSG registration study on newly diagnosed AML/high-risk MDS, over 3000 patients were registered from 2007 to 2011. Median age of the patients was 63 y.o. and 2-year survival rate was 55% and 24% in under and over 65 y.o., respectively. The former data are similar to those of prospective intervention studies, although the outcome of elderly patients is still poor. In AML-97 and -201 studies recruiting de novo AML under 65 y.o., the incidence of t(8;21) is higher than Western countries (less than 10%), although those of inv(16) (6%) and normal karyotype (45%) were similar. The prognosis of CBF leukemia was favorable nearly 70% in OS, whereas t(9;22), 11q15 translocation, t(10;11), t(6;9), t(16;21) and 8p11 translocations showed worse prognosis in addition to established "adverse" cytogenetics. AML201 study revealed that intensified dose of DNR (50mg/m2/d x 5d) is equivalent to IDA (12mg/m2/d x 3d) regarding to CR rate, RFS and OS, whereas high dose of AraC (2g x 2/d x 5d) is marginally effective in the use of consolidation to CBF leukemia. To improve outcome of AML, an addition of a third drug to 3+7 or newly developed therapies have shown promising initial results. However, the results often become disappointing after larger studies. Allo-HSCT has been so far thought to be a limited choice for poor risk AML. In an ongoing study AML209, AML with FLT3/ITD is allocated to receive allo-HSCT in 1st CR. This paper will discuss the preclinical data and future of new agents including TKI, mTOR inhibitor and STAT inhibitor, and targeting drugs to self-renewal. Combination chemotherapy using these new agents will provide the greatest potential for successful future of AML

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Sunday 30 October **1600-1730**
HSANZ Symposium 4: Acute Leukaemias ***Auditorium B***

Biology and Therapeutic Targeting of High Risk B-ALL

Charles Mullighan

Abstract not received at time of going to print

Sunday 30 October **1600-1730**
ANZSBT Symposium 4: Transfusion Support in Haemoglobinopathies ***Auditorium A***

Transfusion Support for the Alloimmunized Patient

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Alloimmunization in response to transfusion is a rare occurrence if robust procedures are in place to ensure ABO and D compatible blood is used. Only ca.10% recipients make alloantibodies to other blood groups but there is no reliable test to identify these individuals. Unfortunately, patients with several blood group alloantibodies are not uncommon. Clearly prevention of alloimmunization occurring in the first place would be the preferred approach. Automated DNA-based blood grouping is now well advanced and its availability has led some to argue that all blood donors should be typed at multiple blood group loci so that patients can receive blood compatible at many blood group loci and thereby minimise risk of alloimmunization. Apart from the fact that this procedure is unnecessary for 90% transfusion recipients there are significant associated problems. In many countries the distribution of blood groups in the donor population does not match that of the patient population and so it does not follow that donor blood compatible at multiple loci will be available for all patients. Even if the donor and patient populations are identical with respect to blood group distribution it would be necessary to have available a very large inventory of typed donors (>10,000) to meet all patients requirements at multiple blood group loci. A more pragmatic approach would focus this technology on patients already alloimmunized or with diseases requiring regular transfusion support over a long period and therefore highly likely to become alloimmunized, in order to minimise further alloimmunization. For example, a powerful argument can be made for applying DNA-based technology to provide blood compatible at multiple loci for patients with Sickle Cell Disease. For patients with multiple alloantibodies where compatible blood cannot be found the manufacture of red cells ex vivo from patient-derived cells is an intriguing possibility for the future.

Sunday 30 October **1600-1730**
ANZSBT Symposium 4: Transfusion Support in Haemoglobinopathies ***Auditorium A***

Transfusion Support for Patients with Haemoglobinopathies

Mammen Chandy
Tata Medical Center, Kolkata, India

Transfusion and chelation remain the mainstay for most patients with thalassaemia major and an appropriate regimen can ensure normal growth and development. Alloimmunization is reduced if transfusion is initiated before the first year of life and the RBC is pre-storage leucoreduced to a WBC of $<1x\ 10^6$/L. Red cell genotyping should be done before initiating transfusion at least for C ,c, E, e and Kell. Maintaining a pre-transfusion Hb of 9-10.5 gm/dL with a post transfusion Hb not exceeding 15 gm/dL by transfusing 10-15 ml /kg of packed red cells over 3-4 hours, at an interval of 2-4 weeks, remains the most practical regimen. Increased transfusion requirements may be due to red cell antibodies or hypersplenism and the latter should be considered if the antiglobulin tests are negative and the annual transfusion requirement exceeds 200 ml/kg of packed red cells. Splenectomy may be useful in these patients. The decision on whether to put a child with thalassemia intermedia on regular transfusion and chelation is more difficult particularly in a resource poor environment. However a child whose baseline Hb is <7gm/dL will have better growth, reduced skeletal deformity and late life osteopenia if Hb is maintained above 9.5gm/dL by regular transfusion. Stem cell transplantation for thalassaemia can be done with ABO mismatched donors and the optimal management will be discussed. Post transplant patients require irradiated blood products but the duration for which this needs to be continued in the event of rejection is not clear.

Transfusion in patients with sickle cell anaemia has different challenges and is indicated mainly for patients with complications which include sequestration crisis, acute chest syndrome, stroke etc. Exchanges transfusion may be required often since increase in haematocrit may worsen vaso-occlusion. Prophylactic transfusion to keep HBA levels above 30% may help to reduce recurrence of stroke.

Sunday 30 October **1600-1730**
ANZSBT Symposium 4: Transfusion Support in Haemoglobinopathies ***Auditorium A***

How Do I Obtain Rare Red Cells For My Patient?

Susan MacCallum
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Rare red cells are rarely required, but when needed can present a complex clinical and laboratory problem requiring a national and at times, international effort. The definition of what is considered rare varies from country to country, but generally refers to supplying blood to a patient with an antibody to an antigen of high prevalence, with an occurrence rate of less than 1:1000.

This presentation will discuss the management of patients with complex serology with and without transfusion-dependent haemoglobinopathies. Therapeutic options include autologous donation, banking frozen blood, recruiting family members, and searching national and international rare donor registries.

Sunday 30 October **1600-1730**
ASTH Symposium 4: Blood on the Floor: Managing Surgical Bleeding ***Bayside 204***

Perioperative Coagulation Management in Cardiovascular Surgery

Klaus Görlinger[1], Daniel Dirkmann[1], Alexander A. Hanke[2], Markus Kamler[3], Eva Kottenberg[1], Matthias Thielmann[3], Heinz Jakob[3], Jürgen Peters[1]
[1] Department of Anesthesiology and Intensive Care Medicine, University Hospital Essen, Germany. [2] Department of Anesthesiology and Intensive Care Medicine, Medical School Hannover, Germany. [3] Department of Thoracic and Cardiovascular Surgery, University Hospital Essen, Germany

Introduction
Blood transfusion is associated with increased morbidity and mortality. We developed and implemented an algorithm for coagulation management in cardiovascular surgery based on first line administration of coagulation factor concentrates combined with point-of-care thromboelastometry/impedance aggregometry [1].

Methods
In a retrospective cohort study including 3,865 patients we analyzed the incidence of intraoperative allogeneic blood transfusions (primary endpoints) before and after algorithm implementation.

Results
Following algorithm implementation the incidence of any allogeneic blood transfusion (52.5 vs. 42.2%; p<0.0001), packed red blood cells (PRBC) (49.7 vs. 40.4%; p<0.0001), and fresh frozen plasma (FFP) (19.4 vs. 1.1%; p<0.0001) decreased, whereas platelet transfusion increased (10.1 to 13.0%; p=0.0041). Yearly transfusion of PRBC (3276 vs. 2959 units; p<0.0001) and FFP (1986 vs. 102 units; p<0.0001) decreased as did the median number of PRBC and FFP per patient. The incidence of fibrinogen concentrate (3.73 vs. 10.01%; p<0.0001) and prothrombin complex concentrate administration (4.42 vs. 8.9%; p<0.0001) increased as did their amount administered per year (179 vs. 702g; p=0.0008 and 162 x 10^3IU vs. 388 x 10^3IU; p=0.0184, respectively). Despite a switch from aprotinin to tranexamic acid, an increase in use of dual antiplatelet therapy (2.7 vs. 13.7%; p<0.0001), patients' age, proportion of females, emergency cases, and more complex surgery the incidence of massive transfusion (≥10 units PRBC) (2.5 vs. 1.26%; p=0.0057) and unplanned re-exploration (4.19 vs. 2.24%; p=0.0007) decreased. Composite thrombotic/thromboembolic events (3.19 vs. 1.77%;p=0.0115) decreased but in-hospital mortality did not change (5.24 vs. 5.22%; p=0.98).

Conclusions
First line administration of coagulation factor concentrates combined with point-of-care testing was associated with decreased incidence of blood transfusion, massive transfusion, re-exploration, and thrombotic/thromboembolic adverse events.

Reference
Görlinger K, et al. Perioperative coagulation management and control of platelet transfusion by point-of-care platelet function analysis. Transfus Med Hemother 2007; 34:396-411.

Sunday 30 October **1600-1730**
ASTH Symposium 4: Blood on the Floor: Managing Surgical Bleeding ***Bayside 204***

Prevention of Venous Thromboembolism in Spine Surgery. Does Anticoagulant Prevent VTE Without Bleeding Risk?

Takeshi Fuji
Osaka Koseinenkin Hospital, Osaka, Japan

Impositi et al. reported the 199 pulmonary complications out of 1592 spine surgeries. In their series, there are 23 pulmonary thromboembolism (PTE) patients and the incidence of PTE is 1.4%. In Japan, Japanese Spine Research Society reported 18 cases of deep venous thrombosis (DVT) out of 16,157 spine surgeries. The incidence of DVT is 0.1%. Japanese Society of Anesthesiologist (JSE) initiated the survey of symptomatic perioperative PTE in the board member's hospitals. By the survey of JSA, the incidence of PTE in spine surgery is 4 times larger than in body wall operations. The 2008 survey of JSA indicates that the incidence of PTE after spinal surgery is 2.74 per 10,000 operations. My colleague reported the incidence of DVT after spinal surgery, by ascending venography. In lumbar surgery, the incidence of DVT, including non symptomatic, is 26.5% of operations (Spine 2000). Because of the difficulties in treating massive PTE, detecting DVT by clinical symptom, and screening at-risk patients by ultrasonography every day,, adequate prevention of venous thromboembolism (VTE: DVT + PTE) in all spine surgery is imperative. We introduced the venous foot pump (VFP) for prevention of VTE in lumbar spine surgery on October 1998. We investigated the incidence of DVT by ascending venography after lumbar spine surgery before and after introducing VFP (VFP group / non VFP group). In each group, the patient initiates walking at 5 days after the operation. In the VFP group, VFP was applied during surgery, and continued more than 20 hours per day until ambulation. The bilateral venography of the leg was done at 2 weeks after surgery. The incidence of DVT is 26.5% in non VFP group and 17.8% in VFP group. This difference is not statistically significant. From the JSA survey, the mortality rate of PTE by anticoagulant prevention is lower than that of PTE by mechanical prevention. The efficacy of anticoagulant for thromboprophylaxis is usually evaluated from the incidence of all VTE examined by venography, and the safety is evaluated from the incidence of bleeding events. The incidence of major bleeding event is relatively low, so the study, which study group has 100 cases, has not the power to evaluate statistically. Concerning about anticoagulant, higher efficacy means higher risk of bleeding. In spine surgery, the risk of postoperative epidural hematoma is about 0.2-0.5% in Japan. The half-life of anticoagulant is 1 hour with unfractionated heparin (UFH), 72-120 hours with warfarin, 3.2 hours with enoxaparin, and 16.2 hours with fondaparinux. Some anticoagulants have antagonist drugs. Protamine sulfate is the antagonist drug of UFH and partially effective in enoxaparin. Anticoagulant for thromboprophylaxis in spine surgery is still controversial. In spinal surgery, reoperation due to neurological worsening, epidural hematoma, or instrumentation failure is possible. Therefore, in the choice of anticoagulant, shorter half-life is better and neutralising agents are necessary. UFH is an old anticoagulant, which has the risk of heparin induced thrombocytopenia, but it has short half-time and has neutralized agent, protamine sulfate. We use UFH only for the patient with the history of VTE before surgery. Basic thromboprophylaxis in spine surgery is early ambulation, active ankle exercises, elastic compression stockings, intermittent pneumatic compression or venous foot pump. For high risk selected patients, anticoagulants should be recommended with care.

Sunday 30 October **1600-1730**
ASTH Symposium 4: Blood on the Floor: Managing Surgical Bleeding ***Bayside 204***

The Australian Perspective on the Role of Point-of-Care Analysers in Cardiac Surgery

Bruce Cartwright
Affiliation

Point of Care testing of Haemostasis has only recently been adopted into the practise of cardiac surgery in Australia. This talk will explore how a major cardiac surgical centre in Sydney has implemented guidelines to guide transfusion practise and POC testing is used to plan timing of surgery in the presence of significant antiplatelet effect. Future directions in POC testing will also be explored.

Sunday 30 October **1600-1730**
Nurses Symposium 4: Fuzzy Haematology ***Bayside Terrace***

Empirical Bioethics and Haematology

Kimberly Strong
Centre for Values, Ethics and the Law in Medicine – University of Sydney, Sydney, NSW, Australia

There has been an "empirical turn" in bioethics over the past few decades whereby a field once purely concerned with theoretical analysis of ethical issues in medical settings has embraced a range of empirical research methods traditionally used by the social sciences. This emergence of "empirical bioethics" is not without controversy, but for the most part, criticisms tend to lack an appreciation for the assumptions that underpin empirical bioethics or misrepresent what it claims to achieve. The assumptions, strengths and limitations of empirical bioethics will be presented in light of a qualitative study that explored a contentious technological option in haemapoietic stem cell transplantation – the use of preimplantation genetic diagnosis to create a 'saviour sibling'. This study involved semi-structured interviews with clinicians who, given their specialty, could be in a position to facilitate discussions about the technology; and parents who had been involved in making treatment decisions for their children in the haematology setting. In general, the interviews revealed a strong symmetry between concerns expressed by many of the clinicians and the theoretical concerns of bioethics based on rhetoric of harm, but a profound *disconnect* between the concerns of parents and those traditional ethical arguments. Notably, however, the empirical exploration of this contentious topic also revealed a convergence of views regarding the appropriateness of disclosure – that parents should be told about the technology and provided guidance as to its appropriateness in their child's specific scenario. These findings underpin the value of empirical bioethics – that it can elucidate the attitudes, experiences, values and processes involved in decision-making and reveal changes in moral stance that might otherwise be missed by traditional philosophical analysis.

Sunday 30 October **1600-1730**
Nurses Symposium 4: Fuzzy Haematology ***Bayside Terrace***

Fuzzy Faces: Self-portraits of Young People With Cancer

Peter Lewis
Centre for Values, Ethics and the Law in Medicine, University of Sydney, NSW, Australia

Introduction
Health care researchers are seeking novel and creative ways of engaging adolescents and young adults in research more often than ever before. The Growing Up with Cancer study used mixed methods including surveys, interviews, and the creation of digital "self-portraits" to determine the nature and extent of the impact of cancer on young people's transition from adolescence to young adulthood. This paper will describe the process undertaken to create the digital self-portraits. The paper will then link four participants' self-portraits with their interview data in order to show how these two methods of data collection can be used in combination to produce a richer understanding of participants' experiences.

Process
Creative research methods can be used to access previously unattainable information, perspectives, and knowledge from research participants. Creative methods can also facilitate reflection, sharing, and dialogue in ways unavailable in traditional, linear narrative methods. Creative methods, include photography, drawing, painting, poetry, and music, encourage participants to view their experiences with fresh eyes.
The Growing Up with Cancer project used creative methods in a novel way. A digital media artist worked with participants in groups and individually to facilitate the creation of their digital self-portraits. The process required a long period of engagement with participants that was rewarding for participants and researchers and that produced some attractive and meaningful self-portraits.

Meaning
The creative process combined with semi-structured interviews to create a "reflective space" that participants used to explore the meaning of their cancer experience. Fuzzy faces refers to the fluid, unstable state experienced by participants as a result of their cancer illness and treatment. Viewing participants' self-portraits in light of their interview data meant that the research team could develop a thorough and nuanced impression of participants' cancer experience.

Sunday 30 October **1600-1730**
Nurses Symposium 4: Fuzzy Haematology ***Bayside Terrace***

What, When and How – Information Needs: Myeloma Focus

T King[1,2], K White[1], M Stephens[1], N Ferrar[3]
1 Sydney Nursing School, Cancer Nursing Research Unit, University of Sydney.
2 Institute of Haematology, Royal Prince Alfred Hospital; 3 Psycho-Oncology Service, Sydney Cancer Centre, Sydney, NSW, Australia

Background
While Multiple Myeloma (MM) remains an incurable disease, significant advances in the understanding of disease biology, combined with rapid bench to bedside approval of new agents has led to an overall improved survival (OS). Individuals diagnosed with MM are faced with a cancer with no curative intent, significant heterogeneity, increasingly complex treatment schedules and significant morbidities. The benefits of providing information to patients are well-known, including reduced anxiety and depression. Despite this there has been limited research to identify the information needs of this group of patients and their families.
Aim
We undertook a study to 1) identify the information and education needs of those affected by myeloma; 2) specifically examine information needs related to steroids; and 3) develop and deliver resources and programs to meet these needs.
Method
An exploratory descriptive qualitative design utilising individual and focus group interviews was employed. A semi structured interview schedule guided the interviews and focus groups. No one directly involved in patient care was present. Interviews were audio-recorded and transcribed verbatim. Content data analysis was used. 47 participants (35 patients, 12 carers) agreed to participate and four focus groups (3 patient groups, 1 carer group) were held. Interested participants who could not attend the focus groups were offered individual interviews. For patients, range of time since diagnosis was 6 months to 18 years.
Findings
The overriding theme was '*navigation of care'* and significant gaps in access to, and delivery of, information were revealed. Gaps included HCPs' poor communication skills, lack of time and limited recognition of patient psychological needs, as well as inaccurate information on the internet and the lack of information for families or carers. Individually tailored information and improved care coordination manage the increasing amounts of information were required.
Conclusions
The themes identified in the study support the ongoing value of specialist care coordination. An exercise mapping the patient information pathway and signposting individuals to existing resources and services is underway.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session A)
Bayside Gallery B

Hematopoietic Stem Cell Transplantation (HSCT) in the People's Republic of China

Dao-Pei Lu on behalf of CSBMT and China Marrow Donor Program (CMDP)
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The total number of HSCT performed in 2009 in P.R. China is still no more than 2000 cases. A great majority of them had been reported to CSBMT (Chinese Society of Blood and Marrow Transplantation). Among them 1133 received allogeneic HSCT, 284 cases received autologous HSCT. The types of HSCT are shown in Figure1.

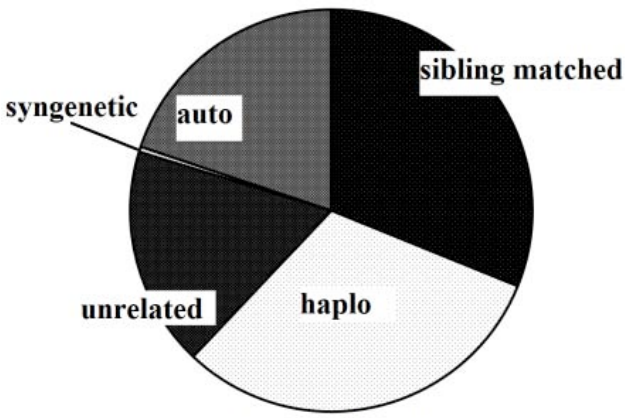

Figure 1. Types of HSCT

A feature of this distribution is that: haplo-identical HSCT consists of a quite large proportion of allo-HSCT. The reasons are partly due to the reduction of family size, and partly due to the development of clinical techniques. The distribution of the transplant numbers are mostly in big cities such as Beijing, Shanghai, Guangzhou, Chongqing, Suzhou, Hangzhou, Tianjin and Wuhan. The indications of allo-HSCT are mostly hematological malignancies in the order of the following: AML (35%), ALL (29%), CML (16%), MDS (6%), Lymphoma (4%), et al. The overall survival of unrelated and syn-HSCT is followed up. The most important risk factor for survival is the stage of the disease. The patient in CR1 had the best disease free survival rate, while the patients in advanced stage had the worst one. The 5-yrs survival rate among the unrelated HSCT recipients at Dao-Pei Hospital and CMDP are 72.2% and 55.9% respectively. The Chinese government has been limiting the number of cord blood bank below 7. However, a few new cord blood banks as well as placental stem cell banks are in the early stage. Unrelated cord blood, although still in use, is less used in clinics because of the expensiveness due to increased platelet transfusion. A patient with very severe aplastic anemia was rescued promptly with autologous stored cord blood. The longest survivor after syn-HSCT has been followed up 47 years after transplant. This is a revelation that engrafted hematopoietic stem cells remain actively proliferating without apparent aging.

No conflict of interest to disclose

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session A)
Bayside Gallery B

The Current Activities of APBMT/WBMT

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As the secretarial office and the data center of Asia-Pacific Blood and Marrow Transplant Group (APBMT), and also as one of the secretarial office of Worldwide Network for Blood and Marrow Transplantation (WBMT), we report the activities of both organizations between autumn 2010 and summer 2011.
Topics of the 15th Annual Congress of APBMT in Phuket: There were 639 registered delegates from 22 countries, 279 (43%) from Thailand and 360 (57%) from foreign countries. 105 abstracts were submitted with 18 oral and 78 poster presentations. In terms of the numbers of participants and the percentage of those from foreign countries, it was the largest APBMT Congress in the past (11th in Nagoya: 207 participants, the percentage of foreigners: 53%, 12th in Beijing: 543, 42%, 13th in Taipei: 454, 23%, 14th Seoul: 472, 38%). It was also the first APBMT Congress where the joint session with WBMT was organized.
APBMT Activity Survey 2009: The 4th survey (HSCT performed by 2008) was done. Fifteen out 16 countries/regions participated (The Philippines submitted the data this year). The items of the disease types and stem cell sources were modified to correspond to the WBMT survey criteria.
WHO-WBMT Vietnam Workshop 2011: The preparatory meetings for the Vietnam Workshop were held in Phuket, Orland, Hawaii and Paris under the attendance of the representatives from Vietnam, WBMT (APBMT/EBMT/CIBMTR/ WMDA etc) and WHO. The goals of the workshop were discussed and were set as followings; 1) Create awareness among government policy-makers about the value of hematopoietic stem cell (HSC) transplantation in developing healthcare systems, 2) Encourage the integration of HSC transplantation within the Healthcare Policy of developing countries, 3) Establish the basic ethical, medical and infrastructure requirements for providing HSC transplantation within a developing healthcare system, 4) Create a model for achieving goals 1) – 3) that can be replicated throughout the WHO regions of the world, 5) Optimize existing transplant programs. Through almost bi-weekly telephone conference, the actual constructions as well as speakers of the workshop have been finalized.
In Asia-Pacific countries, the activities around stem cell transplantation show a steady increase in terms of the numbers of commit specialists and countries/transplant centers and transplant case numbers. Nevertheless, it is also true that the activity has not been captured in many countries and it is expected that the coming Vietnam Workshop 2011, which is organized by Vietnam, APBMT/WBMT and WHO, would contribute to shed light on these countries/regions

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session A)
Bayside Gallery B

Challenges of HSCT and BMT Registry in India

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Allogeneic haematopoietic stem cell transplantation began in India in March 1983 at the Tata Memorial Hospital and in 1986 at the CMCH, Vellore. Thereafter, it took a number of years before more centres came up. A number of factors like, a vast country with a massive population having limited economic resources, fewer trained professionals and inadequate support in the government health sector were responsible for this slow growth. However, with economic liberation of 1990s, availability of adequately trained professionals and emergence of private sector health care, have helped rapid growth in the field in last decade. Currently there are close to 30 active transplant centres in the country. At present, every year about 700 transplants are carried out in these centres with larger institutes like CMCH at Vellore, TMC at Mumbai doing more than 50-100 procedures annually. The larger centres continue to focus on allogeneic transplants. This growth has provided opportunity for development of a country-based transplant registry. Since 2007 an ISCT registry has been initiated with about 80% centres contributing the data on an annual basis. This data finds its way to the APBMT registry. A few centres are continuing to send data to CIMBTR or IBMTR. We believe this registry will provide invaluable scientific information for developing countries. Since 2008, CMCH, Vellore has begun MUD transplants with donors provided through NMDP & German programmes. Subsequently, a few more centres have begun such programmes successfully. There is a serious lack of a functional unrelated marrow donor registry in India. However, a couple of registries led by NGOs like MDRI and DATRI have started registering voluntary donors. We believe, in a year or so these organisations will be able to provide donors for Indian patients as well as for recipients from other countries. This a true challenge for us.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session A)
Bayside Gallery B

BMT Recipient and Donor **Registry** in Korea

Jong Wook Lee
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From 1983 to 2010, a total of 15,848 cases of hematopoietic stem cell transplantation (HSCT) were performed in Korea. The Korean Society for Blood and Marrow Transplantation (KSBMT) has implemented a web-based transplant registration program since April 2005 through the Registry Committee of KSBMT. Of 42 HSCT centre, a total of 28 institutions that are responsible for more than 90% of all transplantations, are currently participating, with a total of 5,666 cases registered up until August 2011. Since May 2008 we have set forth minimum data requirements for registration, similar to the least minimum data (LMD) requirements of APBMTR. Although the number of registered cases is increasing, half of all transplants are currently registered, emphasizing the need for improved registration rates. A summary of cases registered through KOSTIA compared to the total number of cases from 2005 to 2009 is as follows: 623/1139 (55%) in 2005, 753/1315 (57%) in 2006, 768/1382 (56%) in 2007, 818/1459 (56%) in 2008, and 537/1459 (37%) in 2009. The registration program is currently being updated to allow for immediate retrieval of APBMT's LMD data.

KONOS (KOrean Network for Organ Sharing) has grown to 220,000 donors. The vast number of transplants are performed using donors who are already fully tissue typed (i.e. HLA-A, B, DR typed) at the time of registration as a volunteer donor. Every year 19,000 new volunteer donors joined the KONOS. Since 1994, two donor centres, KMDP & CHSCB, have facilitated more than 3,200 transplants from unrelated donor with 430 transplants per year. Matching rate is getting higher up to 87% for the Korean patients.

This accomplishment has been facilitated by the efforts of 42 transplant centres, and 2 donor centres, 5 volunteer donor recruit centres, 9 cord blood banks, 6 HLA typing laboratories, and cooperative international registries.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session A)
Bayside Gallery B

Challenges of Establishing Bone Marrow Transplant Services In Vietnam

Nguyen Tan Binh, Tran van Binh, Tran van Binh, Huynh Nghia, Tran Quoc Tuan, Huynh Van Ma, Bao Minh Hien
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The paper describes the authors' experience in setting up a Bone Marrow Transplantation program at the Ho Chi Minh City Blood Transfusion and Hematology hospital over a period of 15 years from 1996 to 2011. Ninety-eight transplants were performed (in a total of 147 cases in 6 centres throughout the country), with sources from bone marrow, peripheral blood stem cell (both Allogeneic and Autologous), and from cord blood.

Many difficulties, both human and material, were overcome by setting up techniques and facilities appropriate to the country's available condition. Indications were Thalassemia major (7 cases, 7.3%), CML (17 cases, 17.5 %), AML (63 cases, 65 %), ALL (6 cases, 6.2%), NHL (3 cases, 3 %), SAA (2 cases, 2 %). The mean age of this patient cohort was 28 (age range 4 – 50). Graff versus host disease was mild: grade 1 - 2 seen in 21 patients (21.4 %). The disease-free survival of this series of patients is 5.1 years (range 0.3- 14 years) and the overall survival 6.7 years (0.3-15 years). This result is comparable to those in similar status achieved in others countries. The mean cost of the operation was around US$20,000 in adult and US$15,000 in children. The cost was partially covered by the medical national insurance.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session B) ***Bayside 104***

Achievements in Hematopoietic Stem Cell Transplantation for Beta-Thalassemia Major

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Aim
Hematopoietic Stem Cell Transplantation (HSCT) is still the only curative treatment for patients with Beta-Thalassemia major. We report our experience in HSCT for Thalassemia, with more focus on GVHD effect on survival and impact of HSCT on hepatic fibrosis.
Methods
From 1991 to 2011, 525 thalassemia patients underwent transplantation (donor types: 492 full matched siblings, 10 mismatched siblings or other relatives, 23 full matched other relatives). The hematopoietic cell sources were 198 bone marrow, 315 peripheral blood, 9 cord blood and 3 bone marrow combined with peripheral blood. 87 patients received mesenchymal cells in addition to peripheral blood or bone marrow. Biopsy specimens from liver were obtained from 10 patients (female:4, male:6) who had undergone HSCT. The biopsies were studied for the presence of donor-derived hepatocytes using FISH and immunohistochemical staining for CD45.
Result
Median time to ANC recovery in BMT/PBSCT patients was 19/13 days (range 8-73/6-53; $p < 0.001$). Median time to platelet recovery was (28/18 days, range 11-92/6-84; $p<0.0001$). The median follow-up was 22 months. Grade I-IIaGVHD/no GVHD has no effect on overall survival (OS) or disease-free survival (DFS), within the first 100 days. While, grade III-IVaGVHD/no GVHD has a significant effect on OS and DFS. (OS HR: 4.97, DFS HR:1.9). Among patients who showed limited cGVHD, protective effect on OS and DFS/no cGVHD wasn't statistically significant. While the effect of extensive cGVHD on OS and DFS/no cGVHD was statistically significant (OS HR:4.38, DFS HR:3.08). XY and XX-positive hepatocytes accounted for 10-25% and 25-80% of cells in histological sections of the biopsy specimens of female and male patients, respectively.
Conclusion
Engraftment time is shorter in peripheral blood stem cell transplant method. Grade I-II aGVHD and cGVHD have positive effect on survival. Circulating stem cells can differentiate into mature hepatocytes in B-Thalassemia major patients who had undergone HSCT.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session B) ***Bayside 104***

T and B Depleted Haploidentical Haematopoietic Stem Cells Transplant (hapHSCT) For Children With Refractory Haematological Diseases

Godfrey Chi-Fung Chan
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For children with no suitable HLA-matched donors or cord blood but requiring haematopoietic stem cells transplant, the use of haploidentical donor is one of the possible options to consider. There are 3 approaches for hapHSCT, including: 1) non-manipulated bone marrow (BM) or peripheral blood stem cells (PBSC); 2) positive *ex-vivo* selected CD34 stem cells from BM or PBSC; & 3) BM or PBSC with negative *ex-vivo* depleted T & B cells. The non-manipulated BM & PBSC hapHSCT mainly used *in-vivo* ATG as T cells depletion. The engraftment rate has been reported to be satisfactory but it is associated with significant high acute and chronic GVHD rate (40 to 60%). The positive CD34 selected donor's graft is associated with much less GVHD but delayed engraftment and high infectious risk due to prolonged immune reconstitution is a concern. The *ex-vivo* depleted CD3 T cells and CD19 B cells or *ex-vivo* depleted CD3 T cells with *in-vivo* CD20 B cells depletion approach has been used by several centres in recent years for children with refractory haematological illnesses. Due to the high stem cells dose from the haploid PBSC and selected depletion of T & B cells only, rapid engraftment; low GVHD rate and rapid immune reconstitution has been reported. We therefore adopted this CD3/CD19 *ex-vivo* depleted hapHSCT for our patients who failed after conventional HSCT. So far 5 children (4 with relapsed leukaemia & one with refractory aplastic anaemia) received CD3/CD19 depleted PBSC grafts from their parents in our unit. All patients engrafted and their neutrophil engraftment occurred within 14 days (range 11-14 days) and platelet engraftment occurred within 28 days (ranged 15-28 days). None developed Gr II-IV GVHD. Except one patient developed adenovirus associated haemorrhagic cystitis, none suffered from severe infection. However, all of these high risk patients relapsed subsequently and most relapse/rejection occurred within 6 months after the transplant. The relapse free survival of such 2nd transplant is lower than the expected 30-40% reported by Handgretinger R, et al. As comparison, we did not use KIR mismatch status as selection criteria and we also did not monitor the graft status weekly after transplant so no prophylactic DLI had been added back in our cohort. This may imply the importance of graft status monitoring especially during the early transplant period with this approach. Future effort should be directed at more specific T cells depletion so GVL effect can be retained.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session B) ***Bayside 104***

Biological Advances and Intervention in Leukemia Relapse Following Allogeneic Hematopoietic Stem Cell Transplantation

He Huang
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Disease relapse is one of the leading causes of death following allogeneic hematopoietic stem cell transplantation (allo-HSCT). The incidence of hematologic malignancy relapse following allo-HSCT varies between 10% and 80%, and is dependent on a number of variables including disease, disease status, host age, donor source, conditioning regimen, HLA disparity, graft-versus-host disease (GVHD) prophylaxis, leukemia genetic basis, leukemia epigenetic feature, resistant leukemia stem cell, and others. Important biological and clinical differences have been identified between diagnostic and relapsed leukemic cells, including the acquistion of new chromosomal abnormalities, gene mutations. In allo-HSCT, donor T lymphocytes and NK cells play critical roles in alloimmune recognition and their ability to detect non-self-antigens can lead to GVHD or contribute to relapse prevention through recognition and elimination of minimal residual disease (MRD). Those factors which are implicated in alloreactivities of T-cell and NK cell affect the risk of relapse and transplant outcome, including killer-cell immunoglobulin-like receptor (KIR) genetic characteristics of a stem cell donor and donor T-cell costimulatory molecule genes polymorphic features. We should determine the optimal frequency for monitoring MRD and chimerism after allo-HSCT and assess the efficacy of interventional strategies based changes in MRD and/or chimerism to prevent clinical relapse. Further evidence for a GVL effect by donor lymphocyte infusion (DLI) is provided. However the clinical benefit is limited to a minority of patients. Strategies to reduce tumor burden before DLI, as well as newer approaches to augment the efficacy of DLI should be investigated. The role of second allo-HSCT has been limited by unacceptable relapse rates and high mortality. Ultimately, understanding the biology of relapse and mechanisms involved in GVL induction will permit more effective and patient specific approaches for relapsed disease. Alternatives to cellular therapies and novel targeted drugs for treating relapse must be considered as well.

Sunday 30 October **1615-1745**
APBMT: Transplant Issues Specific for Asia-Pacific Region (Session B) ***Bayside 104***

Treatment of Cerebral Palsy Using Cord Blood Stem Cells

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National Taiwan University Hospital/College of Medicine, Division of Hematology/Oncology/Department of Paediatrics, Taipei, Taiwan

Background
A pilot study was conducted to determine the safety, feasibility and effectiveness of intravenous administration of autologous umbilical cord blood (CB) in young children with cerebral palsy (CP).
Study Design and Methods
The enrolled patients were those with age over 6 months, diagnosis of cerebral palsy with the severity of GMFCS level III or more, and available cryopreserved autologous CB unit. The enrolled patients received pre- and 6 months post-transplant evaluations including EEG, brain MRI, PET, evoked potentials, gross motor functional measurements and neurocognitive investigations, such as Bayley III and Comprehensive Developmental Inventory for Infants and Toddlers (CDIIT). The age- and severity-matched controls were also recruited for contrast.
Resuts
From May 2009 to May 2010, 12 children received 12 CB infusions. Median age was 2 years 8 months old (ranged from 9 months to 5 years 1 month old). Mean recryopreservation volume was 74 mL, total nucleated cell count was9.04×10^8, and CD34 count was 4.3×10^6. Postthaw sterility cultures were all negative. Three patients had infusion reactions. A total of 11 enrolled patients and 7 controls completed the follow-up investigations. Improvement of brain PET was significantly higher in the CB treated group than the controls. The improvement of motor and neurocognitive functions was comparable in both groups, but inversely correlated with severity of CP in the CB treated group.
Conclusion
IV infusion of autologous CB is safe and feasible in young children with CP. The efficacy of autologous CB is not significant in patients with severe CP. However, further investigation is warranted in children with mild or moderate CP.

Monday 31 October **0830-1000**
HSANZ Free Communications 1: Lymphoma ***Auditorium B***

O001 0830

PET-CT After Induction Therapy is Highly Predictive of Patient Outcome in Follicular Lymphoma: Central Review of PET-CT Scans in a Subset of PRIMA Trial Participants

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Purpose

The recently demonstrated predictive power of ^{18}F-FDG PET-CT in response assessment for follicular lymphoma (FL) remains unvalidated. In the international PRIMA study, we identified 32/122 (26%) positive PET-CT scans after induction immunochemotherapy using local investigator PET interpretation. PET+ patients had an inferior 42 month PFS of 32.9% vs. 70.7% in PET negative patients, p<.0001*. We undertook central review of these scans

Material and Methods

Scans were assessed by two independent PET physicians using IWC 2007 criteria and the 5 Point Scale. Patient characteristics and outcomes were evaluated

Results

In all, 179/242 PET-CTs performed from 2004-2007 were collected, with 130 suitable for central review. At diagnosis, 54/55 scans were positive (mean SUVmax 11.7, range 4.6-35.6). There was no correlation between baseline SUVmax and outcome.

Of 58 postinduction scans, 53 were interpreted using IWC criteria and 58 using the 5PS. PET status was not significant when applying IWC criteria (HR for progression in PET+ 2.3 (95% CI 0.8-6.4). When applying the 5PS with cut-off ≥4, a single local postinduction PET- patient was reviewed as PET+, due to probable tonsilar inflammation. The patient has not progressed. Six PET+ patients with minimal residual disease on local assessment (SUV range 1.4-2.8) were re-classified as PET-. Three patients, each with mesenteric disease, have progressed. The 42month PFS in PET+ was 25.0% (95% CI 3.7-55.8%) vs. 59.6% (95% CI 43.1-72.7) in PET-, p=0.02, HR 2.8, (95% CI 1.1-7.1).

Conclusion

In this retrospective subgroup analysis, 18F-FDG PET-CT induction response status, using a cutoff of ≥4 on the 5PS, is strongly predictive of outcome after frontline therapy for FL. While not as discriminating as local review it provides a reproducible platform for development of PET response criteria. Criteria for assessing minimal residual uptake, particularly for mesenteric disease, need refinement and are perhaps best interpreted with reference to the baseline scan.

The PRIMA study was supported by Roche. The company had no role in analysing the PET data or preparing the abstract.

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
HSANZ Free Communications 1: Lymphoma ***Auditorium B***

O002 0845

Metadherin Contributes to the Pathogenesis of Diffuse Large B-cell Lymphoma

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Aim
Metadherin (MTDH) has been demonstrated as a potentially crucial mediator of various types of human malignancies. However, the expression and role of MTDH in diffuse large-B-cell lymphoma (DLBCL) have not been reported yet.
Methods
Fifteen fresh and thirty araffin-embedded tissues from DLBCL patients were collected. The tissues from patients of reactive hyperplasia of lymph node were used as a control group. Peripheral blood mononuclear cells from healthy volunteers served as normal control compared with human DLBCL cell lines LY1 and LY8, which were treated with tumor necrosis factor-α (TNF-α, 250pg/ml) and cultured for 48 hours to induce the upregulation of MTDH protein. The expressions of MTDH mRNA and protein in DLBCL and controls were determined by quantitative PCR, Western Blot and immune-histochemistry. The impacts of MTDH overexpression on LY1 and LY8 cells' proliferation and apoptosis were assessed by ^{3}H-TdR incorporation method and flow cytometry.
Results
A remarkable elevation of MTDH on mRNA level was detected in DLBCL tissues ($P<0.001$). MTDH protein was also significantly increased in DLBCL cell lines and tissues in comparison to their counterparts ($P<0.001$). Immunohistochemical analysis showed high expression of MTDH in 23 of 30(76.67%) paraffin-embedded archival DLBCL specimens. Statistical analysis suggested that the overexpression of MTDH was strongly correlated to the clinical staging of patients with DLBCL ($P<0.05$). The expression of MTDH protein in LY1 and LY8 cells was upregulated after treated with TNF-α ($P<0.05$). Furthermore, we determined that the increase of MTDH in DLBCL cells could distinctly enhance cell proliferation and inhibit cell apoptosis ($P<0.05$).
Conclusion
MTDH was overexpressed in DLBCL and correlated with the clinical staging of patients with DLBCL. The increase of MTDH promoted growth and survival of DLBCL cells. This novel study suggests that MTDH is involved in the pathogenesis of DLBCL and may contribute to further investigation on the useful biomarkers and potential therapeutic target in DLBCL patients.

No conflict of interest

Monday 31 October **0830-1000**
HSANZ Free Communications 1: Lymphoma ***Auditorium B***

O003 0900

Monocytes Are Associated With Impaired T-cell Immunity and Residual Interim-PET/CT Avidity After 4 Cycles of CHOP-R In Patients With High-Risk DLBCL

Frank Vari[1], Mark Hertzberg[2], Erica Han[1], John F Seymour[3], Rodney Hicks[4], Devinder Gill[5], Colm Keane[1,5], Pauline Crooks[1], Kristen Radford[6], Maher K. Gandhi[1,5]
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Purpose
To establish the relationship between systemic inflammatory/immune cells and avidity of interim PET/CT, during chemo-immunotherapy for diffuse large B-cell lymphoma (DLBCL).
Patients and Methods
We prospectively analysed circulating immunity in 34 patients (mean 53yrs range 31–69; F35%, IPI3-5 65%, 'B' 51% 'B', bulk 35%) with untreated high-risk DLBCL, enrolled in the ALLG NHL21 study. They received #3 CHOP-R-14, then after #4 underwent interim-PET/CT between days 17-20. 10/34 remained interim-PET/CT avid. Blood was taken pre-treatment and day 21 (post-cycle 4).
Results
$CD3^+/8^+/4^+$ T-cells were all reduced pre-treatment compared to 23 healthy subjects. After 4 cycles, $CD4^+$ lymphocytes declined further (p=0.0003). T-cell proliferation was equivalent to controls at both time-points, indicating no intrinsic functional defect. Monocytes were elevated at pre-treatment compared to healthy subjects (p=0.007), with a predominantly $CD14^+HLA\text{-}DR^{-/lo}$ phenotype. Pre-treatment values for monocytes (p=0.004), $CD14^+HLA\text{-}DR^{-/lo}$ monocytes (p=0.03), mean fluorescent intensity of HLA-DR on monocytes (p=0.025) and $CD3^+$ T-cells (p=0.037), but not conventional clinical factors including international prognostic index, were associated with persistent interim-PET/CT avidity. Post-cycle 4: monocytes, $CD3^+$ T-cells and lymphocyte/monocyte ratio (LMR) were also related to interim-PET/CT avidity. Strikingly, patients achieving interim-PET/CT negativity had a marked rise in LMR (p=0.0006), whereas the LMR in those remaining PET/CT positive stayed unchanged. Depletion of patient but not healthy monocytes enhanced proliferation of $CD3^+/8^+/4^+$ T-cells. Post-cycle 4 monocyte HLA-DR expression was up-regulated from pre-treatment (p=0.01), accompanied by an increase in *ex-vivo* blood myeloid dendritic cells (BMDCs; p=0.044), suggesting the $CD14^+HLA\text{-}DR^{-/lo}$ phenotype is associated with an impaired ability to differentiate into BMDCs *in-vivo*. Post-cycle 4 plasma arginase fell relative to pre-treatment (p=0.0035), implicating arginine metabolism in immunosuppression.
Conclusions
The relevance of interim-PET/CT has generated much interest. To our knowledge this is the first study to find an association between biological markers of inflammation/immunity and interim-PET/CT avidity in uniformly treated high-risk DLBCL patients.
No conflict of interest to declare.

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
HSANZ Free Communications 1: Lymphoma ***Auditorium B***

O004 0915

Lymphoma-Specific But Not Non-Specific Cell-Free Circulating DNA Can Be Used to Monitor Disease Response in Lymphoma

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Aim
Recently, non-lymphoma specific circulating DNA was shown to be elevated in a broad-range of lymphomas, implicating a role as a potential biomarker. Epstein-Barr virus' (EBV) presence within a proportion of lymphomas implies EBV-DNA has potential as a lymphoma-specific disease response biomarker. However application would be restricted to EBV-associated lymphomas. No detailed comparison has been performed of lymphoma-specific versus non-specific DNA as biomarkers. Nor have the kinetics of circulating DNA during treatment been established, and the optimal methodology remains unknown.
Method
We prospectively evaluated DNA levels and clinical response of 63 newly diagnosed lymphoma patients (36 Hodgkin Lymphoma, 13 Post-Transplant lymphoproliferative disorder, 9 Diffuse Large B-cell Lymphoma, 4 Lymphomatoid Granulomatosis and 1 Angioblastic T-cell Lymphoma, mean age: 44 years, range: 18-89; female/male ratio 33/30). Only histologies in which EBV was known to be potentially associated were chosen. DNA was measured in paired serum, plasma and cell samples at five pre-determined time-points taken prior, during and following treatment. Results were stratified by EBV-tissue status, and correlated with clinical / radiological response.
Result
Both cell-free (c-f) circulating EBV-DNA (in EBV-associated lymphoma) and non-specific c-f DNA levels (in all lymphomas) were discriminatory at presentation compared to healthy controls. Within EBV-associated lymphomas, there was a strong correlation between specific and non-specific circulating c-f DNA ($r=0.9$, $p<0.0001$). Non-specific c-f DNA was significantly associated with baseline serum lactate dehydrogenase. However, only c-f EBV-DNA correlated with clinical / radiologic response. Serum versus plasma, and single versus multiple-copy EBV-gene targets were equivalent.
Conclusion
Both circulating c-f non-specific DNA and lymphoma-specific DNA are elevated at presentation. However only lymphoma-specific DNA reflected therapeutic response. Lymphoma disease response can be monitored by blood tests, but new lymphoma-specific biomarkers need to be identified to broaden applicability.

No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 1: Lymphoma ***Auditorium B***

O005 0930

MIPI and Early Lymphocyte Recovery Predict Long-term Survival in Patients With Mantle Cell Lymphoma (MCL) Treated with R-HyperCVAD and ASCT

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Aim
Analysis of the impact of MCL international prognostic index (MIPI) and absolute lymphocyte count (ALC) on Day +15 post ASCT on overall survival (OS) of patients with MCL treated with immunochemotherapy and autologous stem cell transplantation (ASCT).

Background
Increasing CR rates and survival has been reported in MCL patients treated with R-HyperCVAD regimen(JCO 23:7013-23). ASCT provides incremental improvement. MIPI predicts outcome in non-ASCT treatment of MCL. ALC recovery after ASCT predicts survival in patients with MCL treated with CHOP/CHOP-like regimens.(BMT 37:865–71)

Method
We retrospectively analysed 25 MCL patients treated with R-HyperCVAD. All except one had Busulphan/Melphalan(Bu/Mel) ASCT. MIPI was calculated from baseline characteristics. ALC on Day+15 was assessed from serial blood counts.

Results
Median follow-up was 54months. R-HyperCVAD achieved a CR of 96%, which improved to 100% post ASCT. At 54 months the actuarial PFS and OS rates were 63% and 92%, respectively. These rates among patients with ALC15>$0.5x10^9$/L (n=14) were 93% and 100%, compared with 38% and 78% respectively in those with ALC15<$0.5x10^9$/L(p=0.008). The actuarial PFS and OS rates at 54 months in patients with low MIPI (n=19) were 80% and 92%, compared with 33% and 83% respectively in those with intermediate/high MIPI score (p=0.003).

Conclusion
R-HyperCVAD with Bu/Mel transplant achieves high CR rates and long-term survival in MCL patients. MIPI is a simple prognostic score whereas ALC15 is a surrogate marker for immune recovery after ASCT. Both these parameters are strongly associated with PFS and OS in patients treated with R-hyperCVAD and ASCT.

No conflicts of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 1: Lymphoma ***Auditorium B***

O006 0945

Intermittent G-CSF Maintains Dose Intensity After ABVD Therapy Complicated By Neutropenia

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Introduction
G-CSF is commonly used to maintain dose-intensity in patients receiving ABVD chemotherapy for Hodgkin lymphoma. However, the need for growth factor support is unclear, with studies suggesting that dose-intensity can be maintained without G-CSF and with minimal incidence of febrile neutropenia. Moreover, G-CSF is expensive (costing approximately $1925 per cycle for pegfilgrastim and $1050 per cycle for 7 days of 300ug filgrastim) and is associated with side-effects including bone pain and increased risk of bleomycin-related lung toxicity. Intermittent G-CSF may be an effective compromise, given that the effect of G-CSF on granulocyte precursors in-vitro persists for 4-5 days after administration and intermittent scheduling was as effective as daily G-CSF in maintaining dose-intensity in breast cancer patients receiving adjuvant chemotherapy. After promising results of a pilot study of intermittent G-CSF in Hodgkin Lymphoma (Grigg et al), this schedule has been used subsequently at physician discretion at RMH as secondary prophylaxis for ABVD complicated by neutropenia.
Aims
To compare the efficacy of daily (including pegfilgrastim) vs intermittent G-CSF protocols between 1996 and 2009.
Methods
Retrospective analysis of the incidence of (a) neutropenia without G-CSF support and (b) febrile neutropenia and treatment delay in patients receiving G-CSF according to these protocols.
Results
848 cycles in 85 patients (M:F 43:42; median age = 32 (range: 14-71) years) with predominantly stage II/III HL were evaluated. The median neutrophil count on d15 (when cycle 1B is due) was 0.9 (range: 0-18.7). Most patients (86%) received G-CSF, generally commencing during cycle 1B (Figure 1). Intermittent G-CSF (typically given on days 4,8,12) was used in 452 cycles compared with 99 cycles for daily or pegylated G-CSF. Febrile neutropenia occurred in 2 and 0 cycles respectively and no treatment delays due to neutropenia occured in either group. After intermittent G-CSF, the median neutrophil count was 7.3 (1.4-47.1x109/L) when chemotherapy was next due, similar to other G-CSF regimens (Table 1). The cost difference between pegfilgrastim for and three doses of 300ug filgrastim per cycle over 11 cycles (i.e. cycles 1B-6B) was A$21,450.
Conclusions
Intermittent G-CSF is effective in maintaining dose-intensity with minimal febrile neutropenia in patients receiving ABVD and results in substantial cost savings.

This research was supported by AMGEN. The company had no role in analysing the data or preparing this abstract.

Monday 31 October **0830-1000**
HSANZ Free Communications 2: AML, PNH & Myeloma ***Bayside 103***

O007 0830

An Insertional Mutagenesis Mouse Model of Multiple Myeloma for Genetic and Functional Studies

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Transposon insertional mutagenesis (IM) is a powerful approach for identifying genetic pathways involved in cancer initiation and progression and assessing their functional significance in vivo. In IM screens the types of tumours that develop are largely determined by the tissue and developmental stage in which the transposase is activated. IM has not previously been targeted to the mature B cell compartment. *Vk*Myc* was the first transgenic mouse model to accurately recapitulate many clinical features of multiple myeloma and show therapeutic fidelity [1]. In this model the *c-MYC* gene was placed under transcriptional control of the Vk promoter, but a stop codon prevented translation(FIGURE 1). The stop codon overlapped with a preferential target sequence for Activation Induced Deaminase (AID), the enzyme responsible for class switch recombination and somatic hypermutation during B cell development. In *Vk*Myc* mice the stop codon was sporadically reverted in a minority of maturing B cells. All mice developed a plasma cell dyscrasia with age.

Aims

To adapt the *Vk*Myc* model to generate two new mouse models in which *PiggyBac (PB)* transposon IM is targeted to the mature B cell compartment.

Methods

In one construct the *MYC* exons in *Vk*Myc* were replaced by *PB* transposase. In the second *Thosea asigna 2A* (T2A) cDNA was used to link *PB* in-frame to *MYC*. The T2A linker hydrolyses soon after translation, generating two separate proteins. Transgenic mice were generated by pronuclear injection and mutagenesis cohorts are being expanded. In these mice *PB* transposase mobilises a transposon cargo that can activate or disrupt gene expression.

Results and Future Work

We have confirmed the transposon is mobilized in DNA collected from blood, bone marrow and spleen from these mice. The mice are being monitored for tumour development. We hope to use these mice to study gene pathways involved in myeloma pathogenesis and to identify genes co-operating with *MYC* in myelomagenesis. *No conflict of interest to disclose*

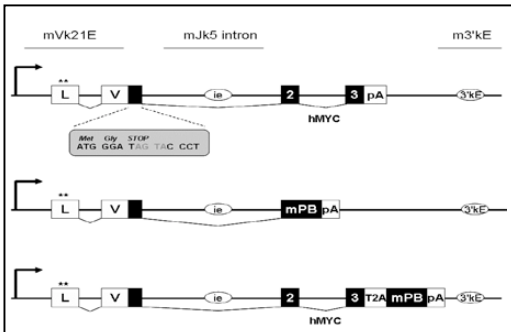

FIGURE 1:Top: Vk*Myc construct [1] The rearranged mouse Vk21 kappa light chain gene in which the Jk5 exon was replaced by a short coding exon containing a Kozak ATG. Human MYC exons 2 and 3 replaced the C1k region. Transcription initiates at the Vk21e proximal promoter, extends to the leader (l) and Vk (v) exons, splices in frame to hMYC and terminates at the polyA signal (PA). Intronic (ie) and 3'kappa enhancers (3'ke) are maintained. The DNA sequence immediately downstream of the Vk21 ATG is depicted. Nucleotides in grey letters fit the DGYW consensus for AID targeting.
Middle/bottom: Our two constructs

1. Chesi, M., et al. Cancer Cell, 2008. **13**(2): p.167

Monday 31 October **0830-1000**
HSANZ Free Communications 2: AML, PNH & Myeloma ***Bayside 103***

O008 0845

A Comparative Study of Dual PI-3K/mTOR and Single mTOR Inhibitors in a Pre-clinical Model of ALL

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The major regulators of stromal-dependent ALL cell growth and survival convey their proliferative and survival signals through the phosphoinositide 3-kinase (PI-3K) pathway. Inhibition of mTOR signalling, downstream of PI3K with RAD001 inhibits proliferation and induces cell death. Although PI-3K and mTOR have overlapping functions, mTOR can be activated independently of PI-3K, and proliferation and survival can be stimulated by PI-3K in an mTOR independent manner. Therefore combining PI-3K and mTOR inhibition is likely to be advantageous over inhibition of either kinase alone.

We demonstrated that dual PI-3K and mTOR inhibition with BEZ235 or BGT226 significantly inhibits ALL proliferation *in vitro*, with a 3 log greater potency in comparison to RAD001. However, while the concentrations of BGT226 required to kill ALL cells is approximately one log lower than RAD001, the IC_{50} for BEZ235 is similar to that of RAD001. Both BEZ235 and BGT226 induced a combination of autophagy and apoptosis. While caspase-3 cleavage was detected this was less than anticipated with BGT226. Reversal of cell death using the pan-caspase inhibitor Z-VAD was partial at best. We also demonstrated in a NOD/SCID xenograft model of human ALL that dual inhibition of PI-3K and mTOR significantly prolonged the survival of mice. However, BEZ235 and BGT226 were superior to RAD001 in only 1 of 4 xenografts and BEZ235 was inferior in 2 and BGT226 in 1 of 4 xenografts. The reasons for the variable effects remain to be confirmed but high basal phosphorylation of AKT was detected in the xenograft most sensitive to the dual kinase inhibitors. The potential synergy of dual kinase inhibitors with conventional chemotherapy drugs and in mTOR inhibitor resistant cases remains to be studied. Overall dual PI-3/mTOR inhibitors show considerable promise but careful patient selection may be required to obtain optimal outcomes.

No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 2: AML, PNH & Myeloma ***Bayside 103***

O009 0900

Down-Regulation miRNA-15a/16 By Interleukin-6 Promotes Myeloma Cells Drug Resistance

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At present, more and more studies identified that loss of 13q14 sequences is not significantly associated with a poor prognosis in myeloma. And the meaning of 13q deletion for myeloma patient was not completely understood. miRNA-15a and -16, 2 of the decreased or totally absent miRNAs in MM which are located in chromosome 13. Previous studies have confirmed that miRNA-15a and -16 regulate proliferation and growth of MM cells in vitro and in vivo. In the present study, we identified that miRNA-15a/-16 down-expression were correlated with proliferation and drug sensitivity of MM cells. miRNA-15a/-16 expression were up-regulated obviously in MM cells after conventional cytotoxic agent melphalan and novel reagent bortezomib treatment. In addition, the microRNA-15a/-16 expression in dexamethasone sensitive myeloma cell MM1S were obviously higher than the resistance one MM1R. However, bone marrow stromal cells of myeloma patients interfered melphalan and bortezomib induced apoptosis with higher level of cytokines secretion. Bone marrow stromal cells could suppress miRNA-15a/-16 expression of myeloma cells. And IL-6 treatment was more effective than VEGF on miRNA-15a/-16 expression suppression with time and dose dependent pattern. miRNA-15a expression up-regulation was obviously than miRNA-16 after cytotoxic agent treatment. Furthermore, restoration of miRNA-15a led to G1/S arrest and decreased proliferation of U266 cells. In conclusion, our data suggest that via suppressing the expression of tumor suppressor gene miRNA-15a and miRNA-16 which located on chromosome 13, IL-6 secreted by MM-BMSCs provide survival support and protect myeloma cells against apoptosis, which may contribute to the development of drug resistant myeloma cells.

No conflict of interest to declare

Monday 31 October **0830-1000**
HSANZ Free Communications 2: AML, PNH & Myeloma ***Bayside 103***

O010 0915

Long-term Clinical Outcomes with Sustained Eculizumab Therapy in Paroxysmal Nocturnal Haemoglobinuria (PNH)

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Background
PNH is a chronic, life-threatening disease associated with increased thrombosis, end-organ damage, poor quality of life (QOL), and premature mortality. The complement inhibitor eculizumab reduces chronic haemolysis, leading to reductions in thrombotic events (TEs) and improvements in chronic kidney disease (CKD) and QOL.

Method
All patients (N=195) participating in the PNH eculizumab clinical trials and extension studies were evaluated for safety, sustained outcomes, and survival.

Result
Median eculizumab treatment duration was 29mo. Lactate dehydrogenase was rapidly reduced from 2,293U/L (~10x ULN) at baseline to 310U/L 1mo post-treatment (P<.0001), and was sustained through 36mo (P<.0001). Thrombotic events were significantly reduced from 52 pretreatment to 10 trial events by matched-time analysis (P<.0005). Among 7 patients with TEs, 5 had a history of TEs and 2 were concomitantly treated with anticoagulation. No TEs were reported among 11 patients who discontinued anticoagulation while on eculizumab. CKD was reduced from 69% at baseline to 31% (n=29) 36mo posttreatment. Significant increases in haemoglobin (mean increase: 9G/L; P<.0001) and significant reductions in transfusion requirements (P<.0001) were sustained over 36mo. Among 87/195 patients receiving ≥36mo of eculizumab, 29% (25/87) became transfusion independent for the entire treatment period. Eculizumab was well-tolerated; 90% (175/195) of patients completed the primary and extension trials. Most adverse events (91%) were mild/moderate in severity. Two cases of meningococcal sepsis were successfully treated without sequelae. Four patient deaths were reported (not related: n=3; possibly related: n=1). Overall probability of survival was 97.64% at 3yr, which was maintained through 5.5yr of ongoing treatment in some patients.

Conclusion
Long-term reduction of chronic intravascular haemolysis after eculizumab treatment in PNH patients is associated with significant improvements in thrombosis incidence, CKD, and other PNH-associated symptoms. Long-term eculizumab treatment also resulted in a high probability of survival that is maintained with ongoing treatment.

This research was supported by Alexion Pharmaceuticals, Inc. The company assisted in data analysis.

Monday 31 October **0830-1000**
HSANZ Free Communications 2: AML, PNH & Myeloma ***Bayside 103***

O011 0930

Difference of Multiple Leukemia Genes and Immunophenotype Between Ages In Untreated Acute Leukemia - 778 Cases Analysis

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Objective
To analyze the characteristics of multiple genes and immunophenotype of untreated acute leukemia.
Methods
The patients' bone marrow cells were detected for 31 leukemia fusion genes (including 124 mRNA breakpoint or splice variants) and HOX11 oncogene by multiplex nested PCR and immunophenotype by flowcytometry. The criteria of diagnosis and classification of acute leukemia was made according to that of WHO (2008).
Results
From 2006 to March 2011, 778 cases were detected in Beijing Daopei Hospital. Most of them came from other parts of China. 71% were ≥14Y with ALL 30%, AML 68%, M/L MPAL 2% separately. 29% were <14Y with ALL 62%, AML 38% separately. In ALL of ≥14Y and < 14Y, T-ALL 22% vs. 8.7%, B-ALL 76% vs. 91.3%, T/B MPAL 2% vs. 0%. In T-ALL of ≥14Y, 11% γδ T type. In T-ALL of <14Y, no γδ T type. In B-ALL of ≥14Y and <14Y, BCR-ABL+ 34% vs. 7.1% and TEL-AML1 0.8% vs. 15.9%. In AML of ≥14Y and <14Y, AML1-ETO 13.2% vs. 30.6%, PML-RARA 6.9% vs. 1.2%. In AML, 45% < 30Y and 70.7% <60Y. In ALL, 79% was <30Y and 98% <60Y.
Conclusion
This is the first large series of patients with acute leukemia from China with analysis of multiple leukemia genes, immunophenotype and their relationship with age. In the cases age >14Y, there were more poor prognostic immunophenotypes (MPAL, T-ALL, γδT-ALL) and genotypes (BCR-ABL +ve) and less favorable prognostic genotypes (AML-ETO +ve, TEL-AML1+ve). The majority of AML cases were <60Y which is different from the reports from Europe and American and merits investigation of the causes.

No conflict of Interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 2: AML, PNH & Myeloma ***Bayside 103***

O012 0945

The Novel MEK Inhibitor GSK1120212b Inhibits HMCL Growth in vitro and Synergises With a Range of Conventional and Novel Anti-myeloma Therapies

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Aim
GSK1120212b, a novel orally bioavailable MEK inhibitor, has been shown to inhibit tumour growth and induce cell cycle arrest in some cancer cells. Here we report the efficacy of GSK1120212b in human myeloma cell lines (HMCL) and primary myeloma (MM) cells when used as a single agent and in combination with conventional and novel anti-myeloma therapies.
Method
HMCL response to variable doses of GSK1120212b (10nM-5μM) was determined by MTS assay. FACS and cell cycle analysis were used to evaluate the profiles of the cells post-MEK inhibition. Mechanistic studies using Western analysis with antibodies against p-MEK, MEK, p-ERK and ERK were performed. Subsequently, the anti-MM activity of GSK1120212b was evaluated in HMCL co-cultured with HS5. Primary samples from relapsed/refractory MM patients were subjected to GSK1120212b treatment and analysed utilising Apo2.7-PE on cells gated to $CD45^{neg}CD38^{pos}$. Finally, combination studies with conventional and novel anti-MM therapies were also performed.
Results
GSK1120212b demonstrated an IC_{50} of 10-5000nM against 3 HMCLs at 72h hours. This correlated with Annexin/PI staining and cell cycle analysis, which demonstrated dose-dependent apoptosis induction and an accumulation of G_0/G_1 cells consistent with cell cycle arrest, respectively. Western analysis revealed increased p-MEK activity while total MEK levels decreased. Moreover, a decrease in p-ERK was observed with no change in total ERK levels. GSK1120212b demonstrated variable ability to induce HMCL apoptosis in HS5 co-culture, with or without transwells. Finally, GSK1120212b exhibited synergism with both conventional and novel anti-MM therapies but was most pronounced with the combination of GSK1120212b and GSK2110183b (Akt inhibitor) and GSK1120212b and LBH589 (HDAC inhibitor).
Conclusion
Considering the potent anti-MM effects of GSK1120212b and its ability to synergise with other anti-MM agents, MEK inhibition demonstrates a promising future avenue for the management of MM and warrants further investigation.

No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 3: Cell Therapies & Transplantation ***Bayside 104***

O013 0830

Autologous Peripheral Blood T Lymphocytes Transduced With an Anti LewisY Chimeric Receptor Gene can be Infused Safely and Persist in Patients With LewisY Positive Acute Myeloid Leukaemia

Amit Khot [1], Stefan Peinert[1], Paul Neeson[1], Tsin Tai, Karen Chen, Dominic Wall[1,2], Dirk Honemann[1], Michael Kershaw[1], Jennifer Westwood[1], Joseph Trapani[1], Mark Smyth[1], Phillip Darcy[1], Andrew Scott[1], Lucy Kravets[1], Rodney Hicks[1], Michael Dickinson[1], David Ritchie[1, 2], H Miles Prince[1, 2]
[1] *Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.*
[2] *University of Melbourne, Victoria, Australia.*

Aim
High-risk multiple myeloma (MM), acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) have a dismal prognosis with available treatments. We evaluated the safety and immunological effects of infusing peripheral blood T-lymphocytes transduced with an anti-LewisY (LeY) chimeric receptor gene in high-risk patients with LeY positive MM, AML or MDS in a phase 1 study.
Method
A chimeric receptor has been engineered consisting of extracellular humanized scFv recognizing the Le^Y Ag, linked to an extracellular CD8 hinge region, a transmembrane and cytoplasmic CD28 signalling domain, and zeta signalling chain. The humanized anti- Le^Y scFv-CD28-ζ receptor has been cloned into the pSAMEN retroviral vector and transfected into the packaging line PG13. The construct is transduced into T-cells harvested from the patient, which are re-infused into the patient after treatment to achieve a minimal residual disease state is completed. Peripheral blood(PB) and bone marrow(BM) samples are analysed by PCR to detect the gene construct.
Results
Adequate numbers of mononuclear cells (1.64×10^9-26.5×10^9) have been harvested in 5 AML patients; 4 patients have been treated with 0.5×10^9-1.3×10^9 T-cells(8-30% transduced T-cells), which met all Good Manufacturing Practice quality criteria, without significant toxicity. Trafficking of transgene positive cells to the BM is observed in all patients by PCR and SPECT scans. Persistence in the BM and PB is seen up to 10 months post-infusion. After adoptive transfer there is an apparent polarization of the LeY-T cells to a Th2 type as IFN-γ and IL-2 secretion is absent in response to stimulation by LeY antigen. Endogenous T-cells from these patients show evidence of mixed Th1, Th2 and Th17 polarization.
Conclusion
LeY-T cells can be safely infused into patients with high risk AML. The cells are persistent for prolonged periods and show polarization to a Th2 phenotype after adoptive transfer.
No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
HSANZ Free Communications 3: Cell Therapies & Transplantation ***Bayside 104***

O014 0845

Acquired Down-Regulation of Effector Mechanisms Leads to Impairment of the NK Cell Anti-Tumour Effect Despite Robust Tumour Localisation; Potential Implications for Adoptive Cellular Therapy

Saar Gill[1], Adrianne Vasey[1], Holbrook Kohrt[2], Mareike Florek[1], David Ritchie[3], Robert Negrin[1]
Department of Medicine, Divisions of Blood and Marrow Transplantation[1] and Oncology[2] Stanford University, Stanford, California, USA. [3]Division of Haematology and Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia

Aim
Natural killer (NK) cells are thought to hold promise in adoptive immunotherapy of malignancy. While early-phase clinical trials show short-term persistence of NK cells infused into patients, convincing evidence is lacking that these cells mediate an anti-tumour effect in vivo. We therefore set out to delineate the barriers to successful NK cell immunotherapy.
Methods
Mice bearing subcutaneous lymphoma received irradiation and a bone marrow transplant in addition to an intravenous infusion of 0.5×10^6 NK cells derived from luciferase-transgenic donors. Tumour growth was monitored by calipers and NK cell trafficking was evaluated by bioluminescent imaging (BLI). At different timepoints tissues were harvested for immune cell functional studies.
Results
NK cells infused into control non-tumour bearing animals homed to lymph nodes, spleen and liver maximally by the end of the second week. By contrast, in tumour-bearing animals NK cells homed to lymphoid organs in the first week, followed by progressive accumulation within the tumour site. Surprisingly, NK cells failed to impact tumour growth and survival. Compared to NK cells isolated from the spleen, intratumoural NK cells were found to exhibit diminished interferon-γ production and lower expression of the activating receptor NKG2D. Cytotoxicity was also diminished among splenic and intra-tumoural NK cells, compared with control IL-2 cultured cells. These observations were related to proliferation and required contact between the tumour and NK cells.
Conclusions
NK cells become dysfunctional shortly after transfer and target encounter. These findings suggest that short term *in vitro* killing assays cannot be used to predict the *in vivo* behaviour of immune effector cells. Furthermore, our results corroborate the low expression of NK activating receptors reported in patients with active acute leukemia. These findings explain why NK cells may not be able to deliver *in vivo* on the *in vitro* promise that their name suggests.
This research was supported in part by Genzyme. The company had no role in analysing the data or preparing the abstract.

Monday 31 October **0830-1000**
HSANZ Free Communications 3: Cell Therapies & Transplantation ***Bayside 104***

O015 0900

HLA-mismatched Unrelated Allogeneic Progenitor Cell Transplantation in Adults: Overall Survival Is Influenced By Underlying Disease Rather Than Degree of HLA-mismatch

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Aims
To determine the outcomes of HLA-mismatched unrelated donor (URD) allogeneic progenitor cell transplants (PCT) performed at our institution.
Methods
HLA-mismatched URD PCT were identified from an institutional data-base. Overall survival (OS), relapse rates, transplant-related mortality (TRM) and aGVHD incidence were determined retrospectively by review of individual patient medical records. All grafts were T-replete, with CsA and day 1-11 MTX used as standard GVHD prophylaxis in all cases. All donor / recipient pairs were typed for HLA A, B, C, DRβ1 and DQ loci.
Results
Between 1995 and March 2011 a total of 40 mismatched URD PCT had been performed. Median age was 47yrs (range 16-65yrs). Underlying disease indication for PCT included acute leukaemia in 25 patients (63%) and non-acute leukaemia in 15 (37%). 28 donor / recipient pairs (70%) were mismatched at 1 loci only, including 19 with low-resolution (17 class I / 2 class II) and 9 with high-resolution mismatches (2 class I / 7 class II). 12 patients had >1 mismatched loci. Non-engraftment occurred in 1 patient (3%). At median follow-up of survivors of 48mths (range 4.6-109mths) OS is 51% with median OS 76mths. The only factor predicting for OS was underlying disease, with OS at 4yrs 64% *vs* 31% for acute leukaemia *vs* non-acute leukaemia patients respectively (p=0.046), due to an excess TRM in the non-acute leukaemia group (TRM 27% *vs* 60% for acute leukaemia *vs* non-acute leukaemia patients respectively; p=0.02). There was a strong trend towards increased risk of aGVHD with increased HLA-mismatch, with grade 3/4 aGVHD occurring in 18% *vs* 50% in single *vs* >1 mismatched loci pairs (p=0.056) and 36% *vs* 8% in low *vs* high-resolution mismatched pairs (p=0.123). Overall relapse rate was 14%.
Conclusions
HLA-mismatched URD PCT is associated with acceptable toxicity and OS. Although degree of HLA-mismatch appeared associated with risk of severe GVHD, underlying disease at transplantation was the only predictor for OS.

No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 3: Cell Therapies & Transplantation ***Bayside 104***

O016 0915

HLA-G+ Acquired by Bone Marrow T-cells Mediates Tumour Escape in Myeloma

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Aim
We investigated the acquisition of HLA-G by T-cells from bone marrow (BM) plasma cells of patients with multiple myeloma (MM). Our aim was to explore this as a possible mechanism of the immunological tolerance present in myeloma and other malignancies.
Methods
Flow cytometry and confocal microscopy were used to determine expression profiles of HLA-G on plasma cells and T cells in BM and blood and to analyse functional inhibitor assays.
Results
Our previous confocal microscopy and flow cytometry studies demonstrated that the acquisition of tumour membrane proteins by trogocytosis was more common in patients with MM than in other B cell malignancies and that the transfer of tumour membrane proteins is greater to T than B or NK cells. HLA-G, CD80 and CD86 were prominent amongst the molecules involved.In this study HLA-G expression on BM plasma cells (CD38++) ranged from 0-100% and high HLA-G expression was associated with a shorter survival (χ^2=12.4; p<0.004). HLA-G expression was up to 6x higher on the myeloma stem cell subpopulation (defined by side population - SP) suggesting that these cells are not only drug resistant but also evade immune surveillance. HLA-G was expressed on 0.02 to 0.56% (mean 0.24%) CD3+ cells in the blood of age-matched controls (n=15). Higher levels of HLA-G were present on CD3+ cells from 20% of patients with MM (n=56; range 0.03 to 1.12%; mean 0.31%; F=3.7; p<0.01). HLA-G expression was significantly greater on BM T-cells than blood T-cells (U=38; p=0.016). Expression of HLA-G on BM plasma cells correlated with BM T-cells (r=0.87; p<0.001) but not with peripheral blood T cells. *In vitro* mixing studies demonstrated that HLA-G was acquired by T-cells from BM plasma cells. Both flow-sorted HLA-G+ plasma cells and HLA-G+ T-cells significantly inhibited HLA-G- T-cells in CFSE-tracked proliferation assays (p<0.001). The HLA-G+ T-cells were CD25- and thus not the phenotype of nTregs.
Conclusion
These studies demonstrate that T-cells acquire additional molecules during contact with malignant plasma cells in the BM. Acquisition of HLA-G can change effector T-cells into acquired regulatory T-cells which inhibit the normal immune response and thus potentially promote tumour escape.
No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 3: Cell Therapies & Transplantation ***Bayside 104***

O017 0930

Generation of Influenza-Virus Specific T-cells for Immune Reconstitution in Stem Cell Transplant Patients

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Influenza viruses cause potentially fatal respiratory infections in stem cell transplant patients. Specific T-cells provide long-lived host adaptive immunity to influenza viruses and the potential for generating such cells for clinical use was investigated.
The inactivated influenza vaccine (Fluvax) was used as the antigen source. Only reagents and culture medium approved for clinical manufacture were used. Monocyte-derived dendritic cells (MoDC) pulsed with Fluvax were used to stimulate autologous PBMC at a responder to stimulator ratio of 10:1. On Day 7, a second stimulation was performed. 20U/ml IL-2 was added from Day 7 and 50U/ml IL-2 from Day 14 onwards. Media exchanges were performed as required using fresh medium containing IL-2. Over 21 days of culture, a mean fold increase of 26.3 in cell number was observed (n=7). Cultures were mainly T-cells (mean 92.9%) with effector and central memory phenotypes, contained a low percentage of NK cells (mean 7.7%) and no B cells or monocytes (both <1%). While CD4 cells dominated (mean 78.1%), there were a sizeable percentage of CD8 cells (mean 14.9%). Following expansion, CD154 was expressed on 8.1% of CD4 cells. Both CD4 (mean 26.3%) and CD8 (mean 3.2%) cells produced one or more Th1 cytokines IFNγ, TNFα and/or IL-2 when restimulated with Fluvax pulsed MoDC. Most responding CD4 cells produced all three cytokines simultaneously while CD8 cells primarily produced IFNγ. A mean of 11.1% CD4 and 9.1% of CD8 cells mobilized CD107 when restimulated with Fluvax (n=6). Th1 cytokines were produced in response to stimulation with antigens derived from individual viruses H1N1, H3N2 and Brisbane strains by the CD4 subset in all of 6 cases and CD8 subset in 2 of 6 cases. In addition, both CD4 and CD8 cells expanded when co-cultured with MoDC pulsed with Fluvax or individual strains of influenza (n=2). In 3 of 6 cultures, IL-21 was also produced by up to 1% of CD4 cells following restimulation with influenza viruses. In conclusion, we demonstrate a clinically applicable method that yields high numbers of highly reactive T-cells specific for influenza-viruses. Resultant cells express a phenotype characteristic of activated antigen specific cells capable of B cell helper function. We propose that reconstructing host immunity through adoptive transfer of influenza virus specific T-cells possibly combined with early post-transplant influenza vaccination will reduce the frequency of influenza-related deaths in the period of severe immune suppression that follows stem cell transplant.
No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 3: Cell Therapies & Transplantation ***Bayside 104***

O018 0945

Donor Factors Determining Outcome in Reduced Intensity Allogeneic Stem Cell Transplantation

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Aim
To assess the impact of donor factors on outcomes in reduced intensity (RIC) matched sibling allogeneic haematopoietic stem cell transplantation (allo-HSCT) in patients (pts) with hematologic malignancies.
Methods
Between 1/03-1/10, 56 pts undergoing allo-HSCT and their donors were identified at our centre. Median donor age was 54 yrs (16-71) with 27% donors ≥60 yrs. 55% were female. All donors were fully matched siblings and underwent PBSC mobilisation and harvest following G-CSF administration. Donor age, gender, parity, CMV status and cellular content of the PBSC product including CD34, total CD3, CD4, CD8, CD16/56 and CD19 cells infused were assessed and correlated to a number of transplant outcomes.
Results
Donors mobilized >2 and >5 x 10^6/kg CD34+ cells in 93% and 50% cases respectively. Neutrophil and platelet engraftment occurred in 100% and 93% of recipients respectively.
3 yr.DFS and OS was 68% and 54%. Transplant related mortality (TRM) was 18%. Rates of aGVHD and cGVHD were 43% (24/56) and 57% (32/56) respectively. When compared to allo-HSCT using younger donors, donors aged $\geq$60 harvested significantly fewer CD34+ cells (5.5$\pm$4.92 vs 3.4$\pm$2.29 vs, p= 0.012); were associated with significantly longer time to neutrophil engraftment (18$\pm$2.91 vs 13.5$\pm$3.15 days, p=0.0001), and were associated with a trend towards an increasing rate of cGVHD (73.3% vs 48.8%, p=0.09). The use of PBSC product from female vs male donors was associated with higher transplanted total CD3 (3.1$\pm$1.01 vs 2.3$\pm$1.26, p=0.03) and CD4 cells (1.97$\pm$0.74 vs 1.76$\pm$0.81, p=0.009); and higher rates of aGVHD (50% vs 25%, p=0.05). Donor age or gender did not adversely affect TRM, DFS or OS in our cohort.
Conclusion
Donor factors, in particular older donor age and gender may play a significant role in determining the quality of harvested PBSC product and outcomes in RIC allo-HSCT. Future studies will be directed at assessing whether manipulation of PBSC product from high risk donors may improve transplant associated outcomes following RIC allo-HSCT.
No conflict of interest to disclose

Monday 31 October **0830-1000**
HSANZ Free Communications 4: Myeloma & Fungal Infections ***Bayside 105***

O019 0830

The Discovery and Assessment of n-Methyl-2-Pyrrolidone (NMP) as a Biologically Potent Anti-Myeloma IMiD® Subunit

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Aim
NMP is an industrial solvent and drug vehicle used in depot injections, orthopaedic cements and transdermal systems. It is also commonly used to deliver kinase inhibitors in preclinical cancer models. We detected unexpected antineoplastic activity in NMP-treated controls using the immunocompetent Vk*MYC multiple myeloma (MM) model. As NMP is a parent compound of thalidomide, we hypothesised NMP has IMiD®-like activity.

Methods
NMP's mechanistic activity was defined by benchmarking against lenalidomide (LEN) using *in vitro* assays of IMiD® activity on human PBMCs and human MM cell-lines (HMCL). *In vivo* characterisation was performed in an LPS-model of endotoxaemia and by comparison of Vk*MYC MM responses in immunocompetent *vs.* immunodeficient mice.

Results
At physiologically relevant doses (1 – 10μM) with equimolar potency to LEN, NMP exhibited key hallmarks of IMiD® activity: suppression of LPS-induced monocyte TNFα production, T-cell costimulation, Th1 cytokine skewing and enhanced NK activity. NMP (but not LEN) upregulated DNAM-1 and NKG2D ligands on HMCLs thereby sensitising them to NK-mediated lysis. When compared to LEN *in vivo,* NMP showed superior suppression of inflammatory cytokines (IL-6 and TNFα) following LPS-challenge and protected mice from endotoxic death. LEN was ineffective at controlling Vk*MYC myeloma growth; whereas NMP induced disease stabilisation or regression in all transplanted Vk*MYC myelomas tested (n = 3). The *in vivo* anti-myeloma activity of NMP was abrogated by concurrent T-cell immunosuppression (with mTOR inhibitors) and absent in $RAG2^{-/-}\gamma c^{-/-}$ transplant recipients.

Conclusion
NMP is an IMiD® subunit with superior activity over LEN in immunocompetent sepsis and myeloma models. This is the first description of an active IMiD® fragment, and has major implications for drug development. Extensive scientific literature citing NMP as a preclinical drug delivery vehicle requires re-evaluation. Occupationally and iatrogenically exposed persons should be evaluated for immune-mediated side effects. Finally, current clinical trials combining IMiDs with mTOR inhibitors could be detrimental, due to the mutually antagonistic activities of these agents.

Monday 31 October **0830-1000**
HSANZ Free Communications 4: Myeloma & Fungal Infections ***Bayside 105***

O020 0845

Dysregulated Gene Expression of Heat Shock Protein (Hsp) Family Members Correlates With Poor Clinical Outcome and Drug Resistance in Multiple Myeloma (MM)

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2. Malignant Haematology & Stem Cell Transplantation Service, The Alfred Hospital, Melbourne Australia

Hsp inhibition is an emerging therapeutic paradigm in cancer. In MM, Hsp gene expression and its clinical significance are yet to be defined.

Aims

Human myeloma cell lines (HMCLs): To evaluate Hsp27 gene expression in relation to CD45 expression and IL-6, and the impact of Hsp90 inhibition following Hsp27 silencing. Patient samples: To evaluate Hsp gene expression in malignant and normal plasma cells (PC), and correlate expression with clinical outcome (OS, PFS) in MM patients.

Methods

PCs from 47 BM aspirates (n=41 MM, n=6 normal) were isolated. Total RNA was extracted from HMCLs and PCs. Relative gene expression of Hsps 27, 70, 90α and 90β was quantitated using RT-PCR. HMCL CD45 expression was established by flow cytometry. HMCLs were cultured with/without IL-6. Hsp27 was silenced by siRNA and then HMCLs were treated with Hsp90 inhibitor NVP-HSP990. Clinical outcomes (OS, PFS) were compared for expression of individual [high ($\geq 75^{th}$ centile) vs. low ($\leq 25^{th}$ centile)] and multiple Hsp genes [0-1 vs. 2-4, high (>median) vs. low (≤median)].

Results

Hsp27 gene expression was inversely proportional to CD45 expression (p=0.01), doubled 24 hours following IL-6 withdrawal (p=0.0051) and Hsp27 silencing enhanced killing 4-fold with an Hsp90 inhibitor (p=0.0018). Relative gene expression of all Hsps was significantly increased in MM PCs compared with normal, in both early (preceding first relapse/progression, n=23) and advanced cases (n=18): Hsp27 p=0.0001, Hsp90α p=0.037; and advanced only: Hsp70 p=0.046, Hsp90β p=0.0064. Increased gene expression correlated with worse clinical outcome: patients with high Hsp70 gene expression had worse PFS [median PFS 943 vs. 1228 days (p=0.028)], patients with high Hsp90β gene expression had worse OS and PFS [median OS 1439 vs. 3373 days (p=0.0032)], median PFS 761 vs. 1439 days (p=0.0015). Patients who over-expressed 2-4 Hsps had a worse PFS than those with 0-1 overexpressed (p=0.04).

Conclusion

Hsp gene expression is dysregulated in MM and correlates with poor clinical outcomes. We hypothesise that Hsp27 plays a cytoprotective role in MM and contributes to drug resistance.
No conflicts of interest to disclose.

Monday 31 October **0830-1000**
HSANZ Free Communications 4: Myeloma & Fungal Infections ***Bayside 105***

O021 0900

Immunological Biomarkers in 10 year Survivors of Multiple Myeloma

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Aim
Investigate immune mechanisms which may contribute to long-term survival in multiple myeloma (MM). The presence of expanded T-cell clones in MM predicts for a positive outcome. Increased Treg and decreased Th17 and absolute Slan-DC counts have been demonstrated in MM and may impair immune-mediated disease control. All 10 year MM survivors attending RPAH were identified and the status of these biomarkers was determined.

Methods
Peripheral blood samples were analysed for the presence of CD3+ T-cell receptor Vβ restricted Tcell clones (BetaMark Kit), the number of CD3+CD4+CD25h+CD127- Tregs, CD4+IL-17+ Th17 cells and $CD16^{+}CD14^{low}$ $M\text{-}DC8^{+}$ Slan-DCs. Results were compared with local data collected from two large MM cohorts and age-matched controls.

Results
26 patients had survived for 10 years and 22 were available for testing. Median age at diagnosis was 59. 92% were ISS 1. 73% had end-organ effects. 58% had received cytotoxic chemotherapy; 27% novel agents; 38% autologous transplants; 8% allogeneic transplants and 31% were untreated. Expanded T-cell clones were documented in all (100%) of the 10 year survivors, a marked increased compared with 54% (n=144) and 48% (n=120) in our previous MM cohorts (χ^2=43.6;p<0.001). Mean Treg number was 5.7% of CD4+ cells in the 10 year survivors which was significantly less than 8.9% in the all-MM cohort (t=3.1; p< 0.005) but similar to 6.5% in age-matched controls. Th17 cell numbers were significantly greater in the 10 year survivor group (3.3% of CD4+ cells) than our previous MM cohort (0.72%;U= 78; p< 0.005) and mean absolute Slan-DC numbers were significantly higher (p<0.05).

Discussion
Analysis of immunological biomarkers in 10 year survivors of MM demonstrated a statistically significant increase in T-cell clones, Th17 cells and Slan-DC's and decrease in T-regs when compared to a large MM cohort. This emphasizes the importance of an immunological mechanism in long term disease control.
No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
HSANZ Free Communications 4: Myeloma & Fungal Infections ***Bayside 105***

O022 0915

Sirtuin Dysregulation in Myeloma Correlates with Poor Clinical Outcome

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Introduction
The sirtuin (SIRT) family of deactylases consists of seven mammalian members (SIRT1-7). SIRT1 and SIRT2 are frequently altered in malignancies, with expression correlating to poorer outcomes in a range of solid tumours. Hence, they represent potential novel targets for anti-cancer therapy, but are not inhibited by presently available deacetylase inhibitors. In multiple myeloma (MM) their pattern of expression and impact on patient outcome has not yet been determined.

Aims
(i) Determine and compare *SIRT* expression levels in purified MM cells and normal plasma cells,
(ii) Correlate *SIRT* expression in MM samples to overall survival (OS – diagnosis to death) and progression-free survival (PFS – diagnosis to first relapse/progression).

Methods
Bone marrow aspirates from MM and normal patients were obtained following consent. Total RNA and cDNA were prepared from purified normal (n=6) and MM plasma cells (n=41) and quantitative RT-PCR performed for *SIRT1-7*. The OS and PFS in MM samples with high gene expression levels ($\geq 75^{th}$ centile) compared to low levels ($\leq 75^{th}$ centile) were derived utilising Kaplan-Meier survival plots.

Results
Quantitative gene expression analysis revealed that *SIRT1-3* and *SIRT5-7* were elevated in MM plasma cells compared with normal plasma cells ($p<0.01$). When *SIRT* expression levels were correlated to patient outcome, it was observed that patients with elevated *SIRT5* compared to lower levels display decreased PFS (median survival: 658 days vs. 1224 days; $p=0.0085$) and OS (844 days vs. 2272 days; $p=0.01$). Although samples with high *SIRT* expression were predominantly from patients with advanced MM (ISS stage II or III) at diagnosis, this observation was statistically insignificant.

Conclusion
SIRT levels are augmented in MM patients providing the rationale for the exploration of SIRT inhibitors as anti-MM agents. Increased *SIRT5* expression correlates with poor clinical outcome indicating that *SIRT5* may represent a novel predictor of adverse outcome in newly diagnosed MM patients.

No conflict of interest to declare

Monday 31 October **0830-1000**
HSANZ Free Communications 4: Myeloma & Fungal Infections ***Bayside 105***

O023 0930

Incidence of Invasive Fungal Infection in Adult Patients with Acute Lymphoblastic Leukaemia

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Aim
To define the rate of invasive fungal infection (IFI) during treatment for acute lymphoblastic leukaemia (ALL) in adult patients treated at The Royal Brisbane and Women's Hospital (RBWH).
Method
All adult patients treated for ALL at RBWH between May 2005 and December 2010 were identified from an institutional data-base. IFI rates and outcomes were determined retrospectively via review of medical records. Unit policy throughout this time period was for use of Fluconazole as primary antifungal prophylaxis in this patient cohort. The revised EORTC / MSG guidelines were used for defining specific incidence of IFI (CID 2008; 46: 1813).
Results
In total 38 patients were diagnosed and treated for ALL at our institution during this time period. 37 were treated with HyperCVAD and one patient received (palliative) Vincristine and Dexamethasone. In total 10 patients (26%) required treatment with broad spectrum antifungal therapy, including 1 patient who received broad spectrum antifungal therapy twice. Of these 10 patients, 9 were receiving Fluconazole as primary prophylaxis at IFI diagnosis, and all 10 received Voriconazole as their initial broad spectrum antifungal treatment. 3 patients suffered proven IFI, including 2 cases with *Scedosporium prolificans* and one case of *Candida tropicalis* sepsis. Although 7 patients suffered possible IFI (none with positive mycological criteria), all 7 clinically and /or radiologically improved post commencement of Voriconazole. 2 of the 10 patients died from their IFI (1 patient each with *S. prolificans* and *C. tropicalis*).
Conclusion
Rates of IFI are not insignificant in adult ALL patients receiving Fluconazole prophylaxis. The incidence of IFI complicating treatment of ALL is comparable to previously published rates of IFI in treatment of myeloid malignancies (NEJM 2007; 356: 348). The high rate of IFI in adult ALL should raise consideration for exploration of alternative prophylactic strategies in this patient group.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
HSANZ Free Communications 4: Myeloma & Fungal Infections ***Bayside 105***

O024 0945

Posaconazole Therapeutic Drug Monitoring: Experience at a Tertiary Referral Centre

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Aim
Multiple factors have been shown to affect the absorption and bioavailability of posaconazole, a potent antifungal agent. In this study we retrospectively reviewed all patients receiving posaconazole since the introduction of therapeutic drug monitoring (TDM) at Alfred Health in September 2010, to determine the prevalence of low posaconazole levels (PL) and identify risk factors for low PL.
Method
61 patients with acute leukaemia (*n* = 27) or after haematopoietic stem cell transplant (HSCT) (*n* = 34) receiving posaconazole after 1 September 2010 were included. All PL for the 61 cases were abstracted. A low PL was defined as <0.5 mg/L. Additional data collected included demographic data, liver function test results and details of graft-versus-host disease, proton pump inhibitor (PPI) use and total parenteral nutrition (TPN) use.
Results
56 patients received posaconazole as prophylaxis and 5 received it as therapy for invasive fungal infection (IFI) for a median of 89 days (range, 2-378 days). A total of 271 PL were performed, with a median of 4.0 (range, 0-23) per patient. 12 patients (20%) did not have any PL performed.
84 (31%) of 271 PL were low. 27 (32%) low PL instigated a change in management, *i.e.*, an increase in dose (*n* = 18) or a switch to a different antifungal (*n* = 7). Low PL were more frequent in patients on PPI (34% vs. 16%; $p = 0.0081$), with albumin <30 g/L at commencement (37% vs. 25%; $p = 0.0263$) or on TPN (61% versus 26%, $p < 0.0001$).
Hepatotoxicity (defined as ALT or GGT more than five times the upper limit of normal) occurred in 7 patients (11%), resulting in the discontinuation of posaconazole in one patient. No patients on prophylactic posaconazole developed an IFI.
Conclusion
These data suggest that posaconazole TDM is essential in at-risk haematology patients to prevent therapeutic failure. An algorithm to optimise TDM and clinical use of posaconazole needs to be developed, implemented with education and prospectively evaluated.
No conflict of interest to disclose

Monday 31 October **0830-1000**
ANZSBT Presidential Symposium ***Auditorium A***

O025 0830

Pacific Transfusion Leaders Program: Building Leaders for Safe Blood - A Long Term Mentoring Approach

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Background
Amongst transfusion professionals, leaders are crucial in driving transfusion policy and improving technical capability at institutional, national and regional levels. Traditional approaches to development in transfusion have focused on in-country short or long-term technical support and training of individuals, with moderate success. The Pacific Transfusion Leaders Program (PTLP) takes a regional approach to transfusion system improvement, building a regional network of leaders with increased confidence, critical thinking and technical skills through a mentoring and peer networking approach.

Aims
Develop and sustain a regional leadership network with increased capacity to understand and analyse a broad range of transfusion-related policy and content issues, and the leadership skills to drive improved policy and practice, leading to safer collection, processing and clinical use of blood products.

Methods
PTLP brings together ten Fellows from nine Pacific countries with key roles (scientific, nursing, medical), identified as leaders and nominated by their Ministries of Health. Fellows train with specialist agencies, including Australian Red Cross Blood Service, Nossal Institute for Global Health and hospital transfusion laboratories, to develop capacity in four key areas: technical knowledge, leadership, research capability and transfusion policy and practice. Fellows will develop their understanding of requirements for effective blood systems, identify and conduct projects in areas of need and develop leadership skills to drive change in public health agenda. Peer-networks and on-going mentoring will sustain momentum. Fellows will build relationships with Australian organisations and raise awareness of Pacific blood systems within the regional transfusion community.

Conclusions
Development and support of leaders with improved technical knowledge and enhanced relationships with governments and other stakeholders will result in improved safety and sufficiency of blood supplies, safer and more effective transfusion practice and reduced health and financial risks faced by transfusion systems in Pacific countries.

PTLP is supported through an Australian Leadership Awards-Fellowships Grant from AusAID.

Monday 31 October **0830-1000**
ANZSBT Presidential Symposium ***Auditorium A***

O026 0845

Identification of Genetic Polymorphisms That Predict Host Immune Response to Rhesus D Antigen

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Background
Haemolytic disease of the newborn (HDN) occurs after an RhD-negative woman becomes immunised to foetal RhD-positive red blood cells (RBCs). HDN cases have dropped significantly since the successful application of prophylactic anti-D. The Blood Service embarked on a national RhD Immunisation Program where RhD-negative individuals were immunised with RhD-positive RBCs in order to produce anti-D for national supply. However, up to 35% of enrolled donors do not respond to immunisation and are therefore unnecessarily exposed to risks associated with RBC transfusion. This study sought to examine the host genetic factors in RhD sensitisation, with the aim of establishing a simple genetic test to predict responses to RhD antigen in potential donors.
Method
Initial genotyping studies were performed for host factors such as HLA-DRB1, TLR2, TLR4, CD14 and FCgRIIA with in-house PCR-restriction fragment length polymorphism assays. Further in-depth genotyping analysis was conducted at the Australian Genome Research Facility with an Affymetrix 9K ImmunoInflammatory Single Nucleotide Polymorphism (SNP) Chip.
Result
An initial study of 157 RhD immunised donors indicated an association between TLR2-753 and HLA-DRB1-07 genotypes and a high response to RhD (X2 P-value = 0.019, OR = 0.09, 95% CI = 0.01 – 0.07). This haplotype excluded 97% of non-responders, but only 25% of responders were identified. Further analysis of 184 RhD immunised donors identified 17 significant SNPS using logistic regression models. A response classifier was built using 7 SNPs giving an AUC of 89.3% on the true validation set. Three of these SNPs are associated with immune response pathways that regulate T cell and B cell receptor signal transduction.
Conclusion
Based on recently identified SNPs associated with responsiveness to RhD immunisation, the development of an RT-PCR-based screening assay could provide a means to advise prospective donors of their likely response to immunisation before exposure to transfusion-associated risks of RhD immunisation.
No conflict of interest to disclose

Monday 31 October **0830-1000**
ANZSBT Presidential Symposium ***Auditorium A***

O027 0900

Transfusion Reactions and Junior Medical Officers' Understanding of Transfusion Medicine

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Aim
Reports to Queensland incidents in transfusion (QiiT) for the first annual report demonstrated variability in the management of transfusion reactions (TRs). The aim of this survey was to appreciate the exposure of junior medical officers to transfusion medicine (TM).
Methods
QiiT is a voluntary haemovigilance system utilised by both Public and Private healthcare facilities in Queensland. Reports received by QiiT up to January 2010 were analysed for the first annual report. Junior medical officers (JMO) were surveyed using a voluntary electronic survey distributed via postgraduate medical centres.
Results
The reports to QiiT demonstrated heterogeneity in the management and investigation of transfusion reactions. There was inconsistency in the investigation of febrile reactions, and validated cases of TRALI were all misreported as alternative reactions.
Of 225 JMO respondents, only 42% recall undergraduate training in TM. However, their ranking of the risks of five TRs was accurate and correct patient identification ranked top in ensuring safe transfusions. Laboratory pre-transfusion testing was ranked as the second key area in their routine clinical practice. However, there was a discrepancy between this perception and their understanding of issues relating to ABO compatibility of fresh blood components. Management of TRs ranked 4th in ensuring safe transfusions, yet ranking of the likeliest fatal transfusion reactions was inconsistent with reported data. Of the JMO surveyed 20% had registered with Bloodsafe and 21% had seen NHRMC guidelines on the use of fresh blood components.
Conclusions
JMO manage TRs as part of their daily clinical practice. Yet their knowledge and exposure to TM appears highly variable. This is reflected in the management of reactions as documented by QiiT and the survey responses. Interestingly, there are no national guidelines on the management of TRs. Development of national guidelines would be a first step towards addressing these issues.
No conflict of interest to disclose

Monday 31 October **0830-1000**
ANZSBT Presidential Symposium ***Auditorium A***

O028 0915

Positive, Partial, Weak, Weaker and Weakest: Where is the Divide Between RhD Positive and Negative for r'r and r"r Blood Donors?

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Background
DEL phenotypes are below the detection levels of automated Rh(D)-testing and are reported as Rh(D)neg. Identification of Rh(D)neg donors is important to make Rh(D)neg blood available and prevent RhD allo-immunisation.
Aim
To determine the frequency of DEL in donors serotyped as r'r and r"r.
Method
QLD donations typed r'r or r"r (n = 250) were tested by an adsorption-elution procedure using anti-D (clone MCAD6) to detect the DEL phenotype, and genotyped for RHD exons 4, 5 and 10. DEL-seropositive or RHD-genotype positive donors were further investigated by SNP mapping.
Results

Group	DEL-serotype	*RHD*- Exon 4 & 5	*RHD*- Exon 10	N	SNP Genotype
P&G,NEG	Neg	Neg	Neg	225	
G,E10	Neg	Neg	Pos	12	6 *RHD-CE*(3-8/9)-*CE*
G,487del4	Neg	Pos	Pos	1	1 *RHD*(487del4)[a]
G,DBTII	Neg	Neg	Pos	1	1 DBT II[b]
G,E4-5&10	Neg	Pos	Pos	2	1 *RHD*(IVS3+1G>A)[c]
					1 *RHD*(IVS3+2T>A)[c]
P&G,DEL+	Pos	Pos	Pos	3	1 *RHD* 94insT (FS, 35X)[c]
P,DEL+	Pos	Neg	Neg	6	Unresolved P&G mismatch

[a] A known Rh(D)neg. [b] A known partial D type. [c] Known DELs

The basis of the Rh(D) negative phenotype for 90% of donors was *RHD* gene deletion (Group P&G,NEG). Group G,E10 represents a significant source of phenotype genotype anomaly if only exon 10 typing was considered. The Group G,487del4 donor carries a non-functional *RHD* gene. *RHDEL/partial* genotypes were detected in 6 donors (2.4%), although use of MCAD6 anti-D failed to detect the *RHDBTII* donor who was expected to type DEL-seropositive, and *RHD*(IVS3+1G>A) and *RHD*(IVS3+2T>A) donors known to be DEL. For Group P,DEL+, the basis of DEL-seropositive tests without evidence of *RHD* gene inheritance is not yet resolved.
Conclusions
Transfusion of r'r or r"r components that are *RHD* gene-deletion negatives or *RHD-CE*(3-8/9)-*CE* to Rh(D)neg recipients is expected to be uncomplicated. The divide appears to be for the 5 donors with *RHDEL* genotypes and the *RHDBTII* donor. There are reports of immunisation following transfusion with products of these types. *RHD* genotyping will minimise risk of exposure by identifying weak Rh(D) types, and consideration should be given to excluding donors with these forms of very weak Rh(D) expression from the Rh(D)neg panel.
No conflict of interest to disclose

Monday 31 October **0830-1000**
ANZSBT Presidential Symposium ***Auditorium A***

O029 0930

Experience with Thromoboelastometry (ROTEM) Guided Transfusion Therapy in Patients Undergoing Orthotopic Liver Transplantation

David Roxby, Romi Sinha, Shaba Vakalia
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Aim
Orthotopic liver transplantation (OLT) can be associated with critical haemorrhage, microvascular bleeding and coagulopathy. Bleeding commonly occurs during dissection of the native liver, graft reperfusion or vascular anastomosis. The aim of this study was to examine the clinical effectiveness and efficacy of visco-elastic functional clotting assays (thromboelastometry) [ROTEM] to guide intra-operative transfusion therapy in OLT patients.
Methods
Two groups of patients were included, those whose intra-operative transfusion requirements were guided by routine coagulation studies (INR, aPTT, fibrinogen and platelet count [non-ROTEM Group]) and those whose requirements were guided solely by ROTEM. Samples were taken at the beginning of the dissection (pre-anhepatic); anhepatic and reperfusion phases (reperfusion of the graft liver) and whenever there was diffuse bleeding. Product usage [red cells (RBC), fresh frozen plasma (FFP), platelet and cryoprecipitate) was compared between the two groups using the Kruskal-Wallis test.
Results
Thirty seven patients were included in each group. Overall, patients received a median of 4 RBC (IQR 2.-7), 4 FFP (IQR 2-7), 1 platelet (IQR 0-2) and 2 batches of cryoprecipitate (IQR 0-2). There was a 15% reduction in RBC use (158 vs.186) and 40% reduction for FFP (137 vs. 229) transfused between the ROTEM and non-ROTEM groups. Patients in the ROTEM group received less FFP during OLT [3 (IQR 1.5-6) vs. 5 (IQR 3-9) p=0.04]. This change was more evident during the reperfusion phase in the ROTEM group [2 (IQR 0.2) vs. 3 (IQR 2-4) p=0.01]. In the first 24 hours post-operatively, there was no significant difference in product usage between the two groups.
Conclusion
ROTEM guided transfusion therapy resulted in significant reduction of FFP transfused in OLT and particularly during the reperfusion phase. The reduced use of FFP did not result in increased bleeding or increased demand for blood products post-operatively.

No conflict of interest to disclose

Monday 31 October **0830-1000**
ASTH Free Communications 1: Coagulation Science ***Bayside 204***

O031 0830

Mechanism of Lateral Self-Association of Von Willebrand Factor

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Von Willebrand Factor (VWF) is a multimeric plasma protein that chaperones coagulation Factor VIII and binds platelets to an injured vascular wall. VWF molecules are secreted from vascular endothelial cells as variable numbers of disulphide-linked homodimers. When exposed to the shear forces found in flowing blood, VWF molecules undergo lateral self-association that results in a meshwork of VWF fibres that bind platelets. Plasma VWF contains unpaired cysteine thiols and lateral association is inhibited by thiol-alkylating agents, implying that this process involves thiol/disulphide exchange between VWF molecules. A recombinant C-terminal fragment of VWF was expressed in mammalian cells and examined for unpaired cysteine thiols using tandem mass spectrometry. The Cys2431-Cys2453 disulphide bond in the VWF C2 domain was shown to be reduced in approximately 75% of the molecules. Fragments containing all three C domains or just the C2 domain formed monomers, dimers and higher-order oligomers when expressed in mammalian cells. From mutagenesis studies, both the Cys2431-Cys2453 and nearby Cys2451-Cys2468 disulphide bonds were found to be involved in oligomer formation. The findings imply that lateral VWF dimers form when a Cys2431 thiolate anion attacks the Cys2431 sulphur atom of the Cys2431-Cys2453 disulphide bond of another VWF molecule. Trimers and higher-order oligomers can also form when the Cys2451 thiolate anion of one of the VWF molecules in the dimer attacks the Cys2431-Cys2453 disulphide bond of another VWF molecule. These observations provide the basis for exploring defects in lateral VWF association in patients with unexplained hemorrhage or thrombosis.

No conflict of interest to disclose

Monday 31 October **0830-1000**
ASTH Free Communications 1: Coagulation Science ***Bayside 204***

O032 0845

Analysis of Platelet Receptor Expression in ITP

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Aim

The aim of this study was to assess platelet receptor expression in patients with immune thrombocytopenia (ITP) before and during steroid treatment. The platelet-specific collagen receptor, glycoprotein (GP)VI, is associated with the Fc receptor γ-chain (FcRγ). GPVI/FcRγ is coassociated on platelet surface with the GPIb-IX-V complex; GPIbα of GPIb-IX-V binds von Willebrand factor and other ligands. Our previous studies showed engagement of platelet FcγRIIa by antiplatelet antibodies induced ectodomain shedding of GPVI, generating soluble ectodomain (sGPVI) in plasma. However, apart from one individual with an anti-GPVI antibody, whether anti-platelet antibodies associated with ITP affect GPVI/GPIb expression/shedding has not been addressed.

Method

In this study we used flow cytometry and a sGPVI ELISA to assess 1) whether patients with ITP had dysregulated expression/shedding of GPVI or GPIbα, and 2) whether platelet receptor expression changes prior to recovery of platelet count in individuals undergoing treatment for ITP.

Results and Conclusions

In 9 ITP patients (mean age=48.6, range 29-79; 6 female) with platelet count 61±9 x 10^9/L (range, 33-122), GPVI surface expression (GeoMean±SE, 137±17) was lower than healthy controls (274±26; *n*=17; platelet count 247±13), and sGPVI in patient plasma was significantly higher (39±4 ng/mL) compared to 17 healthy donors (19±3 ng/mL) (*P*=0.0006). In longitudinal samples analysed at weekly intervals during 2-month treatment with steroids, decreased GPVI surface expression and increased sGPVI in plasma remained essentially unchanged as the platelet count normalized, consistent with persistent anti-platelet antibody. However, while levels of intact platelet GPIbα were significantly reduced in ITP compared to healthy donors (*P*=0.0053), they approached healthy levels within 1 week of treatment, preceding improvement in platelet count. GPIbα is implicated as a regulator of platelet clearance, and the proteolytic status of GPIbα may be a reliable early marker for evaluating response to treatment in ITP.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
ASTH Free Communications 1: Coagulation Science ***Bayside 204***

O033 0900

Anti-platelet Antibodies Induce Apoptosis, Inhibit Megakaryocyte Maturation and Impair Pro-platelet Production

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Aim
Immune thrombocytopenia and drug-induced thrombocytopenia are conditions that may cause severe and occasionally, life-threatening decline in platelet numbers. Thrombocytopenia is caused by auto-antibodies that recognize abundant platelet receptors such as GPIb/IX and GPIIb/IIIa. Antibody-coated platelets are usually removed by macrophages or destroyed by complement-mediated lysis. Here we explore the effects of anti-platelet antibodies on platelet precursor cells, i.e. megakaryocytes (Mks). This study seeks to understand the cellular pathogenesis of immune thrombocytopenias.
Methods
Highly pure Mks were produced *in vitro* from purified human $CD34^+$ cells. To evaluate the effects of anti-platelet antibodies, Mks were cultured in the presence of antibodies and assayed for antibody interaction, proliferation, viability, expression of megakaryocytic markers, induction of apoptosis, ploidy pattern, cell size distribution and pro-platelet production. In addition, the apoptosis-inducing properties of specific anti-platelet antibodies were evaluated in CHO cells expressing the GPIb/IX receptor.
Results
Incubation of Mks with anti-platelet antibodies resulted in strong physical interaction as determined by flow cytometry and confocal microscopy. This interaction led to a substantial reduction in the expression of late megaryocytic markers (GPIX and GPIb-alpha) and to a marked decrease in cell viability. Treated Mks presented apoptotic characteristics as assessed by Annexin V binding and caspase activation. Nevertheless, the ploidy pattern and cell size distribution were not affected in treated Mks. In contrast, the presence of anti-platelet antibodies led to a greatly reduced pro-platelet production capacity.
Conclusion
These results suggest that the severity of thrombocytopenia caused by anti-platelet antibodies may be partly due to induction of apoptosis in Mks, which, in turn, results in Mk death, impairment of Mk differentiation and the inhibition of pro-platelet production.
No conflict of interest to disclose

Monday 31 October **0830-1000**
ASTH Free Communications 1: Coagulation Science ***Bayside 204***

O034 0915

CDI-1 is a Novel Marker of Platelet Apoptosis

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Background and Aims
Apoptosis plays a pivotal role in regulation of platelet lifespan, however understanding the role of apoptotic platelets in haemostasis has been hampered by the overlap between markers of apoptosis and platelet activation. CDI-1 (cell death imager 1) is a tripeptide trivalent arsenical that can image cell death in mammalian cells. We aim to determine if fluorescent tagged CDI-1 can identify apoptotic platelets and if the fluorescent compound is retained in the cytoplasm of microparticles released from apoptotic platelets.

Methods
Flow cytometric analysis was performed on washed platelets with and without treatment with the proapoptic drug ABT-737 and comparison made with thrombin treated platelets. Platelets were exposed to fluorescent tagged CDI-1 or a chemically inert control compound and co-stained with apoptotic markers (fluorescent caspase 8 marker, Annexin V) and activation marker (P-Selectin). Flow cytometric analysis was also performed on microparticles released from platelets treated with ABT-737 and exposed to fluorescent tagged CDI-1 or control.

Results
In platelets treated ex vivo with pro-apoptotic drug ABT-737, fluorescent tagged CDI-1 identified a platelet population that also stained for fluorescent caspase 8 marker (98% concordance). Dual staining with platelet activation markers: Annexin-V and P-Selectin demonstrated two distinct populations: (1) apoptotic population (dual labelled with CDI-1 and Annexin V) and (2) activated population (negative for CDI-1 and positive for P-Selectin). This indicates that CDI-1 can be used to distinguish between apoptotic and activated platelet populations in vitro. Flow cytometric analysis of microparticles from apoptotic platelets identified by size-gating and Annexin-V staining demonstrated a population positive for CDI-1 and negative for its control compound.

Conclusion
Fluorescent tagged CDI-1 identifies apoptotic platelets and microparticles. This will be valuable in investigating the role of platelet apoptosis in *in vivo* mouse models of thrombosis, allowing direct visualisation of apoptotic platelets and microparticles in fluorescent intravital systems.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **0830-1000**
ASTH Free Communications 1: Coagulation Science ***Bayside 204***

O035 0930

Effect of 7.5% NaCl Adenocaine® and Mg2+ on the Properties of Blood Coagulation Before and During Haemorrhagic Shock in the Rat Model

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Aim
Haemorrhagic shock is responsible for a majority of trauma deaths with acute traumatic coagulopathy occurring early in the process. Our aim was to examine the effect of 7.5% NaCl and Adenocaine® (adenosine and lidocaine) and Mg2+ on activated partial thromboplastin times (APTT) and prothrombin times (PT) following 60 minutes of shock and resuscitation in a rat haemorrhagic shock model. Previously, we showed the fluid could resuscitate mean arterial blood pressure (MAP) to a permissive hypotensive range after 40% and 60% blood loss.
Method
Haemorrhagic shock was induced by phlebotomy in anaesthetised, non-heparinised male fed Sprague-Dawley rats (444 ± 10g, n=64) until MAP was 35-40 mmHg and continued for 20min for ~40% blood loss. After 60 minutes of shock (34˚C), a 0.3mL IV bolus (Adenocaine® plus Mg2+) was injected and MAP monitored for 60 minutes. APTT and PT clotting times were measured during haemorrhagic shock and resuscitation at 60 min in untreated, vehicle control, and Adenocaine®/Mg2+ treated rats.
Result
The untreated, control rat plasma had APTT of 17 ± 0.5 sec (n=8). After 20 min bleeding (~40% blood loss), the APTT clotting time increased to 63 ± 21 sec (n=8), and after 60 min shock increased further to 217 ± 31 sec (n=8) before resuscitation. After 60 min resuscitation with 7.5% NaCl Adenocaine® plus Mg2+, the APTT returned to 24 ± 1.7 sec (n=8). The 7.5% NaCl-treated rats (vehicle control) showed an APTT 262 ± 50 sec (n=7). Prothrombin times (PT) followed a near identical pattern.
Conclusion
We showed that hypocoagulopathy occurred early after bleeding and shock and was almost completely reversed after 60 min of 7.5% NaCl Adenocaine/Mg2+ hypotensive resuscitation. Our preliminary in vivo rat data suggest that this resuscitation therapy may have coagulation restorative properties which together with anti-inflammatory properties could reduce early trauma-related mortality and morbidity.

NP, HL, LM - No conflict of interest to disclose. GD – is a consultant for Hibernation Therapeutics Global (HTG) and inventor of 9 patents on Adenocaine®.
This research was supported by an internal James Cook University grant.

Monday 31 October **0830-1000**
ASTH Free Communications 1: Coagulation Science ***Bayside 204***

O036 0945

Effect of Low Dose Omega-3 on Fibrin and Thrombin generation in healthy volunteers and patients with cardiovascular disease

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Aim
Hypercoagulability plays a significant role in the development of cardiovascular disease (CVD). High dose omega-3 has been shown to modify coagulation parameters, but low dose therapy has not been well studied. The aim of this study was to investigate the effect of low dose omega-3 on fibrin and thrombin generation in healthy volunteers and patients with CVD over a 4 week period.
Methods
Twenty-nine healthy volunteers (mean age 30.3yrs; range 22-64yrs), with mean BMI 23.63 kg/m^2 (range 18.93-35.92 kg/m^2) and fifteen patients with CVD (mean age 68.1yrs; range 50-83yrs) with mean BMI 26.99 kg/m^2 (range 20.42-32.53 kg/m^2) were tested pre- and post-omega-3 (DHA 520mg, EPA 120mg daily) for four weeks. Global coagulation activity was measured by overall haemostatic potential (OHP) and thrombin generation (CAT). Statistical analyses were performed using Wilcoxon's matched pair tests and T-Test.
Results
Changes in clot formation were observed in the global clotting assay OHP for both healthy subjects and patients with CVD. This was evident as increased delay to fibrin formation (8:55±2:46 vs. 9:38±2:04min, P=0.021) and decreased maximum velocity (156.7±35.1 vs. 134.27±31.1 maxOD/min, P=0.010). There was also reduced overall coagulation potential (OCP) (41.0±7.1 vs. 38.2±6.9, P=0.053) and OHP (8.7±3.2 vs. 7.4±2.6, P=0.009). In patients, there were significant reductions in OCP (56.4±8.6 vs. 48.4±10.8, P=0.002), OHP (23.5±12.8 vs. 19.0±11.0, P=0.011) and maximum velocity (218.3±73.9 vs. 180.9±60.8 maxOD/min, P=0.010). In healthy volunteers the CAT assay showed no change in thrombin generation while the CVD patients showed a significant increase in lagtime (4.52±1.83 vs. 4.96±2.34min, P=0.009).
Conclusion
The results of this study suggest that low dose omega-3 fish oil has a mild hypocoagulant effect in both healthy subjects and those with CVD. This may be a mechanism for its cardioprotective effect.

No conflict of interest to disclose

Monday 31 October **0830-1000**
APBMT Symposium 4: Special Challenges in HSC Transplant (Joint Symposium with Nurses) ***Bayside Gallery B***

Choosing the Optimal Donor

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Considerations when selecting the optimal graft for allogeneic transplantation include: donor relationship (related versus unrelated); donor-recipient HLA-matching; and, cell source (bone marrow vs peripheral blood versus cord blood). HLA-identical siblings are generally accepted as the preferred donor unless donor co-morbidities preclude donation. If an HLA-identical sibling is not available, an unrelated donor search may be done. Best results with unrelated adult donor transplantation are obtained when donor and recipient are matched at high-resolution for HLA-A,-B,-C, and -DRB1 though transplantation with a single mismatch may give acceptable results in some situations. In the latter case, matching at low-expression alleles (DQ, DP, DRB3/4/5) may be beneficial. Some data suggest that peripheral blood transplantation may be more permissive of HLA mismatching than bone marrow transplantation. If an adult unrelated donor is not available in a timely manner, umbilical cord blood mismatched at one or two loci may be used, with current data suggesting results similar to results with unrelated adult bone marrow or peripheral blood transplantation. The choice of an adult donor versus cord blood is influenced by graft factors such as HLA-match and cell dose but also by patient factors such as age, weight, type of disease and urgency of the clinical situation. Haploidentical related donors may also be effective graft sources; a recent study suggests results similar to cord blood transplantation. With an increasing array of graft source options, transplantation should be considered early in the disease course of patients with diseases potentially benefited by allografting to allow appropriate testing of potential donors and to avoid undue delay when transplantation becomes necessary. Decisions about when and with which graft source to perform transplantation should be based on a several factors, including donor and graft characteristics but also patient age, performance status and disease.

Monday 31 October **0830-1000**
APBMT Symposium 4: Special Challenges in HSC Transplant (Joint Symposium with Nurses) ***Bayside Gallery B***

Exploring the Potential of Nutritional Support in HSCT

Shigeo Fuji
Department of Hematology & Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

Hematopoietic stem cell transplantation (HSCT) has now become an integral part in the treatment of hematological malignancy. After HSCT, oral intake can be insufficient due to the intestinal damage by the conditioning regimen and GVHD. Hence, parenteral nutrition is frequently required to keep the caloric intake. In these situations, the professional intervention by nutritional support team (NST) is indispensable. However, the management strategy of nutritional support in the field of HSCT has not been well established due to the scarcity of clinical trials.

Therefore, we performed several retrospective studies. We demonstrated that insufficient caloric intake was associated with the significant loss of body weight and muscle weight after allogeneic HSCT. We also demonstrated that hyperglycemia during the early phase after allogeneic HSCT was associated with the significant increase of non-relapse mortality.

Considering these results into account, we performed the prospective clinical trial assessing the feasibility of intensive glucose control after allogeneic HSCT. We found that intensive glucose control can be performed safely after allogeneic HSCT and could reduce the risk of infectious diseases comparing to the historical control.

We are now conducting several prospective clinical trials relating nutritional support after HSCT in Japan. To expand our experience and perform larger clinical trials, we established a Working Group in APBMT meeting last year. Now, we are preparing the protocol for the clinical trials.

I will present the current status and future plan of research in this field.

There is no conflict to declare.

Monday 31 October **0830-1000**
APBMT Symposium 4: Special Challenges in HSC Transplant (Joint Symposium with Nurses) ***Bayside Gallery B***

Sexual Health Post-allograft - Helping To Make a Difference

Yvonne Panek-Hudson
Dept of Cancer Medicine. Peter MacCallum Cancer Centre, Melbourne, Australia

Improvements in supportive care have contributed to increased survival rates post Allograft. There is significant interest in late effects and follow up in the mid-long term post Allograft however there is inconsistency in assessment and management of sexual health issues.

Despite the personal nature of sexual health reviews, most patients are willing to engage in these conversations and many are relieved to discover they are not experiencing issues in isolation.

This presentation will discuss common sexual health issues reported by patients post Allograft. It will provide an overview of current recommendations for sexual health assessment, screening and clinical management of potential and identified sequelae.

Monday 31 October **0830-1000**
Lab Haematology 1 ***Bayside 202/203***

Advances in the Phenotypic Analysis of Haematological Neoplasms

Wendy N Erber
Pathology and Laboratory Medicine, University of Western Australia, Crawley, WA, Australia

Immunocytochemistry and flow cytometry are phenotypic techniques that utilise antibodies (monoclonal or polyclonal) to identify cellular antigens for the analysis of haematological malignancies. These techniques can be performed on blood and bone marrow (both aspirate and trephine) specimens. Cells of interest can be identified by their morphological appearance or light scatter properties and their phenotype determined. The major applications are determining the phenotype (lineage and stage of differentiation) of neoplastic cells, identifying disease-associated phenotypes to meet diagnostic criteria as specified by the WHO and lymphoma staging. In recent years phenotypic applications have expanded to include prognostic prediction, identifying potential immuno-therapeutic targets, rare event analysis and disease monitoring. In the past decade there has been an increase in the number and repertoire of detectable cellular molecules beyond cell lineage and differentiation stage. New targets include adhesion molecules, cell signalling and cell cycle molecules, cell enzymes and oncogene products. Phenotyping can be applied to identify products of up- or dysregulated gene products and to predict the presence of genetic abnormalities. Technical, instrumentation and software developments have made imaging flow cytometry possible. This technology incorporates standard flow cytometry with cell identification by digital image analysis: cells can be morphologically identified and targeted molecules localised within the cell of interest. Another phenotyping technique with increasing relevance in the assessment of haematological malignancies is fluorescence immunophenotype and interphase cytogenetics as a tool for investigation of neoplasms (FICTION). The simultaneous assessment of phenotype and genomics enables specific chromosomal abnormalities (assessed by FISH) to be assessed in cells identified by their antigen expression profile (using immunofluorescent labelling). These and other novel developments in the cellular analysis of haematological neoplasms based on phenotype will be presented.

Monday 31 October **0830-1000**
Lab Haematology 1 ***Bayside 202/203***

Staging Bone Marrow in Non-Hodgkin Lymphoma

Dipti Talaulikar
Department of Haematology, Canberra Hospital, ACT, Australia

Follicular lymphoma (FL) and Diffuse Large B-cell Lymphoma (DLBCL) are the commonest subtypes of Non-Hodgkin Lymphoma. Bone marrow (BM) involvement is one of the factors considered in determining prognostic indices such as International Prognostic Index for DLBCL and Follicular Lymphoma International Prognostic Index for FL. A major limitation of relying on light microscopic diagnosis of bone marrow involvement is poor precision. Ancillary studies performed on staging bone marrows are therefore used to aid histological diagnosis including immunophenotyping using flow cytometry on BM aspirates and immunohistochemistry on BM trephines. Additionally, cytogenetic and molecular studies can also be performed as required. The clinical implications of the increased sensitivity of detection of bone marrow involvement are often unknown, particularly as ancillary investigations demonstrate a lack of concordance with histological diagnosis to a varying degree depending on the subtype of lymphoma. The evidence for the routine clinical use of these ancillary tests in the initial staging of subtypes of Non-Hodgkin Lymphoma will be discussed including the possible implications of the routine use of these investigations on prognostic indices.

Monday 31 October **0830-1000**
Lab Haematology 1 ***Bayside 202/203***

Current Trends in the Laboratory Monitoring of Monoclonal Gammopathies

Peter Mollee
Pathology Queensland, Princess Alexandra Hospital, Brisbane, Qld, Australia

Plasma cell disorders such as myeloma, MGUS, AL amyloidosis and Waldenstrom's macroglobulinaemia are characterised by the production of monoclonal intact immunoglobulin or free light chains which can be measured in the serum and urine. While new tests such as flow cytometry, molecular assays, bone turnover markers and bone lesion imaging are being investigated, serum and urine protein electrophoresis and immunofixation and serum free light chain assays remain the backbone of disease diagnosis and monitoring. This session will summarise recent recommendations for the standardised reporting of protein electrophoresis in Australia and New Zealand which may impact local laboratory reports. Rationale for the optimal report formats and the cumulative reporting of results will be reviewed and illustrative examples will be given. Multiple guidelines regarding the diagnosis and monitoring of the plasma cell dyscrasias have recently been produced. The evidence outlining the role of protein electrophoresis and serum free light chain assay in this process will be outlined. Clinicians should be aware of advantages as well as clinical and laboratory pitfalls of these techniques. In particular, the advantages and disadvantages of the serum free light chain assay and newer generation free light chain assays will be reviewed. The influence of renal impairment and inflammatory disorders on the serum free light chain assay as well as laboratory issues that can occasionally produce misleading results will be discussed.

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **1030-1130**
HSANZ Symposium 5: Myelodysplastic Syndromes ***Auditorium B***

Gene Mutations and SNP Analysis in the Diagnosis and Prognosis of Myelodysplastic Syndromes

Ghulam Mufti

Abstract not received at time of going to print

Monday 31 October **1030-1130**
HSANZ Symposium 5: Myelodysplastic Syndromes ***Auditorium B***

Myelodysplastic Syndromes – How Can We Build on Hypomethylating Agents?

Melita Kenealy
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Azacitidine is now established as standard of care for patients with higher risk myelodysplastic syndromes (MDS), with improved responses and overall survival when compared to conventional care regimens. Optimising single agent hypomethylating therapy requires careful patient selection and consideration of comorbidities, and a greater understanding of mechanism of action to enable establishment of an informative early biomarker of response and optimal formulation, dose and scheduling. In addition, the role of demethylating agents in the context of allogeneic transplant needs to be further explored. It is likely that combination strategies will be utilized to maximize efficacy of hypomethylating agents. A number of novel agents are currently in clinical trials as single agent or in combinations for high risk MDS. These include a range of histone deacetylation inhibitors (HDACi), immunomodulatory agents such as lenalidomide or thalidomide, clofarabine and thrombopoietin mimetics. The rationale for these agents and combinations, and clinical results to date will be described. Finally, it is well recognized that the prognosis of high risk patients after azacitidine failure is poor, with a median overall survival under 6 months, so novel agents and combinations are required in this setting.

No conflict of interest to disclose

Monday 31 October **1030-1130**
ANZSBT Symposium 5: Complications of Transfusion – Human Error Issues ***Auditorium A***

Background to Errors – The Psychology of Errors

Shelly Jeffcott

Abstract not received at time of going to print

Monday 31 October **1030-1130**
ANZSBT Symposium 5: Complications of Transfusion – Human Error Issues ***Auditorium A***

The Key Incident Monitoring and Management System ("KIMMS") External Quality Assurance Program for Pre-analytical Pathology Incident Monitoring

Penny Petinos[1], Ken Sikaris[1, 2]
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2 Melbourne Pathology, Private Bag 5, Collingwood Vic 3066, Australia.

Pathology in Australia has been a leader in the introduction of accreditation and quality assurance for pathology laboratories to demonstrate competence and continual improvement.

Recent studies in many countries however, have shown that the majority of adverse patient incidents occur in the non-analytical phase of the test-request-report cycle. In order to minimise the risk of errors and incidents in pathology, the pre- and post-analytical phase of testing need to be measured and monitored.

KIMMS aims to monitor the pre- and post-analytical phase of the laboratory quality system. The program is designed to provide pathology practices with the tools for continuous measurement and monitoring of key incident indicators. KIMMS offers participating laboratories a mechanism for external peer comparison. Data collection is based on various categories which allows for comparison, not only within your organisation but also against other participating organisations and laboratories.

Reports are sent to participants after each 3 month data collection cycle, allowing review of performance and a focus on areas where improvement may be needed. It also allows participants to track their incidents over a period of time to see if improvement measures that have been introduced are helping reduce errors. In 2011 a Harm / Risk matrix has been introduced which will allow participants to focus on areas with the greatest risk.

By highlighting the areas in both the pre- and post-analytical areas of pathology where improvements can be implemented and a "**get it right the first time**" attitude prevails, patients will receive a much greater level of care, by providing the correct results on the correct patient. This will in turn free up time and money in Pathology and allow a greater focus on patient care.

RCPA QAP thanks the Department of Health & Ageing for supporting this project.

Monday 31 October **1030-1130**
ASTH Symposium 5: Stop the Clot – Prophylaxis for Inpatients ***Bayside 204***

VTE Prophylaxis in the Hospital Setting

AK Kakkar

Abstract not received at time of going to print

Monday 31 October **1030-1130**
ASTH Symposium 5: Stop the Clot – Prophylaxis for Inpatients ***Bayside 204***

Prevention of Venous Thromboembolism Using Anticoagulants in Orthopaedic Surgery. Prevention Focus of Next Generation Anticoagulants in Japan

Takeshi Fuji
Osaka Koseinenkin Hospital, Osaka, Japan

Patients undergoing orthopaedic surgery are at high risk of venous thromboembolism (VTE). Without thromboprophylaxis, deep vein thrombosis (DVT) is reported to occur approximately 40% to 60% following total knee arthroplasty (TKA), total hip arthroplasty (THA) and hip fracture surgery (HFA).Therefore, the administration of appropriate pharmacologic prophylaxis for VTE by risk stratification in orthopaedic surgery has been recommended by several guidelines for thromboprophylaxis.

As anticoagulants in prophylaxis for VTE, the indirect factor Xa (FXa) inhibitor, fondaparinux, and low-molecular-weight heparin (LMWH), enoxaparin, have been mainly prescribed. We have the placebo controlled clinical experiences of enoxaparin and fondaparinux in Japanese orthopaedic surgery. However, these anticoagulants require parenteral administration and more convenient ones, which are oral agents with a rapid onset of action, predictable pharmacokinetics and pharmacodynamics, and a low propensity for food or drug interactions, have been sought.

Currently, new oral anticoagulants are being developed and several oral anticoagulants are in advanced stages and approval process. Dabigatran (direct thrombin inhibitor) and Rivaroxaban (direct FXa inhibitor) have already been marketed in orthopaedic surgery in the EU and the US. Edoxaban, which is developed by DAIICHISANKYO, is an oral direct FXa inhibitor and has been approved by thromboprophylaxis for VTE following TKA, THA and HFS in Japan this year.

Edoxaban has a rapid onset of action with a t-max of 1-3 hours, and predictable and linear pharmacokinetics across the dose range. Edoxaban demonstrated well thromboprophylaxis for VTE and the incidence of major and clinically relevant non-major bleeding was similar compared to enoxaparin.

The purpose of this paper is critically reviewing the available clinical data with regard to the newer oral anticoagulants. Particularly, we would like to introduce the comparison to the design and results of each clinical trial, STARS (Edoxaban), RECORD (Rivaroxaban) and ADVANCE (Apixaban).

Monday 31 October **1030-1130**
Nurses Free Communications 1 ***Bayside Terrace***

O037 1030

Innovative Practice in Haematology – A Nurse Led Chronic Transfusion Program

Jacqui Jagger, Clare Harris
Cancer Services, Central Coast Local Health Network, Gosford, NSW, Australia

Aim
To improve coordination and access to services for patients with a diagnosed haematological condition requiring chronic transfusion support.
Background
Prior to 2009 patients requiring regular blood product transfusion for Myelodysplasia (MDS) or Myelofibrosis (MF) were managed between haematologists, GPs, secretaries and clinic staff. The fragmented service often resulted in patients not attending for, or not receiving, their booked transfusion. Reasons for not attending were due to poor communication, incorrect timing of pathology or the patient had already received the transfusion via an emergency department/inpatient following deterioration in condition.
Method
In July 2009 the Haematology Cancer Nurse Coordinators mapped the flow of MDS/MF patients requiring regular transfusions throughout Gosford/Wyong Hospitals. Consultation of key stakeholders, including patients, occurred to identify the problems. In October 2009 the Chronic Transfusion and MDS nurse led clinic (CTMDS NLC) commenced. The service aimed to provide transfusions to patients with MDS/MF in a timely fashion, an appropriate setting and to maintain a pre-transfusion haemoglobin suitable to each patient. The service would be firmly patient centred ensuring appropriate assessment/referral for ongoing needs as functional ability and medical condition changed.
Result
The service has resulted in a marked reduction in hospital admission for transfusions, improved occupancy of clinic resources and individualised care of 40 patients with deteriorating medical conditions.
Conclusion
Providing a service to the specific needs of a patient group can have far reaching positive results. Within the present day climate of bed pressures, limited finances and outpatient resources, a nurse-led chronic transfusion service can more appropriately utilise existing resources and stream line the patient journey.

No conflict of interest to disclose

Monday 31 October **1030-1130**
Nurses Free Communications 1 ***Bayside Terrace***

O038 1045

Expanding Nursing Roles in Transfusion into Rural Areas

Jo Perillo, Lisa Stevenson, Linley Bielby and Erica Wood on behalf of the Blood Matters Program
Blood Matters Program, Quality, Safety and Patient Experience Branch, Department of Health, Victoria and Australian Red Cross Blood Service, Victoria

Background
The Blood Matters program is a collaborative undertaken by the Department of Health (the department) and the Australian Red Cross Blood Service. Approximately one third of all red cells are used to treat cancer and blood disorders, and the program receives support from the Victorian Cancer Action Plan to fund roles to assist with developing the cancer workforce and improving the transfusion care of patients in regional Victoria. This role is known as Transfusion Trainer (TT).
Aim
To describe the development and the introduction of the TT role into 25 rural hospitals in Victoria and identify similarities and differences of this role to the Transfusion Nurse (TN) role based in metropolitan or regional hospitals.
Method
Hospitals were identified as being eligible for funded positions based on numbers of transfusion events over a 12 month period and complexity of care. TT position descriptions were developed; TTs were oriented to the role, given a resource toolkit and have been supported with education forums and a virtual network amongst themselves and TNs. They have now been employed for 12 months and an evaluation of their achievements was performed against key performance indicators (KPIs), the major one relating to education within their hospital. TTs were surveyed to identify expectations of the role, barriers and challengers faced and overcome, and where support and education was sourced.
Result
All TTs participated in the evaluation and all had met KPIs set in their position descriptions. All TTs and TNs were invited to participate in a survey to compare their responses to role expectation, challenges and education perceived necessary and support networks utilised to support their role and development.
Conclusion
The development and introduction of the TT role has developed in the rural setting by improving staff education, reporting and participating in quality activities by meeting KPIs. Similarities and differences between the TT and TN role have been identified.
No conflict of interest to disclose

Monday 31 October **1030-1130**
Nurses Free Communications 1 ***Bayside Terrace***

O039 1100

A Safer Place for Jehovah's Witnesses

Helen Atkinson
Royal Hobart Hospital, Hobart, Tasmania, Australia

Background
The accidental transfusion of blood products to Jehovah's Witness (JW) has occurred at RHH. In 2011, Red Alerts and religious fields were removed from identification labels, making bedside identification more difficult.
Aim
To analyse the cause of these incidents and develop and strategies to reduce them.
Method
All incidents were reviewed. Meetings were held with key stakeholders to discuss potential strategies for improvement.
Results
Key factors noted in the incidents included: (1) The majority of incidents occurred with blood products that patients did not recognise as "blood". (2) The consent policy only included fresh blood products. (3) Alerts were missing from patient histories. (4) Many assumptions were made by both JW patients and hospital staff regarding Jehovah's Witnesses and blood transfusions.
Followup
The following measures were implemented:
1) Change to the Consent Policy and Form.
2) Daily checking of admission lists and adding alerts to the records.
3) Consultation with the JW representative re consent to access records. This involved accessing histories of persons not in hospital. Over 300 consents were received and history review commenced. This revealed that two-thirds of this population have no alerts.
4) Increased education for staff and JW population
5) Access by Jehovah's Witness patients to Transfusion Nurse.
Discussion
Incident review indicated multiple contributing factors. The multipronged preventative approach has involved patients, the Jehovah's Witness community, the TNC and hospital staff: ensuring the consent form covered all blood derived products; increased staff education about Jehovah's Witnesses; involvement of the Jehovah's Witness community; proactive updating of alerts; and increasing the opportunity for Jehovah's Witnesses to see the Transfusion Nurse Consultant.
Conclusions
Success will be measured by the decrease in adverse events. Improved relationships between the Transfusion Nurse Consultant and the JW representative will allow increased staff and Jehovah's Witnesses engagement and information and knowledge gained will encourage hospital staff in the use of non blood alternatives in all patients which will improve overall transfusion safety.
No conflict of interest to disclose

Monday 31 October **1030-1130**
Nurses Free Communications 1 ***Bayside Terrace***

O040 1115

Keeping the Faith: Successful AML Induction Without Blood Transfusions or Religious Compromise in a Jehovah's Witness

Yvonne Gonet
Haematology Royal North Shore Hospital, Sydney, NSW, Australia

Granulocytic sarcomas are rare extramedullary tumours consisting of primitive granulocytic cells. They may evolve "de novo" or be associated with other haematologic disorders such as acute myeloid leukaemia (AML) myelodysplasia or myeloproliferative disorders.

A young male aged 19 years with Downs Syndrome presented with acute abdominal pain thought to be due to small bowel obstruction. Laparotomy revealed an intestinal Granulocytic Sarcoma, with five of eleven mesenteric lymph nodes infiltrated with tumour. Postoperative leukaemic staging was completed and a bone marrow biopsy demonstrated 20% myeloblasts.

Due to intellectual impairment this young man was completely dependent upon his family (assessed chronological age: 5-6 years). The whole family followed the teachings of Jehovah's Witness (JW) and despite wanting curative-intent treatment for their son, directed that **no** blood products were to be administered during the AML therapy.

Induction chemotherapy for AML is predictably toxic and a clinically precarious time for the patient requiring supportive care in the form of antibiotics and blood transfusions. The use of any blood product (including autologous) is fundamentally contra-indicated in the JW belief structure.

Taken into account the challenges of this case, I will describe how the treating team collaborated to evolve some innovative strategies for the holistic and safe care of this young man whilst in the haematology unit. Forward planning with pre-admission iron infusions, the use of colony stimulating factors (CSF), EPO, Peg CSF, vitamins and limiting the number and volume of blood tests resulted in a successful AML induction followed by two consolidation therapies without ANY red cell transfusions.

No conflict of interest to disclose

Monday 31 October **1030-1130**
APBMT Symposium 5: Immunotherapy & Gene Therapy ***Bayside Gallery B***

Functional Subsets of Human CD8$^+$ T-cells: Implications for Adoptive T-cell Therapy of Cancer

Stanley R Riddell, Carolina Berger, Michael Hudecek, Cameron J Turtle
Fred Hutchinson Cancer Research Center, Seattle, WA, USA

A variety of phenotypic subsets of CD8$^+$ T cells are present in peripheral blood, and this heterogeneity ensures appropriate responses to neo-antigens and to antigens to which the host has been previously exposed. The extraordinary specificity of T cells makes them an attractive cancer therapeutic. However, the rarity of tumor-reactive T cells in cancer patients, the difficulty isolating them in sufficient numbers for adoptive immunotherapy, and the unpredictable persistence of transferred cells have been obstacles to broad application. Technologies that enable genetic modification of T cells have been refined and are being used to redirect the specificity of T cells to tumor antigens. An issue is how the phenotypic and functional heterogeneity in T cells that could potentially be genetically modified might be capitalized upon to enhance the efficacy and safety of cancer immunotherapy. CD8$^+$ T cells can be broadly divided into CD45RA$^+$ naïve T cells (T_N) that contain the greatest T cell receptor diversity, and express CD62L$^+$ and CCR7$^+$ to enable their transit through lymph nodes; and CD45RO$^+$ memory T cells that have clonally expanded in response to antigen, and can be subdivided into CD62L$^+$ central memory (T_{CM}) and effector memory (T_{EM}) subsets. In humans, the CD45RO T_{CM} and T_{EM} subsets have recently been shown to also contain major populations of CD161^{hi}, IL-18Ra^{hi} T cells that differ dramatically in function, gene expression profile, and specificity from CD161^{lo} counterparts. All of these subsets can be genetically retargeted to recognize tumor cells, however studies in vitro and in animal models demonstrate profound differences in the functional properties of T cells from each of these subsets and in their ability to eradicate tumors. These results will be discussed in the context of efforts to use genetically modified T cells for cancer immunotherapy in the clinic.

Monday 31 October **1030-1130**
APBMT Symposium 5: Immunotherapy & Gene Therapy ***Bayside Gallery B***

Haematopoietic Stem Cell and T-cell Delivered Gene Therapy for HIV

Geoff Symonds
St Vincent's Centre for Applied Medical Research and Calimmune, St Vincent's Hospital

HIV is a disease not solved and current anti-retroviral therapy has side effects that can be severe. An alternate approach is described that uses haematopoietic stem/progenitor cells (HSPC) and/or CD4+ T-cells to deliver anti-HIV genes. These introduced cells will then repopulate the individual's immune system to supply a population of cells resistant to infection and the cytopathic effects of HIV. Results will be presented showing proof of principle of this approach in tissue culture and an animal model, where HIV replication is inhibited and cell viability increased. The major active principle that we are using is a short hairpin RNA to the important HIV co-receptor, CCR5. This approach mimics a natural mutation found in approximately 1% of Caucasians which functionally deletes CCR5 and results in resistance to HIV infection.

Monday 31 October **1030-1130**
Lab Haematology 2 ***Bayside 202/203***

FISH in Haematological Malignancies

Lynda J Campbell
Victorian Cancer Cytogenetics Service, St. Vincent's Hospital, Fitzroy, VIC Australia

Fluorescence in situ hybridization (FISH) has become an important component of cytogenetic analysis. It plays a critical role in several areas. 1) As an adjunct to conventional cytogenetics to clarify chromosome abnormalities observed by G-banding, it is particularly useful to unravel complex variants of common translocations such as a masked or three-way Philadelphia translocation in chronic myeloid leukaemia or with subtle rearrangements in the setting of poor chromosome morphology: for example, the inversion 16 in acute myelomonocytic leukaemia or MLL translocations in myeloid disorders. 2) FISH can identify cryptic abnormalities that cannot be visualized by conventional cytogenetics: the t(12;21) that is a common finding in paediatric acute lymphoblastic leukaemia, the deletion of 4q12 resulting in *FIP1L1-PDGFRA* fusion in hypereosinophilic syndromes or the t(4;14) that is an indicator of poor prognosis in plasma cell myeloma. 3) FISH can also be used to determine the presence of non-cryptic abnormalities when dividing cells are not available, such as when cultures fail to produce metaphase spreads or when culturing of cells is not feasible as the only tissue available has been embedded in paraffin. The identification of an exceptionally poor prognosis class of lymphomas with "double-hit" translocations has increased markedly the demand for FISH for the presence of *MYC*, *BCL2* and *BCL6* translocations in lymphomas. 4) FISH is being used extensively to detect the presence of copy number abnormalities in chronic lymphocytic leukaemia and plasma cell myeloma to determine prognosis: loss of TP53 has been shown to indicate a poor prognosis in both disorders and loss of ATM in CLL is also an indicator of poor prognosis. 5) Finally, using probes that detect the presence of X- and Y-chromosomes, FISH is an effective method of establishing the presence of engraftment in the setting of a sex-mismatched stem cell transplant. FISH is thus an integral part of the testing algorithms for an ever increasing number of haematological malignancies.

Monday 31 October **1030-1130**
Lab Haematology 2 ***Bayside 202/203***

Does Flow Cytometry Have a Role in Myelodysplastic Syndromes?

Kate Burbury
Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia

Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms — biologically and clinically — characterised by dysmaturation, ineffective hematopoiesis and genetic instability, with a propensity for leukemic transformation. With the increasing availability of effective disease-modifying therapies, efforts to develop treatment strategies require standardised criteria for diagnosis, classification and prognosis as well as the ability to effectively evaluate treatment response.

This has been achieved in part by the revised 2008 WHO classification; the development of prognostic scoring systems, such as the international prognosis scoring system (IPSS) and the WHO classification-based prognostic scoring system (WPSS); the consensus guidelines outlining both refined definitions and standards in the diagnosis of MDS; as well response assessment criteria. Conventional criteria, in particular haematological and morphological, however, are inherently subjective lacking both sensitivity and specificity. Although cytogenetics is more objective, it is specific in only a minority and cannot focus on cellular subpopulations.

Multiparameter flow cytometry (FC), however, can be used real-time, is a highly reproducible and objective way of assessing the pattern of expression of multiple antigens on defined subpopulations. By comparing antigen expression with that identified on the equivalent normal cell populations, abnormalities can provide a diagnostic indication of clonality or at least stem cell dysmaturation. With the capacity to provide a more objective and dynamic assessment, FC can not only contribute to the refinement of the diagnosis, sub-classification and prognostic stratification but also and importantly, sequential assessment after therapeutic intervention.

There is now increasingly robust data demonstrating the capacity of FC to discriminate MDS from non-clonal cytopenias and dysplasia, as well as contributing to refinement of disease classification and prognostication. Recognising this, the consensus guidelines for the diagnosis of MDS has included FC as co-criterion. This data and the potential role of FC in MDS will be reviewed.

No conflict of interest to disclose

Monday 31 October **1130-1230**
Carl de Gruchy Oration ***Auditorium B***

From Alexandria to Melbourne: A Voyage of Discovery

Hatem Salem

Abstract not received at time of going to print

Monday 31 October **1330-1500**
HSANZ ASTH Combined Presidential Symposium **Auditorium B**

O041 1330

Differential Niche and Wnt Requirements During Acute Myeloid Leukemia Progression

Steven W Lane*[1,2], Yingzi J Wang*[1,3], Cristina Lo Celso*[4,5], Christine Ragu[5], Lars Bullinger[1,8], Stephen M Sykes[5,6], Francesca Ferraro[5], Sebastian Shterental[6], Charles P Lin[7], D Gary Gilliland[6,9], David T Scadden[5], Scott A Armstrong[1], David A Williams[1]

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**These authors contributed equally to this work*

Aim

Haematopoietic stem cells (HSC) engage in complex bidirectional signals with the haematopoietic microenvironment and there is emerging evidence that leukaemia stem cells (LSC) may utilize similar interactions. We used a syngeneic retroviral model of MLL-AF9 induced acute myeloid leukaemia (AML) to evaluate LSC-microenvironment interactions in vivo.

Methods

Retroviral transduction of haematoietic stem and progenitor cell populations was used to generate acute myeloid leukaemia in vivo and in vitro. In vivo homing to the haematopoietic microenvironment was determined by live 3-dimensional confocal microscopy. Statistical analysis included Student's t-test (2 tailed) and Kaplan-Meier survival analysis.

Results

Two different stages of leukaemia progression were identified. The first was propagated by "pre-LSC" comprised of immortalized haematopoietic stem and progenitor cells that give rise to leukaemia in vivo with variable latency, presumably due to the gradual accumulation of additional genetic hits. In contrast, LSC derived from mice with established leukaemia give rise to fully penetrant, short onset leukaemia in secondary recipients. The homing properties of these distinctive entities were compared to that of normal HSC and progenitors. The homing and micro-localization of pre-LSC was most similar to long-term HSC and was dependent on cell-intrinsic Wnt signaling. In contrast, the homing of established LSC was most similar to that of committed myeloid progenitors and distinct from the pattern observed with HSC. While osteoblast-derived Dickkopf-1 (Dkk1), a potent Wnt inhibitor known to impair HSC function, dramatically impaired normal HSC localization within the bone marrow, it did not affect pre-LSC, LSC homing or in vivo AML disease latency or penetrance.

Mechanistically, cell-intrinsic Wnt activation was observed in human and murine AML samples, explaining the independence of MLL-AF9 LSC from niche-derived Wnt signals.

Conclusion

These data identify differential engagement of the haematopoietic microenvironment associated with leukaemic progression and identify a LSC niche that is physically distinct and independent of the constraints of Wnt signaling that apply to normal HSC.

SWL, CLC, YW, CR, LB, FF, SMS, SS, CPL, SAA and DAW have no relevant conflicts of interest to declare. D.T.S is a shareholder of Fate Therapeutics and consultant for Genzyme, Hospira and Fate Therapeutics. D.G.G. is now an employee of Merck.

Monday 31 October **1330-1500**
HSANZ ASTH Combined Presidential Symposium **Auditorium B**

O042

1345

GATA2 is a New Predisposition Gene for Familial Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML)

Hamish Scott,[1,2,3,4], Christopher Hahn,[1,2,3], Catherine Carmichael[4], Ella Wilkins[4,5], Chan-Eng Chong[1,2], Peter Brautigan[1,2], Xiao-Chun Li[1,2], Milena Babic[1,2], Ming Lin[1,2], Amandine Carmagnac[4], Carolyn Butcher[6,7], Kathryn Friend[8], Paul Ekert[9], Chung Kok[6,7], Anna Brown[2,7], Ian Lewis[2,3,7], Bik To[2,3,7], Andrew Timms[10], Jan Storek[11], Sarah Moore[1], Meryl Altree[12], Robert Escher[4,13], Peter Bardy[7], Graeme Suthers[12], Richard D'Andrea[2,3,6,7], Marshall Horwitz[10]

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Aim

Autosomal dominant familial predisposition to MDS/AML can be mediated by mutations in the transcription factor genes, *RUNX1* and *CEBPA*, but it is clear that other genes await discovery. Identification of these genes will help in the diagnosis, prognosis and treatment of both familial and sporadic MDS/AML.

Method

Candidate gene sequencing was used to identify germline mutations in families predisposed to MDS/AML. Functional studies were then performed to ascertain those "hallmarks of cancer" (*e.g.* proliferation, differentiation, survival) perturbed by the mutations, and the mechanism of action.

Result

We identified novel mutations in the *GATA2* gene in 4 MDS/AML families. A novel p.Thr354Met mutation was observed in 3 families and a p.Thr355del mutation in 1 family, all of which segregated with multigenerational transmission of MDS and/or MDS/AML. These mutated threonines reside in the DNA-binding, protein-protein interacting second zinc finger (ZF2) of GATA2. Pathogenic *GATA2* coding mutations were not seen in 695 normals or 268 sporadic MDS/AML patients or germline samples from 35 other families predisposed to AML and various other hematological malignancies. Structural modeling demonstrated that both p.Thr354Met and p.Thr355del disturb the ZF2. Consistent with this, DNA binding and reporter transactivation ability was reduced for both mutations. In stable promyelocytic cell lines expressing regulatable GATA2 (WT, p.Thr354Met or p.Thr355del), p.Thr354Met alone inhibited differentiation and apoptosis while allowing proliferation even under the powerful stimulus to differentiate with all-*trans* retinoic acid. Various functional assays including microarrays showed these mutations are mainly loss-of-function mutations but may be dominant negative in some contexts.

Conclusion

Discovery of *GATA2* mutants in MDS/AML predisposed families provides new tools for probing the mechanism of GATA2-induced leukaemogenesis, and clarifying its role in "stemness" maintenance of haematopoietic stem cells. It may also facilitate more effective diagnosis, prognosis, counselling, selection of related bone marrow transplant donors, and development of therapies.

No conflict of interest to disclose

Monday 31 October **1330-1500**
HSANZ ASTH Combined Presidential Symposium **Auditorium B**

O043 1400

Diagnosis of Amyloidosis Subtype by Laser-Capture Microdissection (LCM) and Tandem Mass Spectrometry (MS) Proteomic Analysis

Patricia Renaut[1], Peter Mollee[2], Samuel Boros[1], Dorothy Loo[3], Michelle Hill[3]
1 Anatomical Pathology Dept, Pathology Queensland, Princess Alexandra Hospital, Brisbane, QLD, Australia. 2 Haematology Dept, Pathology Queensland, Princess Alexandra Hospital, Brisbane, QLD, Australia. 3 The University of Queensland Diamantina Institute, Brisbane, QLD, Australia

Aim
Correct identification of the protein that is causing amyloidosis is absolutely crucial for clinical management. Current diagnostic methods, including antibody-based amyloid typing, have limited ability to detect the full range of amyloid forming proteins. We assessed a new technique combining specific sampling of amyloid deposits by LCM and analysis of tryptic digests by tandem MS proteomic analysis.
Methods
We studied five cases of well characterised amyloidosis and two cases in which the amyloid subtype was unable to be diagnosed with confidence. For all specimens, 10µm sections of formalin-fixed paraffin embedded tissue were stained with Congo Red using a standard technique. LCM was performed using an Arcturus XT instrument with an infrared capture laser. Fixation of proteins was reversed using FFPE Protein Extraction Solution (Agilent) with heating. Proteins were digested with trypsin and peptides were analysed by nano-liquid chromatography-coupled tandem mass spectrometry using a Chip CUBE-QTOF. Database searching was performed using Spectrum Mill (Agilent) with the NCBInr human protein database.
Results
The subtype of amyloidosis was able to be determined in all seven cases analysed. Proteins identified included immunoglobulin light chain (localised amyloid n=1, systemic AL n=2), transthyretin (senile amyloid n=1, hereditary ATTR n=2) and serum amyloid A2 (AA n=1). One of the diagnostically challenging cases had: extensive gastrointestinal amyloidosis and no evidence of clonal light chain disease; negative kappa, lambda, SAA and transthyretin immunohistochemistry; and negative genetic studies for known hereditary variants. Tandem MS revealed the amyloid to be of immunoglobulin lambda light chain type. The second diagnostically challenging case had: isolated renal amyloidosis; positive AA stain, equivocal kappa and lambda stains; and the presence of kappa restricted serum light chains. Tandem MS revealed the amyloid to be composed of serum amyloid A2 protein.
Conclusion
Use of LCM and tandem MS is likely to improve the ability to correctly type amyloid deposits in clinical biopsy samples. It may become the new “gold standard” diagnostic technique.
No conflict of interest to disclose

Monday 31 October **1330-1500**
HSANZ ASTH Combined Presidential Symposium **Auditorium B**

O044

1415

The Detection of More Than One Low-Level BCR-ABL1 Mutation in Individual Imatinib-Resistant CML Patients Predicts Poor Response to Second-Line Tyrosine Kinase Inhibitor Therapy, Regardless of the Resistance Profile of the Mutations

Wendy Parker[1], Musei Ho[1], Hamish Scott[1,2,3], Timothy Hughes[2,4], Susan Branford[1,2]
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Aim
Specific imatinib-resistant BCR-ABL1 mutations also confer clinical resistance to nilotinib and/or dasatinib (i.e. T315I, Y253H, F317L and others); therefore mutation analysis is recommended for chronic myeloid leukemia patients after imatinib failure to facilitate selection of appropriate therapy. However, over 30% of patients without these nilotinib/dasatinib resistant mutations also fail second-line therapy. We investigated the impact of having >1 mutation (multiple mutations) on subsequent response to nilotinib or dasatinib in imatinib-resistant patients.
Method
Mutation analysis was performed by direct sequencing (detection limit 10-20%) and sensitive mass-spectrometry (31 mutations; detection limit 0.05-0.5%) in samples of 220 imatinib-resistant patients collected before commencing nilotinib/dasatinib. We previously demonstrated that detection of low-level nilotinib/dasatinib resistant mutations by mass-spectrometry was associated with failure of these inhibitors, therefore patients with these mutations detected by either method were excluded because response is already known to be poor in these cases (n=45).
Results
Multiple nilotinib/dasatinib sensitive mutations were detected in more of the 175 remaining patients by mass-spectrometry (34/175, 19%; 2-9 mutations per patient) than sequencing (16/175, 9%; 2-3 mutations per patient), *P*=.009. The subsequent response of patients with zero/one mutation (n=141) was compared to patients with multiple mutations (n=34). Multiple mutations were associated with lower rates of complete cytogenetic response (21% versus 50%, *P*=.003) and major molecular response (6% versus 31%, *P*=.005), and a higher incidence of acquiring new nilotinib/dasatinib resistant mutations (56% versus 25%, *P*=.0009).
Conclusion
Sensitive mutation analysis using an assay that can simultaneously detect large numbers of BCR-ABL1 mutations identified a poor-risk subgroup with multiple mutations not identified by standard direct sequencing. These patients would not otherwise be classified as being at risk of poor response on the basis of their mutation status. This poor-risk subgroup may warrant closer monitoring or experimental approaches to reduce the high risk of treatment failure and progression after imatinib resistance.
No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **1330-1500**
HSANZ ASTH Combined Presidential Symposium **Auditorium B**

O045 1430

The 2.9 Å Structure of Full Length Human Glu(1)Plasminogen Reveals its Mechanism of Activation in Fibrinolysis

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[2]*Australian Centre for Blood Diseases, Monash University, Melbourne*

Aim
To understand the structural basis of plasminogen activation in fibrinolysis
Methods and Results
The physiological activation of fibrinolysis occurs at sites of vascular injury by secretion of tissue plasminogen activator (tPA). A similar mechanism is initiated within tissues by urokinase plasminogen activator (uPA) on the cell surface and is an important mechanism of cell motility. tPA and uPA cleave and activate the zymogen plasminogen to yield the potent serine protease plasmin which is the key mediator of fibrin clot breakdown or tissue matrix degradation. Full length Glu(1)-Plasminogen is a complex multi-domain molecule that comprises a N-terminal Pan-apple domain, five tandem kringle domains and a C-terminal chymotrypsin-like serine protease domain. The molecule exists in at least two conformational states, termed open and closed. Glu(1)-Plasminogen must be cleaved to yield active enzyme and the conformational state of the molecule represents an important activation control point.
We have crystallized full length human Glu(1)-plasminogen and determined the 2.9 Å resolution structure. Two molecules are present in the asymmetric unit with one of these being in the closed and the other is in the open conformation. In the closed conformation of plasminogen, lysine binding kringles 2, 4 and 5 are blocked by interaction with the Pan-apple and serine protease domains while kringle 1 remains free. Analysis of the other plasminogen molecule reveals a massive conformational change, with the structure adopting a completely different open conformation. The lysine binding sites of krigles 1,2, 4 and 5 are all now unblocked and accessible. The structure reveals the physiological mechanism of activation of Glu(1)-Plasminogen gives insight into its interaction with uPA, tPA and fibrin.
Conclusion
The structure of Glu(1)-Plasminogen has major implications for the development of new therapeutic fibrinolytic agents.

No conflict of interest to disclose

Monday 31 October **1330-1500**
HSANZ ASTH Combined Presidential Symposium **Auditorium B**

O046 1445

Development of a Microfluidic Device to Monitor Platelet Aggregation Dynamics and Function in the Context of von Willebrand Disease

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3 Haematology Department, The Alfred Hospital, Melbourne, Victoria, Australia.

Background
Haemodynamic factors are central to normal haemostasis and pathological thrombus formation. Recent seminal studies within our laboratory have identified a key role for micro-scale blood shear gradients (shear micro-gradients) in driving the earliest stages of platelet thrombus formation, independent of soluble agonists. These studies gave impetus to the development of a novel “lab-on-chip” microfluidics device that may provide rapid clinical screening of platelet function under shear micro-gradient conditions using small volumes of anticoagulated blood.
Aim
To perform a small proof-of-principle characterisation study of the microfluidic platform and to assess its ability to identify differences in platelet aggregation dynamics in anticoagulated whole blood taken from control subjects and patients with diagnosed von Willebrand disease (vWD).
Method
25-30 patients with vWD were recruited from the Alfred Hospital’s Ronald Sawers haemophilia centre. Blood from consenting donors was pre-treated with well-characterised antagonists of the platelet amplification signalling pathways and subsequently perfused at a specified flow rate through a well-defined shear micro-gradient characterised by an entry shear rate of 1,800.s-1, a peak shear rate of 50,000.s-1, returning to an exit shear rate of 1,800.s-1.
Results
Preliminary studies demonstrate that platelet aggregation within the device is strictly dependent on engagement of both the platelet adhesion receptors GPIb/V/IX and the integrin αIIbβ3, suggesting a requirement for von Willebrand factor (vWF). Comparison of normal platelet function with vWD subjects demonstrates that platelet aggregation in the device correlates directly with measured vWF antigen levels. Furthermore, addition of exogenous purified vWF was able to rescue the platelet aggregation deficit demonstrated in patients with vWD. Thus this assay provides a sensitive measure of functional vWF levels.
Conclusion
Platelet aggregation under the defined haemodynamic flow conditions within our prototype device is critically dependent on blood vWF levels, thus providing proof-of-concept supporting utility for the device as a screen for vWD.
No conflict of interest to disclose

Monday 31 October **1330-1500**
ANZSBT Symposium 6: Infectious Threats to the Blood Supply ***Auditorium A***

Hong Kong Experience with SARS and H1N1

Che-Kit Lin
Hong Kong Red Cross Blood Transfusion Service, Hong Kong SAR

SARS

During March to July 2003, Hong Kong (HK) had experienced an epidemic of Severe Acute Respiratory Syndrome (SARS), which started out as an outbreak of community acquired pneumonias of unknown aetiology. It spread rapidly throughout hospital patients and healthcare workers, with high morbidity and mortality. The outbreak has led to a boom in the art and science of infection control and health protection. Precautionary measures, such as frequent cleansing of utilities, personal hygiene and protective gears have become important. The provision of public healthcare services was re-prioritized. The pattern of blood usage changed. The demand for blood dropped 12.8% and the availability of donations decreased by 16.9% during the period. As the SARS pathogen was essentially unknown initially, we implemented a donor deferral policy based on history of contact to ensure blood safety. Our experience also showed that the application of healthcare informatics to capture SARS patient database was useful for screening donors/donations. To save patients, we had successfully collected SARS convalescent plasma for clinical treatment of those in critical condition.

H1N1

Since SARS, HK has implemented a territory-wide epidemic alert system including the necessary infection control and protective measures. Consequently, we were more effective in dealing with the H1N1 epidemics in 2009 in all aspects. The impact of H1N1 on blood donation and utilization was far less than that of SARS, although hospitals' admission had exceeded normal capacity leading to increase use of red cells. While the majority of illnesses have been mild to moderate uncomplicated disease, approximately 10% to 30% of hospitalized patients required admission to intensive care units and fatal outcomes had been reported. To help those who did not respond well to antiviral therapy and were critically ill, we launched a programme to collect and supply convalescent plasma for treatment of selected patients. Some 276 liters of the collected plasma were sent for fractionation into hyperimmune intravenous immunoglobulin for a randomized control trial.

Monday 31 October **1400-1530**
ANZSBT Symposium 6: Infectious Threats to the Blood Supply ***Auditorium A***

ICU Experience of Patients with H1N1 Influenza

Ian Seppelt

Abstract not received at time of going to print

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **1400-1530**
ANZSBT Symposium 6: Infectious Threats to the Blood Supply ***Auditorium A***

Update on Pandemic Threats

Kerry Chant

Abstract not received at time of going to print

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O047 1330

Adverse Events in Three Sibling Bone Marrow Stem Cell Donors

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Background
St. Vincent's Hospital performs approximately 10 sibling donor and 14 unrelated donor bone marrow or stem cell harvests yearly. Consistent with published literature these are generally straightforward and well tolerated by the donors. The World Marrow Donor Association (WMDA) provides detailed guidelines to protect donors' interests and regulatory bodies such as Foundation for the Accreditation of Cellular Therapy (FACT) require written criteria to ensure donor safety. Three case reports of adverse events in sibling donors are presented.

Case 1: Female peripheral blood stem cell donor, 20 years required central venous access to facilitate stem cell collection via apheresis. She developed keloid scarring at the site of the central venous catheter insertion and required referral to dermatology and injection of steroids.
Case 2: Female bone marrow donor, 63 years, had an autologous unit of blood taken 72 hours before planned bone marrow harvest this unit was unavailable due to mishap. Another unit was taken 24 hours prior to harvest, and was transfused intra operatively. However she was profoundly anaemic in the post operative phase and required intravenous iron and increased inpatient stay.
Case 3: Female bone marrow donor, 51 years, became hypotensive, anaemic and experienced vasovagal episodes and urinary retention in the post operative phase, this led to increased inpatient stay. She required 2 units of cross matched blood in addition to having an autologous unit of blood transfused intra operatively. Post operative pain was a significant issue for this donor and she required extended sick leave from work, additional medical review and analgesia.
Conclusion
Despite meeting stringent donor safety requirements and receiving optimal care stem cell or bone marrow harvest is not without risk to the donor.

No conflict of interest to disclose

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O048 1343

Attendee Satisfaction with a Multidisciplinary Model of Survivorship Care Within A Dedicated Late Effects Clinic For Long-Term Survivors Of Stem Cell Transplantation

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Aim
Stem cell transplantation (SCT) can cure haematological malignancy, however, survivorship often carries a burden of ongoing treatment-related morbidity. The Alfred Malignant Haematology and SCT Service has established an innovative multidisciplinary Late Effects Clinic (LEC) dedicated specifically to SCT survivorship (≥2 years post-SCT in ongoing remission) aimed at:

- Risk-based health review addressing physical and psychosocial concerns
- Survivor empowerment through targeted education.

This study aims to assess LEC attendee satisfaction, particularly regarding educational material provided during annual LEC review.

Method
A 12 question satisfaction survey covering demographics, overall experience, referral pathway, informational needs, and recommendations for improvement was developed and mailed to survivors attending the LEC between May 2008 and 2011. A 'reply-paid' envelope was included to facilitate return of the completed questionnaires.

Results
78 surveys were mailed and 67% completed and returned. LEC referral was most frequently by SCT coordinator (51%) or consultant (41%). 96% rated their overall LEC experience as 'good' (12%), 'very good' (37%) or 'excellent' (48%). 83% found the educational information received useful, while 94% reported it 'met their needs'. Gratifyingly, 92% of participants would recommend the Alfred's LEC to other SCT survivors. The most useful information received during LEC attendance was commonly reported as "I am not alone" and "health maintenance advice". Emerging from the questionnaire was a desire to network with other survivors to improve health outcomes. 85% indicated interest in receiving a LEC newsletter containing health check reminders and general Late Effects information. The majority of respondents had no suggestions for LEC improvement - "no - service was excellent".

Conclusion
Determining survivors' concerns and needs is critical to patient-centred care. These results validate the success of our chosen multidisciplinary care model in delivering high quality survivorship care. This survey serves to inform future innovative approaches to SCT survivor support and educational information delivery.

No conflict of interest to disclose

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O049 1356

The Challenge of Achieving and Maintaining a Zero Central Line Bloodstream Infection Rate in a Haematology / Blood and Marrow Transplant Unit

Karen Maddock
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With episode funding here to stay in Australia one of the challenges for Haematology/Bone Marrow Transplant units, whose patients are most often long stay patients, is to increase throughput and reduce the length of stay of patients. If we are to be able to do this within the finite resources available preventable central line associated blood stream infections (CLABSI) must be reduced with the aim to eliminate these altogether.
In the first six months of 2008 our service was experiencing central line blood stream infection rates of 15.07 up to 38.81 per 1000 line days this was way above the benchmark in the literature at the time. A review of the literature showed that as well as insertion “bundles of care” a uniform approach to post insertion line care resulted in significant decreases in infection rates.
The types of central venous access interlink devices available, chlorhexidine for disinfecting the hub prior to lumen manipulation, volume of fluid for flushing the lumens were reviewed and new practices /devices were adopted. A cancer services specific central line care protocol was written and implemented and team of motivated nurses enlisted to assist the implementation.
The above interventions have resulted in a marked decrease in catheter related blood stream infections (CRBSI).The unit has achieved infection rates of between zero and 3.6 in the last twelve months the challenge now is to achieve and maintain a zero infection rate permanently.

No conflict of interest to disclose

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O050 1409

A Practice Variation of Hematopoietic Stem Cell Transplantation (HCT) Nursing in Japan

Sakiko Kondo[1], Naomi Numa[2], Kumiko Takasaka[3], Tatsuo Ichinohe[4], Kunio Yano[5], Takahiro Fukuda[6], Takanori Teshima[7], Shinichiro Okamoto[8] for the Japan Society for Hematopoietic Cell Transplantation
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Background
Significant changes in the field of HCT necessitated updating the 2000 JSHCT guideline regarding infection control early after HCT. Published studies also continue to add to the evidence regarding supportive care. Identifying gaps in the literature and inconsistencies in HCT practices is an important first step in revising the guideline that can be used to standardize practice and link best practices to improved patient outcomes.
Objectives
This cross-sectional descriptive survey aimed to identify practice variation in HCT nursing in Japan.
Methods
Questionnaires were developed with regard to infectious control practices across stem cell transplantation setting. In this survey we focused on protective environment, education of patients and care givers, food safety, and management of CV line. The questionnaires were sent to 96 transplant teams which were selected by taking account of its location, size of HCT, and patient population (adult vs. pediatric). Fifty one teams (53%) responded.
Results
Descriptive statistics revealed minimal practice variation regarding the management of CV line, educating HCT recipient and their care givers, and the screening of both staffs and visitors for contagious infections. Practices regarding implementation of restrictions on patients' hygiene, diet, and social interactions varied by the teams and the phase of HCT. More than 50% of teams allowed recipients to take vegetables and fruits early after HCT. Regarding the personal protective equipment, more than 50% of teams still maintained the strict reverse isolation technique using gown and surgical masks irrespective of the type of HCT.
Conclusions
Although published standards are under consideration, practice variation exists across transplantation centers. This suggests that HCT nurses should take an active role in refining current HCT practice.

No conflict of interest to disclose

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O051 1422

Outcomes in Older Patients Undergoing Autologous Stem Cell Transplantation: Experience at Peter Mac

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Introduction
Autologous stem cell transplantation (ASCT) is part of standard therapy for patients under the age of 65 years who are medically fit, with multiple myeloma (MM), relapsed/refractory lymphoma. With the increasing age and more therapeutic options, the number of patients presenting for ASCT over 65 years of age has and is likely to continue, to increase. ASCT poses a set of unique challenges in the elderly patients because of the presence of co-morbidities and reduced physiologic reserve. We present our experience of delivering ASCT to patients over the age of 70 years with myeloma and lymphoproliferative disorders.
Method
We identified 27 patients ≥70 years and 74 patients 65-69 years of age (as a comparator cohort) who had an ASCT performed at PeterMac between 1999-2011. Retrospective data regarding patient and disease characteristics, as well as transplant-related outcomes were recorded. Safety and efficacy outcomes in those patients ≥70 were compared to the comparator cohort.
Results
Of those ≥70 years: 21 were male, median age 73 years (range 70-79). 15 had MM and the remainder lymphoproliferative disorders. The conditioning regimens included Melphalan, StanBCNU, BEAM, TBI-based. Median CD34+ progenitors reinfused were 4.60 x 10^6/kg. (range: 2.06 – 11.48). All patients experienced febrile neutropenia and there were two transplant related mortalities, Day +29 and Day + 84. Median days to neutrophil and platelet recovery was 11(range: 8-23) and 12.5 (range: 9-23) respectively. Median inpatient days was 18.5 (range: 12-79)
Implications
ASCT in patients ≥70 years did not result in excess mortality, delayed engraftment or longer inpatient stays. This small retrospective study suggest that ASCT in older patients is feasible and safe, however further studies are required, that include this age cohort, to further define the role of ASCT in the elderly.

No conflict of interest to disclose

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O052 1435

Integrating Palliative Care in the Haematology and Bone Marrow Transplantation Unit

Nicole Gavin[1,2], Elise O'Dell[1], Ron Middleton[1]
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Aim
A literature review to explore the integration of palliative care (PC) in the haematology and bone marrow transplant setting.
Method
A literature search of available studies was undertaken searching the Cochrane Database of Systematic Reviews, The Cochrane Library Issue 2 2011; Ovid MEDLINE 1996 to June Week 1 201; CINAHL 1982 to March Week 2 2011 and PubMED to June Week 2 2011.
Result
One study found that 26% of haematology patients received curative treatment until death, another that patients with leukaemia are less likely to be referred to PC compared to patients with lymphoma and another that only a third of patients with a haematological malignancy are referred.
One study which introduced PC into the out-patient unit increased the number of referrals and reduced patient anxiety about referral whilst another found that increasing numbers of PC referrals were made for psychosocial support, but patients also had significant symptom burden.
Four studies showed that communication with patients and families about transitioning to PC occurred very late in the illness trajectory, if at all and would improve preparation for death. One study asking doctors and nurses working in bone marrow transplant found that doctors were more likely than nurses to think that the timing of referral to PC was appropriate and another asking haematologists about their referral patterns found that a small number would refer as part of their initial management.
The two case studies described positive experiences of early integration of PC for the patient and their family. One of these case studies informed development of a model for end-of-life care.
Conclusion
The literature reviewed from a variety of methodologies (retrospective, qualitative, mixed methodology and case studies) supports the integration of PC into haematology and bone marrow transplantation. There is a need for prospective trials to assess the effectiveness of integrating PC into standard haematology care.

No conflict of interest to disclose

Monday 31 October **1330-1500**
Nurses Free Communications 2: Blood and Marrow Transplant ***Bayside Terrace***

O053 1448

A Reflection on Post Transplant Care to Improve Patient Outcomes

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Introduction
The Royal Brisbane and Women's Hospital performs approximately 80-85 allogeneic transplants per year. This case review provides the opportunity to critically reflect upon the care of the post transplant patient and has been the catalyst to identify if any strategies can improve patient care.

Case Report
A 51 year old lady with acute myeloid leukaemia presents with relapsed disease, marrow and central nervous system involvement. The patient achieved a complete remission and was admitted for a transplant with cyclophosphamide and total body irradiation conditioning with cranial irradiation. The unrelated donor was a C mismatch, cytomegalovirus (CMV) positive, male. The patient was considered a high risk for graft versus host disease (GVHD). Worsening mucositis and liver function required a change of immunosuppression management on day +6 and the commencement of steroids due to a compromised airway. Engraftment occurred on day +9. The patient was diagnosed with stage II GVHD skin at day +29. The patient was then discharged day +32.
Five months after transplant the patient presented with a history of 7 days of GI symptoms at home and was treated with 5 days of antithymocyte globulin 6 days after admission. CMV reactivation coupled with worsening liver function led to death two months after admission.

Conclusion
This review has identified critical areas in the care of the post transplant patient which need to be improved. The inpatient unit has commenced post transplant education interviews for patients and carers. A dedicated education program for nurses in the ambulatory care setting is currently being developed. Our nursing model of care is being evaluated to include a clinical nurse to care for post bone marrow transplant patients in the ambulatory setting. Future plans include a need to develop an assessment tool for use by the patients and staff in the ambulatory setting to triage GVHD symptoms and provide timeframes for patients to present.

No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 1 ***Bayside Gallery B***

O054 1330

Neurologic Improvement by Intravenous Administration of Cryopreserved Autologous Cord Blood Mononuclear Cells in Children with Cerebral Palsy

Young-Ho Lee[1,5], Hyun-Joo Jun[1], Hye-Ryeong Kang[1], Mi-Jung Kim[2], Kyung-Vin Choi[1], Se-In Oh[2], Hyungsun Kim[2], Jangsoo Um[2], Jin-Hwa Moon[1], Yun-Young Choi[3], Young-Jun Lee[4], Hee-Jin Kim[5], Jong-Hwa Lee[6], Su Min Son[7], Soo-Jin Choi[8], Wonil Oh[8], Yoon-Sun Yang[8]
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Aim
We conducted a pilot study of intravenous autologous cord blood (CB) infusion in children with cerebral palsy to determine the safety and feasibility of the procedure as well as the potential efficacy for improving neurological impairment.
Method
Patients who diagnosed as cerebral palsy were enrolled in this study and were eligible if their parents had elected to bank their CB at birth. Cryopreserved CB units were thawed and infused intravenously over 10~20 minutes. We tried to assess potential efficacy for 6 months by using brain MRI-DTI (diffusion tensor image), brain perfusion SPECT using Tc-99m ECD, brainstem auditory evoked potential (BAEP), and various evaluation tools for motor and cognitive functions.
Result
Twenty patients (8 male, 12 female) received autologous CB infusion and were evaluated. Median age and body weight at infusion was 55(23~91) months and 13.9(7.2~21.4)Kg, respectively. The types of cerebral palsy were as follows: 11 quadriplegic, 6 hemiplegic, 3 diplegic. Infused TNC was 5.5±3.8 (0.6~15.65)×10^7 cells/kg. Infusions were generally well tolerated, even though 3 patients temporarily experienced nausea and hemoglobinuria, and another 2 patients respectively experienced hemoglobinuria or urticaria during intravenous infusion. Five patients (25%) showed improvement of diverse neurologic domain in developmental evaluation tools as well as of fractional anisotrophy (FA) value of brain MRI-DTI. The efficacy of neurologic improvement was significant in patients with diplegia or hemiplegia rather than quadriplegia (p=0.008)
Conclusion
This study presents the safety and feasibility as well as potential efficacy of autologous CB infusion in young children with neurologic deficits. Upcoming randomized controlled clinical trials of autologous stem cell therapy for children with cerebral palsy will formally assess the effectiveness of this approach.
No conflicts of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 1 ***Bayside Gallery B***

O055 1345

MRI T2* Finding of Iron Status in B-Thalassemia Major Undergoing Hematopoietic Stem Cell Transplantation

Amir Ali Hamidieh, Maryam Behfar, Fatemeh Moeininia, Mahdi Jalili, Ahmad Reza Shamshiri, Kamran Alimoghaddam, Ardeshir Ghavazadeh
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Aim
The excess iron acquired during years of thalassemia eliminate slowly after hematopoetic stem cell transplantation (HSCT). T2* relaxation time approach could be used as an appropriate and non-invasive method in the assessment of myocardial and liver iron overloaded after HSCT. To assess the changes of myocardial and liver iron concentration after successful HSCT by T2*MRI.
Methods
Fourteen beta-thalassemic patients were assessed for tissue iron overload by serum ferritin level, liver biopsy and T2* MRI of heart and liver before HSCT and at the end of sixth months of loss of a thalassaemic phenotype. Chelation was not started in this time.
Results
Myocardial T2* (mean 23.47ms ±10.91 before, vs 20.03ms±9.77 SD,after) and liver iron concentration estimated by MRI (mean 5.23mg/g±3.33 before, vs 5.34 mg/g ±3.13 SD, after) and liver iron score were not changed significantly after 6 months ex-thalassaemia feature. There was no significant correlation between myocardial T2* values and liver iron concentrations either before or after HSCT. Although serum ferritin levels and liver T2* 6 months after HSCT were correlate significantly (p<o.oo1), it was not true for myocardial values.
Conclusions
T2*MRI allows monitoring of iron deposition in a non-invasive way after HSCT and could be even used instead of liver biopsy before HSCT in beta-thalassemia patients. Serum ferritin is an inaccurate parameter to guide iron chelation after transplantation but T2* values before HSCT are usually enough for early treatment.

No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 1 ***Bayside Gallery B***

O056 1400

Unrelated Umbilical Cord Blood Transplantation for Hematological Malignancies Using Fludarabine/BUCY2 Conditioning Regimen

Zimin Sun[1], Huilan Liu[1], Liangquan Geng[1], Xingbing Wang[1], Kaiyang Ding[1], Yun Wu[1],Chenyang Zhou[1],Juan Tong[1], Kaidi Song[1], Baolin Tang[1], Hongjun Liu[2],Chun Li[2], Zuyi Wang[1]
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Aim
A standard non-TBI based regimen of Cord blood transplantation (CBT) has not been firmly established. We investigated a strategy using Fludarabine (FLU)/BUCY2 regimen in CBT for patients with hematologic malignancies.
Method
We here report 17 patients(children 16,adult 1) with hematologic malignancies who underwent single-unit CBT with a conditioning regimen comprising FLU 120 ㎎/㎡, intravenous busulfan (BU) 12.8㎎/㎏ and cyclophosphamide (CY)120 mg/kg (FLU/BUCY2). All patients were given a combination of cyclosporine A and mycophenolate mofetil for graft-versus-host disease (GVHD) prophylaxis. Seventeen patients with acute leukemia (n=13), chronic myelogenous leukemia (n=4) were treated, thirteen of whom were high risk diseases and two were advanced-stage at CBT.
Result
Seventeen patients with a median age of 8 years (range,2.5–46 years) received the median number of nucleated cells and $CD34^+$cells infused were 5.70×10^7/kg (range: $3.15\text{-}9.60\times10^7$/kg) and 3.84×10^5/kg (range:$1.27\text{-}5.24\times10^5$/kg), respectively. The cumulative incidence of primary donor engraftment was 94% (16 patients). Median time to neutrophil$\geq0.5\times10^9$/L was 17 days (range 12-30) and platelet engraftment ($\geq20\times10^9$/L) was 35 days (range 14-56). Preengraftment syndrome (PES) developed in 71% of the patients at a median of 7days (range: 5-13).9 cases developed acute GVHD (56%), more than grade Ⅱ in three cases. Two of fourteen patients who survived more than 100 days developed chronic GVHD. 12 cases are alive at a median follow-up of 7 months (range 3~ 11). Two cases had extramedullary relapsed. The probability of overall survival at 100 days and 1 year are 88.2% and 67.9%, respectively.
Conclusion
Preliminary evidence of the small study suggests successful engraftment and decreasing relapse rate following FLU/BUCY2 regimen for CBT in patients with hematologic malignancies.
No conflict of interest to disclose

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Monday 31 October **1330-1500**
APBMT Free Communications 1 ***Bayside Gallery B***

O057 1415

Encouraging Results of Reduced-Intensity Conditioning followed by Hematopoietic Stem Cell Transplantation from an Alternative Donor for Relapsed Acute Lymphoblastic Leukemia in Children

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Aim
An alternative strategy to intensified conditioning for high-risk leukemia in children is avoiding TRM and enhancing graft-versus-leukemia (GVL) effect by RIST from an alternative donor. Therefore, we evaluate outcomes of children with relapsed ALL who underwent RIST from an alternative donor in our institute to clarify its feasibility and effectiveness.
Method
We retrospectively analyzed 11 children with ALL who underwent RIST from an alternative donor in a second remission (CR2) or beyond CR2 between 2001 and 2008 in our institute. Patients with Ph-ALL were excluded.
Result
All of 11 patients are B-cell precursor ALL. The median age at HSCT was 9 years (range two years - 19 years) old. All patients underwent HSCT followed by Flu and LPAM containing RIC. Three patients underwent BMT from an unrelated donor. Eight patients were transplanted from a parent. Death of TRM was observed in 4/11(36.4%). Acute GVHD grade IV wasn't observed and grade II, III occurred in 2/11 (18.2%), 6/11(54.5%), respectively. Chronic GVHD was seen in 5 out of 7 (71.4%) evaluable patients. Four patients (36.3%) out of 11 are surviving free of relapse after transplant for 40 - 110 months, as of May 2011. Two (40 %) out of five patients who had been on chemo-resistant relapse after myeloablative transplant underwent RIST from a parent are maintaining CR.
Conclusion
These results of RIST from an alternative donor for relapsed pediatric ALL seem to be comparable to those of conventional myeloablative transplantation.

No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 1 ***Bayside Gallery B***

O058 1430

Quantitative Monitoring of EBV Viral Load in 222 Hematopoietic Stem Cell Transplant Patients: Risk Analysis and Development of EBV-associated Post-transplant Lymphoproliferative Diseases (PTLD)

J-L Tang[1,2], M Yao[2], M-Y Lu[3], H-C Wu[4], B-S Ko[2], S-Y Huang[2], C-C Lee[1], C-T Lin[1], H-A Hou[2], S-J Wu[2], C-Y Chen[2], S-C Hsu[2], W-C Chou[2], Woei Tsay[2], Y-C Chen[2], H-F Tien[2], S-T Jou[3], Y-L Yang[3], D-T Lin[3], K-H Lin[3]
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Background and Purposes
Epstein-Barr virus (EBV)-associated PTLD is a life-threatening complication following hematopoietic stem cell transplantation (HSCT). Independent risk factors include use of ATG, acute GVHD, CMV antigenemia, T-depleted graft, and unrelated donor in our previous study. Quantitative real-time polymerase chain reaction (Q-PCR) was developed for early detection and intervention of EBV reactivation to prevent PTLD-related mortality.
Patients and methods
Between Apr 2004 and Oct 2010, EBV viral load in plasma was monitored by Q-PCR in 222 HSCT patients (total 2945 samples) in NTUH. EBV reactivation was defined as > 500 copies/mL in two consecutive assays or > 10-fold elevation than baseline level.
Results
EBV reactivation occurred in 50 (22%) patients. The cumulated incidence of EBV reactivation was 28% at 1-year and 32% at 2-year. Median time to EBV reactivation was 40 days (ranges, 26-406) after SCT and median peak EBV-viral load, 10888 copies/ml (ranges, 948–$3x10^7$). The risk of EBV reactivation was significantly higher in patients receiving ATG (52% vs. 13%, p< 0.001), use of TBI (58% vs. 26%, p<0.001), mismatched donor (69% vs. 27%, p<0.001), CMV reactivation (44% vs. 19%, p<0.001) in univariate analysis. In multivariate analysis, independent risk factors include: ATG use (HR 5.44, p<0.001), TBI (HR 3.52, p<0.001), and Fludara-based conditioning (HR 3.16, P=0.01). PTLD developed in 8 patients (5%) who did not monitor EBV viral load regularly, and two were dead.
Conclusions
Monitoring of EBV viral load is a sensitive and useful tool in the surveillance of EBV-reactivation. Frequent monitoring in high-risk patients is important to prevent occurrence of EBV-PTLD.
No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 1 ***Bayside Gallery B***

O059 1445

Non Invasive Assessment of Acute Graft vs Host Disease of the Gastrointestinal Tract Following Allogeneic Haemopoietic Stem Cell Transplantation Using FDG PET

M Cherk[1], S Patil[2], S Avery[2], T Walker[2], A Wei[2], A Schwarer[2], S Roberts[3], W Kemp[3], G Brown[3], R Lanauze[3], P Urquhart[3], V Kalff[1], A Spencer[2]
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Background
Acute graft versus host disease (GVHD) of the gastrointestinal tract (GIT) often complicates allogeneic hameopoetic stem cell transplantation (AHSCT). FDG PET scanning is known to detect active inflammation and may be a useful non-invasive screening test for acute GIT GVHD and assessing treatment response.
Aims
To evaluate the diagnostic accuracy of FDG PET as a non-invasive technique for assessing patients with clinically suspected acute GVHD of the GIT following AHSCT.
Methods
15 AHSCT patients with clinically suspected acute GVHD of the GIT underwent FDG PET scanning prior to gastroscopy and colonoscopy within 7 days (mean 2.8 days). Endoscopic biopsies of 4 upper and 4 lower GIT segments were obtained for histology, with 2 biopsies obtained per segment if normal macroscopically and on PET and 4 biopsies if abnormal macroscopically or on PET. The degree of PET GIT uptake in each bowel segment was graded 1-4 relative to liver, with patients considered positive for acute GVHD if >3 of 8 bowel segments had grade 3/4 FDG uptake.
Results
4/15 (27%) patients had biopsy proven acute GIT GVHD. PET was positive in 3/4 of these patients (Sensitivity 75%). Of the 11/15 biopsy negative patients, PET was falsely positive in 2 patients (Specificity 81.8%), with 1 of these patients subsequently confirmed as having Norovirus GIT infection. Positive predictive value of PET for acute GVHD was 60% and negative predictive value (NPV) 90%. Of 116 bowel segments evaluated, PET was concordant with endoscopic macroscopic findings in 94/116 (81%) and biopsy in 93/116 (80%) GIT segments.
Conclusions
FDG PET demonstrates significant potential as a non invasive technique for evaluation of suspected acute GIT GVHD. Its high NPV suggests it is a useful technique to exclude GVHD however when positive further invasive biopsy to determine aetiology of active bowel inflammation is still required.

No conflicts of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 2 ***Bayside 105***

O060 1330

Alloantigen Presentation by Recipient Non-Professional Antigen Presenting Cells Induces Lethal Acute GVHD

Motoko Koyama[1], Rachel D Kuns[1], Stuart D Olver[1], Neil C Raffelt[1], Yana A Wilson[1], Alistair LJ Don[1], Katie E Lineburg[1], Renee J Robb[1], Kate A. Markey[1], Antiopi Varelias[1], Bernard Malissen[2], Günter J Hämmerling[3], Purnima Bhat[4], Andrew D Clouston[5], Christian R Engwerda[1], Kelli PA MacDonald[1] , Geoffrey R Hill[1,6]
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Aim
The immunological parameters governing the presentation pathways for allogeneic peptides to induce graft-versus-host disease (GVHD) are unclear. This is critical for the development of clinical therapies based on antigen presentation to control deleterious alloreactive responses.
Method and Result
We developed a GVHD model following a bone marrow transplant (BMT) system whereby presentation of a processed recipient peptide within MHC class II can be spatially and temporally quantified. The presentation of peptide within MHC class II by recipient antigen presenting cell (APC) resulted in GVHD mortality within ten days. While donor APC could induce lethal acute GVHD, recipient APC were 100-1000 times more potent. Antigen presentation by recipient APC resulted in accumulation of antigen-specific T-cells within the gastrointestinal tract and severe histopathology. The specific deletion of recipient dendritic cells surprisingly enhanced the expansion of donor alloantigen-specific T-cells and accelerated GVHD mortality due to a failure of activation-induced donor T-cell death. Consistent with this, the use of bone marrow-chimeric recipients demonstrated that professional, hematopoietic-derived recipient APC in isolation were limited in their capacity to induce GVHD. In contrast, non-hematopoietic recipient APC in isolation induced universal GVHD mortality with high levels of alloreactive donor T-cell expansion and inflammatory cytokine generation. Confocal imaging demonstrated that MHC class II is highly expressed in recipient non-hematopoietic tissue within the dermis and intestinal villi.
Conclusion These data challenge current paradigms, demonstrating that lethal acute GVHD can be induced by alloantigen presented within MHC class II by non-hematopoietic recipient APC.
No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 2 ***Bayside 105***

O061 1345

Expression of *WT1* Gene at Initial Diagnosis in AML As a Prognosticator Predicting Not Pretransplant But Posttransplant Outcome

Woo-Sung Min[1,2], Hee-Je Kim[1,2], Hee-Baeg Choi[3], Hyun-Jeong Sohn[3], Jae-Ho Yoon[1,2], Seung-Hwan Shin[1,2], Seung-Ah Yahng[1,2], Sung-Eun Lee[1,2], Yoo-Jin Kim[1,2], Seok Lee[1,2], Chang-Ki Min[1,2], Seok-Goo Cho[1,2], Jong-Wook Lee[1,2], Chong-Won Park[1,2], Tae-Gyu Kim[3]

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The Catholic University of Korea, Seoul, Korea

Aim

Recent studies have shown that some AMLs have an over-expression of Wilms' tumor gene 1 (*WT1*). Furthermore, *WT1* gene expression correlates with the clinical outcome and *WT1* can be used as an indicator for minimal residual disease (MRD). Quantitative assessment of this gene transcript by real-time quantitative PCR (RQ-PCR) may be useful for predicting prognosis in AML.

Method

We investigated prospectively the prognostic potential of the leukemia-associated antigen, *WT1* expression in bone marrow (BM) of 144 adult patients with newly diagnosed AML and 16 healthy normal controls using RQ-PCR. The molecular profiles were then evaluated with multiple parameters, including the known important prognostic indicators such as age, CBC at diagnosis, cytogenetics, receptor tyrosine kinase mutations, aberrant expression of lymphoid markers, and serum ferritin levels at diagnosis. We evaluated whether the expression level of the gene in the patients affected complete remission (CR) and survival rates before and after hematopoietic stem cell transplant (HSCT).

Result

The median follow-up period for all patients who were event-free survivors was 30 months (range: 2-56). The Kaplan-Meier estimated 4-year overall survival (OS) and event-free survival (EFS) rates in the whole population of evaluable patients were 52% (95% CI, 31% – 41%) and 50% (95% CI, 31% – 43%), respectively. The median expression levels of the gene in normal control and patients were 7.4 (range 0.74-30.27), 58.64 (range 0.9-1950.5), respectively. Interestingly, *WT1* levels of expression at diagnosis correlated with EFS after HSCT. However they did not correlate with CR, OS, and EFS obtained initial treatment of AML.

Conclusion

This finding may suggest that initial expressions of *WT1* in the leukemic blasts or possibly in the leukemic stem cells post-transplant can be the critical target of future immunotherapeutic trial.

No conflict of interest to declare

Monday 31 October **1330-1500**
APBMT Free Communications 2 ***Bayside 105***

O062 1400

Quantitative Assessment of WT1 Gene Transcript After Hematopoietic Stem cell Transplantation is a Powerful MRD marker to Predict the Clinical Outcome in Acute Leukemia

Bingrui Zhao, Xiaowen Tang, Jiannong Cen, Song Jin, Xiaolan Shi, Xiaoai Wei, Weirong Chang, Aining Sun, Depei Wu
Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Key Lab of Thrombosis and Hemostasis of Ministry of Health, Suzhou 215006, China

Background
WT1 is a panleukemic marker that is expressed in 90% of acute leukemias and had been used as MRD marker after chemotherapy or transplantation. However there are few reports concerning about the threshold of WT1 which could predict relapse and outcome of acute leukemia (AL) patients post transplantation.
Objective
To evaluate the predictive value of quantitative Wilms' tumor gene (WT1) assessment for relapse following hematopoietic stem cell transplantation (HSCT) in patients of AL).
Methods
The quantitative assessment of WT1 expression by real-time quantitative PCR (RQ-PCR) was measured in 63 AL patients (pts) with 326 bone marrow samples at different time points post transplant. ROC curve was used to determine the WT1 threshold in order to predict clinical relapses. Furthermore, AL patients were divided into WT1 positive and negative groups according to the threshold of WT1. The clinical outcomes of AL patients post transplant were analysed according to their WT1 status.
Results
BM samples from 19 pts relapse post transplanted showed significantly higher WT1 expression levels compared to the samples from 44 pts with remission AL ($P<0.01$), with a median expression level of 1270 (55-47596) and 132 (0-2959) copies $WT1/10^4ABL$ respectively. Ten of 18 relapsed patients died due to recurrence and had higher levels of WT1 expression than the other 8 patients. According to the ROC (Receiver Operating Characteristic) curve, the cut off value of WT1 was 585 copies $WT1/10^4ABL$ which divided 63 patients into WT1-positive group (>585) and the negative group (≤585). The WT1-negative group was found to have longer relapse- free survival (RFS) ($P<0.01$) and overall survival (OS) ($P<0.01$) than that of the positive group. 21 cases with WT1-positive were followed up and the 3 months, 4-6 months, 7-9 months and 9 months cumulative recurrence rates post HSCT were: 8/8, 2/4, 2/4, 3/5 ($P=0.063$) respectively and had poor outcome.
Conclusion
Our data suggest that, in the HSCT setting, the sequential and quantitative analysis of WT1 may be useful as a leukemia marker for monitoring MRD and as a predictor of AL relapse post transplant with cutoff value of 585 copies $WT1/10^4ABL$.
No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 2 ***Bayside 105***

O063 1415

Induction of Hematopoietic Commitment from Adult Human Bone Marrow Mesenchymal Stem Cell-derived Induced Pluripotent Stem Cells with Cytokines

Yulin Xu, Lizhen Liu, Lifei Zhang, Shan Fu, Yongxian Hu, Yingjia Wang, Huarui Fu, Kangni Wu, Haowen Xiao, Xiaohong Yu, Weiyan zheng, He Huang
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Hematopoietic stem-cell transplantation is applied to treat many diseases. However, immune rejection remains a major obstacle to success. Induced pluripotent stem cells (iPSCs) provide a potential platform to gain specific cell types immunologically compatible with patients. Here, to efficiently generate hematopoietic cell lineages from iPSCs and to prevent contamination from unknown substances using mouse stromal cells, we established a stepwise protocol with three groups of defined cytokine cocktails holding function on mesodermal or hematological signaling pathways. We first obtained iPSCs efficiently from adult human bone marrow-derived mesenchymal stem cells (hBM-MSCs), and then carried the stepwise protocol to induce iPSCs to hematopoietic cells. By treatment with the first group of cytokine cocktails including BMP4 and SCF for 5 days, the expression of mesodermal transcription factors Brachyury and GATA2, which correlated with the production of CD34+ cells in the later induction stage, was higher than that in the untreated groups ($P < 0.05$). After co-culture with the second induction cytokines as SCF, Flt3L and IL-3 for another 9-11 days, CD34+ cells, undetectable in iPSCs, increased more 10% than the untreated groups by analysis with flow cytometry and the cells expressed hematopoietic transcription factors TAL-1 and GATA-2. When exposed to semisolid media with the third group cytokines including IL-3, IL-6, EPO and G-CSF, the induced population formed all types of hematopoietic colonies as CFU-E, CFU-G, CFU-GM, CFU-GEMM and hematopoietic cell lineages were identified by phenotype analysis with Wright-Giemsa staining. The system for efficient hBM-MSC-derived iPSC commitment to hematopoietic cells with no contamination from unknown substance gives an opportunity to obtain potential patient-specific cell sources for therapeutic application and provides a useful model for studying mechanisms of haemopoiesis and its involvement in blood diseases.

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Monday 31 October **1330-1500**
APBMT Free Communications 2 ***Bayside 105***

O064 1430

The Effect of KIRs Expression Profile in Donor/Recipient Pairs in HLA-identical Sibling Hematopoietic Stem Cell Transplantation

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Objective
To investigate the distribution characteristics of KIRs (Killer Inhibitory Receptors) expression profile in donor/recipient pairs with acute leukemia (AL) patients receiving HLA-identical sibling hematopoietic stem cell transplantation (sib-HSCT). And to explore the effect of KIRs expression profile in donor/recipient pairs on clinical outcomes and dynamic of donor T cell and NK cell engraftment.
Methods
The genotypes of donor/recipient KIRs were determinated by polymerase chain reaction-sequence specific primer (PCR-SSP) for 80 pairs of donor/recipient receiving HLA-identical sibling hematopoietic stem cell transplantation. The multiple short tandem repeat (STR) PCR was used to evaluate the status of engraftment of donor T cell and NK cell at +14 days, 21 days, 28 days, 60 days and 90 days after transplantation in 24 cases.
Results
In 80 pairs of donor/recipient, we found: (1) 57.5% of donor/recipient KIRs were completely identical; (2) 13.75% of the donor KIRs genotype containing the recipients'; (3) 17.5% of the recipients KIRs genotype containing the donors'; (4) 11.25% were completely different. The KIR matching showed that 75% was matched for graft versus host (GVH) . The percentage of group donor B/X and group donor A/A was 50%.
Comparing the patients that were KIR-matched and mismatched for GVHD, the incidence of aGVHD was 60% and 30% respectively(*P*=0.0222). And 2 year OS was 62.96% and 94.12% respectively(*P*=0.0492). The aGVHD rate of KIR-matched group was higher than that of non-KIR matched group(15% vs 0%).
Donor B/X group had a higher 2 yrs OS and a higher 2 yr relapse-free survival (RFS) compared with donor A/A group (89.23% vs 49.57%, *P*=0.0159 and 90% vs 59.71%, *P*=0.0239). Patients with three or less aKIRs were associated with a lower 2 years OS(58.9% vs 92.44%, *P*=0.0338) and a lower RFS (65.14% vs 92.59%, *P*=0.0398) compared with patients with more aKIR.
Sequential monitoring of chimerism status of donor NK-cells in 24 cases revealed that on day+14, the percentage of full donor NK cells chimerism patients (85.7%) in non-KIR matched patients was higher than that of KIR matched patients(52.9%)(*P*=0.0456).
Conclusions
The results suggested that donor KIR genotype may have an impact on the aGVHD, OS and RFS, and may be useful in predicting the outcomes of HLA-identical sib-HSCT.
No conflict of interest

Monday 31 October **1330-1500**
APBMT Free Communications 2 ***Bayside 105***

O065 1445

Donor Natural Killer Cell Activating Gene Polymorphisms Associated with the Risk of Early Cytomegalovirus Infection after Allogeneic Hematopoietic Stem Cell Transplantation

Haowen Xiao, Yi Luo, Jimin Shi, Yamin Tan, Jingsong He, Wanzhuo Xie, Xiaoyu Lai, Xiaohong Yu, Xiujin Ye, Zhen Cai, Maofang Lin, He Huang
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Aim
Donor T and natural killer (NK) cells play critical roles in infection prevention following allogeneic hematopoietic stem cell transplantation (allo-HSCT). NKG2D, an activating and co-stimulatory receptor expressed on NK and T-cells, plays pivotal roles in cancer immunosurveillance. Recently, it was reported that rs1049174, a single locus featuring a G-C substitution distinguished two haplotypes of the NKG2D gene, the G allele-positive haplotype (high NK activity) and C allele-positive haplotype (low NK activity). The aim of this study was to analyze the clinical risk factors, donor NKG2D gene polymorphisms associated with cytomegalovirus (CMV) infection within 100 days following allo-HSCT.
Methods
The NKG2D gene polymorphism was analyzed in 128 pairs of recipients and their unrelated donors and 102 pairs of recipients and their HLA-identical sibling donors, who underwent HSCT at our center from 2001 to 2009.
Results
(1) 133 patients (55.4%) had experienced early CMV infection with a median onset of 45 days after HSCT. 12.1% developed CMV infection during granulocytopenia and 87.9% after neutrophil recovery. 93.2% developed CMV positive antigenemia without disease, only 9 patients developed CMV disease (7 patients with pneumonia and 2 patients with enteritis). (2) The donor with NKG2D G allele-positive haplotype, a haplotype expected to induce greater NK cell activity, was associated with a significantly reduced incidence of early CMV infection than those with C/C genotype in both the unrelated and sibling transplantation cohorts. (3) Multivariate analysis identified two risk factors for early CMV infection: patients with CMV reactivation pre-HSCT (RR: 0.575, 95%CI: 0.404-0.819, P=0.002) and donors with NKG2D C/C genotype (RR: 527, 95%CI: 0.371-0.745, P<0.001). Unrelated donor is a less significant factor (P=0.072).
Conclusion
Although CMV disease has been reduced in the era of antiviral prophylaxis and preemptive therapy, our findings suggest the incidence of CMV infection remains high and provides the first report of a relationship between donor NKG2D gene polymorphic features and the risk of early CMV infection.
No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O066 1330

A Retrospective Analysis of 344 Fludarabine Melphalan RIC Allogeneic Stem Cell Transplants for Myeloid and Lymphoid Malignancies in Australia and New Zealand 1998-2008

Adam Bryant[1], Elango Subramonia Pillai[2], Glen Kennedy[2], Anna Kalff[3], David Ritchie[3], Biju George[4], Mark Hertzberg[4], Sushrut Patil[5], Andrew Spencer[5], Keith Fay[1,6], Paul Cannell[7], Leanne Berkahn[8], Richard Doocey[8], Ruth Spearling[9], Ian Nivison-Smith[8], John Moore[1]
1. St Vincent's Hospital, NSW. 2. Royal Brisbane Hospital, Queensland. 3. Royal Melbourne Hospital, VIC. 4. Westmead Hospital, NSW. 5. The Alfred Hospital, VIC. 6. Royal North Shore Hospital, NSW. 7. Royal Perth Hospital, WA. 8. Auckland City Hospital, NZ. 9. Christchurch City Hospital, NZ. 10. Australasian Bone Marrow Transplant Recipient Registry

Aim
Fludarabine Melphalan (FluMel) is the commonest Reduced Intensity Conditioning (RIC) regimen used in Australia and New Zealand. This study aims to assess the relative benefit of this regimen in both lymphoid and myeloid malignancies and to further delineate risk factors associated with an improved survival using RIC conditioning.
Method
This was an ABMTRR based retrospective study assessing the outcome of FluMel RIC allografting in 9 Australian and New Zealand Centres between 1998 and 2008. Data was collected from centres using an excel based eCRF emailed to centres. Analysis was performed using Stata software and a p value less than 0.05 was considered significant.
Results
Median follow up was 3.4 years. There were 342 patients with a median age of 54 years (18-68) and 61% were male. 234 patients had myeloid malignancies with AML (n=166) being the commonest indication whereas there were 110 lymphoid patients with NHL (n=64) the main indication. TRM at D100 was 14% with no significant difference between the groups. OS and DFS were similar between myeloid and lymphoid patients (50% and 43% at 5 years respectively). There was no difference in the cumulative incidence of relapse and GVHD between the groups. Multivariate analysis revealed 4 adverse risk factors for DFS: non-HLA identical sibling donor, not in remission at transplant, previous autologous transplant, and recipient CMV +ve. The presence of Chronic GVHD was associated with a better DFS predominantly due to a marked reduction in relapse (HR 0.44, p=0.003).
Conclusions
This is one of the largest analyses of Fludarabine Melphalan RIC transpants performed. This dataset confirms that FluMel provides durable remissions in both myeloid and lymphoid malignancies with 50% overall survival at 5 years. The multivariate analysis provides important clues for improving outcomes when planning FluMel conditioning in patients with haematological malignancies.

No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O067 1345

Long-term Persistence of Oligoclonal Vbeta Populations Despite Adequate Thymic Output in Autologous Stem Cell Transplant for Auto-immune Diseases

John Moore[1], Wu Ong[1], Steve Carlin[1], Sam Milliken[1], John Snowden[1], Peter Brooks[2], Jim Biggs[1], Helen Englert[3], John Isaac[5], Frederique Ponchel[5], Virginia Nink[4], David Ma[1]
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Aim
The aim of this study was to assess long term follow up of patients who had undergone autologous HSCT for rheumatoid arthritis (RA) or systemic sclerosis (SSc) with a particular emphasis on expansion of oligoclonal vbeta populations in the RA patients and functional assessment in both groups.
Method
8 RA patients and 5 SSc patients who underwent autologous HSCT at St Vincents Hospital 10 and 4 years ago respectively, were enrolled in this ethics committee approved study. Patients were required to complete a Health Assessment Questionnaire (HAQ) during the follow up. Samples of blood were processed and run through LSRII flow cytometry to analyse T cell populations.
Results
The median age of RA patients was 51 years (40-64), with a median follow up of 10.8 years whilst the SSc patients had a median age of 38 years (29-48) with a median follow up of 4.12 years. All patients were female. All the RA patients had relapsed and progressed to other therapies whilst all the SSc patients remained in remission from their disease. HAQ scores for the RA group had returned to pre HSCT levels whereas the SSc patients remained functionally well with a significantly lower HAQ score compared to baseline. All RA patients had a significant ($p<0.05$) expansion of CD4 Vbeta subsets 2, 3 and 12 pre HSCT compared to normal controls. In contrast, there were no Vbeta expansions in the SSc patients. Despite adequate thymic output (as measured by TREC) in the RA patients post HSCT, these populations remained expanded for 3,6, and 9 months post HSCT. Surprisingly, 10 years post HSCT these populations remained expanded in the RA patients despite CD4 counts returning to pre HSCT levels.
Conclusion
This study confirms that RA patients suffered poor functional status post HSCT compared to SSc patients, in part, due to a superior response of HSCT in the SSc patients. The data on Vbeta oligoclonal populations suggests that there is very little 'thymic re-education' in HSCT for RA patients despite adequate thymic output and multiple lines of therapy over a 10 year period.

No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O068 1400

Expression of *WT1* Gene at Initial Diagnosis in AML As a Prognosticator Predicting Not Pretransplant But Posttransplant Outcome

Woo-Sung Min[1,2], Hee-Je Kim[1,2], Hee-Baeg Choi[3], Hyun-Jeong Sohn[3], Jae-Ho Yoon[1,2], Seung-Hwan Shin[1,2], Seung-Ah Yahng[1,2], Sung-Eun Lee[1,2], Yoo-Jin Kim[1,2], Seok Lee[1,2], Chang-Ki Min[1,2], Seok-Goo Cho[1,2], Jong-Wook Lee[1,2], Chong-Won Park[1,2], Tae-Gyu Kim[3]
[1]Catholic Blood and Marrow Transplantation Center, [2]Seoul St. Mary's Hospital, [3]Department of Microbiology, College of Medicine, Seoul, Korea
The Catholic University of Korea, Seoul, Korea

Aim
Recent studies have shown that some AMLs have an over-expression of Wilms' tumor gene 1 (*WT1*). Furthermore, *WT1* gene expression correlates with the clinical outcome and *WT1* can be used as an indicator for minimal residual disease (MRD). Quantitative assessment of this gene transcript by real-time quantitative PCR (RQ-PCR) may be useful for predicting prognosis in AML.
Method
We investigated prospectively the prognostic potential of the leukemia-associated antigen, *WT1* expression in bone marrow (BM) of 144 adult patients with newly diagnosed AML and 16 healthy normal controls using RQ-PCR. The molecular profiles were then evaluated with multiple parameters, including the known important prognostic indicators such as age, CBC at diagnosis, cytogenetics, receptor tyrosine kinase mutations, aberrant expression of lymphoid markers, and serum ferritin levels at diagnosis. We evaluated whether the expression level of the gene in the patients affected complete remission (CR) and survival rates before and after hematopoietic stem cell transplant (HSCT).
Result
The median follow-up period for all patients who were event-free survivors was 30 months (range: 2-56). The Kaplan-Meier estimated 4-year overall survival (OS) and event-free survival (EFS) rates in the whole population of evaluable patients were 52% (95% CI, 31% – 41%) and 50% (95% CI, 31% – 43%), respectively. The median expression levels of the gene in normal control and patients were 7.4 (range 0.74-30.27), 58.64 (range 0.9-1950.5), respectively. Interestingly, *WT1* levels of expression at diagnosis correlated with EFS after HSCT. However they did not correlate with CR, OS, and EFS obtained initial treatment of AML.
Conclusion
This finding may suggest that initial expressions of *WT1* in the leukemic blasts or possibly in the leukemic stem cells post-transplant can be the critical target of future immunotherapeutic trial.
No conflict of interest to declare

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O069 1415

Demyelinating Encephalopathy in Patients Following Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective Analysis of Incidence, Risk Factors and Survival

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The incidence of demyelinating encephalopathy complications, their risk factors, and impact on outcome are not well-defined in recipients of allogeneic hematopoietic stem cell transplant (allo-HSCT). We analyzed a large cohort of 1365 patients who underwent allogeneic HSCT during a 6-year period and identified 36 patients who experienced demyelinating encephalopathy at the Institute of Hematology and People's Hospital, Peking University between 2004 and 2009. The cumulative incidences of all demyelinating encephalopathy complications at 6-year was 3.6%. One patient (2.8%) had demyelinating encephalopathy at the conditioning stage, the other 35 patients had them after transplantation，16 [44.4%] at day 0 to day 100, 10 [27.8%] at day 100 to 1 year, and 9 [25.0%] after 1 year. Multivariate regression analysis identified donor type (P=0.031), infection (P=0.009) and acute lymphatic leukemia (P=0.017) as independent risk factors for demyelinating encephalopathy post-transplant. The median survival time of patients with demyelinating encephalopathy was 514 days after transplantation [95% CI: 223-805]. Survival at 6 year was significantly reduced in patients who developed demyelinating encephalopathy complications compared to those who did not (26.6% versus 73.5%, $P < 0.001$). Of the 36 patients experiencing demyelinating encephalopathy complications, 58.3% (n=21) died. The causes of death were GVHD (n=4), underlying disease relapse (n = 3), infections (n =12), and others (n = 2).

No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O070 1430

Evolution of Mixed Chimerism After Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Hematologic Malignancies

Emmanuel Levrat, Eddy Roosnek, Bilal Mohty, Michael Bernimoulin, Monika Nagy-Hulliger, Yordanka Tirefort, Sandra Paiano, Ayse Hulya Ozsahin, Jean Villard, Jakob Passweg, Yves Chalandon
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Aim
To analyze the evolution of chimerism in allotransplanted patients with hematologic malignancies and compare the outcomes of “lineage restricted” mixed chimerism (MC) with full donor chimerism (FC).
Methods
Patients transplanted between 1986 and 2006, for hematologic malignancies, alive without relapse at 1 year post HSCT were analyzed. Transplants were T-cell depleted in patients with low relapse risks. Chimerism was tested using short tandem repeat polymorphisms after separation into mononuclear cells (lymphocytes and monocytes) and granulocytes by Ficoll density gradient centrifugation.
Results
Of 155 patients, 89 had FC, 36 mononuclear cells MC (MNC-MC) and 30 granulocytic with or without mononuclear cells MC (Gran-MC). There was no differences for age, sex, disease, conditioning, donor type, stem cell source and donor lymphocyte infusions between the 3 groups ($p > 0.05$), but there was more MC in patients with T-cell depletion ($p < 0.001$), and less MC in patients with acute graft-vs-host disease (GvHD) ($p = 0.045$), chronic GvHD ($p = 0.009$) and female donor into male recipient transplants ($p = 0.001$). Survival was significantly better in MNC-MC than in Gran-MC patients ($p=0.001$), with FC patients being intermediate. There was more disease relapse in the Gran-MC group but not in the MNC-MC group as compared to FC ($p = 0.026$). MC was stable over prolonged periods in some patients in the Mono-MC and the Gran-MC groups. Of MC patients alive at 10 years, MC persisted in 83% (15/18) in the MNC-MC and 57% (8/14) in the Gran-MC groups.
Conclusion
Mixed chimerism may remain stable over a long time period. In survivors without relapse at 1 year post HSCT, determining lineage specific chimerism may be useful as outcome differs. MNC-MC probably reflects the persistence of T lymphocytes that have survived the conditioning and this is not associated with increased risk of relapse.

No conflict of interest to disclose
I:154

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O071 1445

Epileptic Seizure in Allogeneic Hematopoietic Stem Cell Transplant Recipients: A Retrospective Study of 79 Chinese Cases

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Background
Epileptic seizure in recipients of allogeneic hematopoietic stem cell transplant (allo-HSCT) has been poorly described. No report has systematically studied its possible causes, risk factors, and effect on the prognosis of allo-HSCT.
Procedure
We retrospectively examined the data of 1461 patients who have undergone allo-HSCT at the Institute of Hematology and People's Hospital, Peking University in the last 6.5 years.
Results
The cumulative incidence of all epileptic seizure complications was 7.1%. Of the 79 patients who had epileptic seizure in transplantation, 3 [3.8%] at the conditioning stage, 52 [65.8%] at day 0 to day 100, 20 [25.3%] at day 100 to 1 year, and 4 [5.1%] after 1 year. Multivariate regression analysis identified age of recipient (≤18 years) (P<0.001), donor type (P=0.004), aGVHD (P=0.018) and hyponatremia (P=0.003) as independent risk factors for epileptic seizure in allo-HSCT. The median survival time of patients with epileptic seizure was 246 days after transplantation [range, 18~2170 days]. Survival after 1 year and 6.5 years was significantly reduced in patients who developed epileptic seizure complications compared to those who did not (57.2% versus 75.7% at 1 year, *P* =0.015, and 31.1% versus 71.4% at 5 years, *P* <0.001). Of the 79 patients experienced epileptic seizure complications, 53.2% (n=42) died. The survival rate of these patients is relatively low, and cerebrovascular disorder or CNS infection-related epileptic seizure usually results in high mortality and poor prognosis.
Conclusions
Age at transplantation younger than 18 years, haploid transplant, aGVHD, and hyponatremia are risk factors for epileptic seizure in allo-HSCT recipients. Epileptic seizure in allo-HSCT patients is associated with a poor prognosis.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O072 1330

Successful Management of Refractory/Recurrent Acute Myeloid Leukemia by Allogeneic Hematopoietic Stem Cell Transplantation and Prophylactic Immunotherapy

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Our previous study has shown that donor's dendritic cell-primed cytokine-induced killer cell (DC-CIK) is a safe and effective therapeutic mean in management of early leukemia recurrence after allogeneic hematopoietic stem cell transplantation (HSCT) (JB Wang, et al. ASH 2008). In the present clinical study, the outcome of refractory/recurrent acute myeloid leukemia (AML) salvaged by HSCT and prophylactic immunotherapy is evaluated. From September 2006 to April 2010, a total 45 patients with refractory/recurrent AML were enrolled. The median age was 29 (2 to 51) years old. The median blasts in bone marrow (BM) were 36% (20% to 92%) before conditioning. The donors were identical siblings (6) or unrelated ones (9) or haploidentical family members (30). Conditioning regiments were individualized according to patients' status. The most common regimen was high-dose cytarabine plus BUCY/CYTBI (20 pts). The patients with impaired organ function received the above regimen except using fludarabine instead of cyclophosphamide (17 pts). To reduce leukemia burden, melphalan was added into above regimen or FLAG followed by reduced-intensified BUCY for recipients with >40% blasts in BM (n=2 and 6 respectively). GVHD prophylaxis was as reported previously (DP Lu et al., Blood 2006;107:3065). Prophylactic immunotherapy including DLI, DC-CIK, NK cells, IL-2, IFN-alpha, thymosin was used in the 11 patients with no evidence of GVHD at 120 days post-HSCT. All but two patients attained durable engraftment. The incidence of grade II to IV aGVHD and cGVHD were 34%, 59.1%, respectively. With median follow-up 30 (1-57) months, the relapse rate was 29.2%. Twenty-eight of 45 (60.2%) patients have been in complete remission since salvaged HSCT. Three years disease-free survival and overall survival were 60.2%, 62.6%, respectively. Our clinical results have shown that the combination of salvage HSCT and prophylactic immunotherapy is a promising modality for treatment of refractory/recurrent AML, even with high leukemia burden.

Monday 31 October **1330-1500**
APBMT Free Communications 3 ***Bayside 104***

O073 1345

Comparative Analysis of Single-Institute Transplantation from Unrelated Cord Blood and Related Adult Donors in Adults with Hematologic Malignancies

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Aim
We compared the clinical outcomes of unrelated cord blood transplantation (UCBT) with related bone marrow transplantation (BMT) and/or peripheral blood stem cells transplantation (PBSCT) in adults with hematologic malignancies.
Method
Between Oct. 2001 and June 2011, 152 adults with hematologic malignancies received unrelated cord blood transplantation (CBT, n=51) and related bone marrow transplantation (BMT) and/or peripheral blood stem cells transplantation (PBSCT)(n=101). All patients received myeloablative transplantations from a single center.
Result
Median follow-up was 17 months (range, 0.8-109.6 months) for BMT/PBSCT and 6.2 months (range, 1.0-96.7 months) for CBT recipients. Recipients of unrelated CBT were younger (median, 22 vs. 30 years of age; P<0.05) and had more advanced disease at the time of transplantation (78.4% vs. 34.7%, *P*<0.05) than recipients of related BMT/PBSCT. All related transplants were HLA matched, whereas 21.6 percent of CBT grafts were HLA mismatched (*P*<0.05). The median number of nucleated cells infused was 0.37×10^8 per kilogram of the recipient's body weight for CBT and 5.65×10^8 per kilogram for BMT/PBSCT recipients (*P*<0.05). Neutrophil recovery was significantly delayed after CBT (median, 19 vs. 12 days post transplantation, *P*<0.05).In multivariate analysis, the overall engraftment rates showed lower trend in CBT recipients (94% in CBT and 100% in BMT/PBSCT recipients, *P*=0.062).There was no significant differences in transplantation-related mortality (TRM) (31.4% in CBT and 21.8% in BMT/PBSCT recipients), relapse rate (9.8% in CBT and 14.9% in BMT/PBSCT recipients), the incidence of grades Ⅲ to Ⅳ acute graft-versus-host disease (aGVHD) (6.3% in CBT and 5.0% in BMT/PBSCT recipients), one year leukemia-free survival (DFS)(57.2% in CBT vs. 71.5% BMT/PBSCT recipients) and one year over survival (OS) (57.2% in CBT vs. 71.5% BMT/PBSCT recipients) between both groups, but extensive chronic GVHD was lower in CBT recipients (0% vs. 15.8%).
Conclusion
HLA-mismatched cord blood should be considering an acceptable source of hematopoietic stem-cell grafts for adults in the absence of an HLA-matched adult donor.

This research was supported by the International Cooperation Research Fund of Anhui Provincial Scientific and Technologic Committee (08080703026), the Fund of the Key Medical Project of Anhui Provincial Healthy Department (2010A005) and Anhui Provincial "115" Industrial Innovation Program (2009)

Monday 31 October **1330-1500**
APBMT Free Communications 4 ***Bayside 202/203***

O074 1400

Prospective Multicenter Phase II Study of Myeloablative Conditioning Consisting of Intravenous Busulfan and Fludarabine +/- Total Body Irradiation for Older Patients (55 Years and Older): Interim Analysis of the JSCT FB09 Study

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Aim
Multicenter phase II study has been conducted to investigate whether myeloablative dose of intravenous busulfan (ivBu) can be used for elderly recipients.
Method
This study started in September 2009, and 32 centers participated (Trial identifier: UMIN000002426). Patients aged from 55 to 70 with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) who were planned for allo-SCT (bone marrow (BM), peripheral blood (PB), and cord blood (CB)) were enrolled. Pretransplant conditioning consisted of 30 mg/m^2 of fludarabine (Flu) for 6 days (total 180 mg/m^2) and 3.2 mg/kg of ivBu for 4 days (divided by 4 daily, total 12.8 mg/kg) with or without total body irradiation depending on type of donor cells. Calcineurine inhibitors + methotrexate for BM or PB recipients, and tacrolimus + mycophenolate mofetil were used for CB recipients.
Result
Thirty-eight patients were enrolled, and the study was closed in August 2010. Median age was 60 (55-68), 22 male and 16 female, 31 AML and 7 MDS were included. Donors were 8 matched and 2 1-Ag/allele-mismatched related BM/PB, 8 matched and 4 1-Ag/allele-mismatched unrelated BM, and 16 CB (≤2-Ag-mismatched). In one case, the total dose of ivBu was reduced (11.2 mg/kg) due to neurotoxicity (grade III). Thirty-five achieved neutrophil recovery by day +17 (median, range, 11-45)). One patient died early before engraftment on day +27 due to cerebral hemorrhage, and two failed to engraft (all were CB recipients). There were 2 cases of veno-occlusive disease. At 12 months post-transplant, there were 7 relapse, and 7 non-relapse mortality. Overall and event-free survivals were estimated to be 62 % and 59 % at 1 year post-transplant.
Conclusions
Myeloablative conditioning using Flu/ivBu12.8 mg/kg +/- TBI was well tolerated with acceptable low toxicities and was sufficient to allow donor cell-engraftment post allo-SCT for elderly patients with AML or MDS. *No conflict of interest to disclose*

Monday 31 October **1330-1500**
APBMT Free Communications 4 ***Bayside 202/203***

O075 1415

Allogeneic Haematopoietic Cell Transplantation for Multiple Myeloma Using Reduced Intensity Conditioning Therapy, 1998-2006: Factors Associated with Improved Survival Outcome

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Aim
Although major advances have been made in the treatment of multiple myeloma (MM), this disease remains essentially incurable with the possible exception of those patients receiving allogeneic haematopoietic cell transplants (HCT). This study reports on the outcome of 95 allogeneic HCT using reduced intensity conditioning (RIC) performed for patients with multiple myeloma (MM) in Australia and New Zealand between 1998 and 2006.
Method
Patients were eligible for the study if they received an allogeneic HCT using RIC for MM between 1998 and 2006 inclusive in Australia or New Zealand. Retrospective data on patient characteristics, donor and transplant details and outcome were obtained from customised questionnaires distributed to participating centres.
Results
The median age at HCT was 52 years. Cumulative incidence of transplant-related mortality was 19% at 1 year post transplant. At 5 years post transplant overall survival (OS) was 40% and progression free survival (PFS) was 23% with no apparent survival plateau. Three factors were independently favourable predictors of OS in a Cox regression model: IgG myeloma (hazard ratio (HR)=0.42, 95% confidence interval (CI) 0.24 – 0.75, P=0.004), an HLA-identical sibling donor (HR=0.37, 95% CI 0.18 – 0.74, P=0.005), and less than 1 year between MM diagnosis and RIC HCT (HR=0.27, 95% CI 0.12 – 0.59, P=0.001).
Conclusion
Kaplan-Meier curves did not show a plateau in OS or PFS post-transplant, with high rates of relapse/progression at one and two years post transplant. Patterns of outcome indicate that RIC HCT may offer the potential for cure for only a small group of MM patients, however results may be more favourable for patients with IgG myeloma who have an HLA-identical sibling donor.
No conflict of interest to disclose

Monday 31 October **1330-1500**
APBMT Free Communications 4 ***Bayside 202/203***

O076 1430

Prevention of Relapse Using DLI Can Increase Survival Following HLA-Identical Transplantation in Patients with Advanced-Stage Acute Leukemia: A Multi-Center Study

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 # Wang-Yu and Liu Dai-Hong contributed equally to this work.
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Aim
To evaluate the role of donor lymphocyte infusion (DLI) in the prophylaxis of relapse for advanced stage acute leukemia after HLA-matched sibling donor T-cell-replete allogeneic hematopoietic stem cell transplantation (HSCT).
Method
A total of 123 consecutive advanced-stage, acute leukemia patients undergoing HSCT from HLA-identical sibling donors during the same time period at 3 institutes in China were analyzed. Fifty of the 123 individuals received prophylactic G-CSF primed DLI, and 73 individuals received no prophylactic treatment.
Result
The incidence of grades II–IV acute GVHD was 17% for patients receiving DLI and 23% for patients not receiving DLI (P = 0.35). The incidence of chronic GVHD was 38% for patients receiving DLI and 17% for patients not receiving DLI (P = 0.021). The 2-year cumulative incidence of relapse was significantly lower in patients who received prophylactic DLI (46%) compared with patients who did not receive prophylactic DLI (66%) (P = 0.02). The 2-year cumulative incidence of non-relapse mortality was comparable (20%) and those who did not receive prophylactic DLI (20%) (P = 0.83). The three-year probability of overall survival was higher in patients who received prophylactic DLI (36%) than in patients who did not receive prophylactic DLI (11%) (P = 0.001). The LFS was also higher in patients who received prophylactic DLI (29%) than in patients who did not receive prophylactic DLI (9%) (P = 0.001). Multivariate analysis for relapse showed that use of prophylactic DLI after transplantation was an independent prognostic factor (p=0.003). Higher OS was associated with use of prophylactic DLI (P = 0.02), younger age (P = 0.002) and non-TBI conditioning (P = 0.026).
Conclusion
Our comparisons suggest that the prophylactic use of DLI can significantly increase survival of patients with advanced-stage, acute leukemia who receive HLA-identical sibling HSCT. *No conflict of interest to disclose*

Monday 31 October **1330-1500**
APBMT Free Communications 4 ***Bayside 202/203***

O077 1445

Impact of ATG on New HLA Groups for Unrelated Donor Allogeneic Stem Cell Transplantation

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Aim
The outcomes of unrelated donor transplantation have improved with refinements in HLA testing. Recently, grouping of HLA matching for unrelated donor was suggested, defining by well-matched, partially-matched, and mismatched. In the current study, the role of ATG for each group was evaluated.
Method
A total of 92 patients diagnosed as hematologic diseases and received allogeneic stem cell transplantation (SCT) from unrelated donor were retrospectively analyzed.
Result
Nineteen patients were classified as well-matched, 42 as partially-matched, and 31 as mismatched. Among them, 57 patients received anti-thymocyte globulin (ATG) as graft-versus-host disease (GVHD) prophylaxis. The overall survival (OS) rate was higher for well-matched group (83%) compared to partially-matched (54%) and mismatched (34%, p=0.076). For partially-matched group, the OS was significantly improved with ATG (83.3% vs. 38.6%, p=0.018). But, the OS was not different between groups with or without ATG for well-matched (87.5% vs. 66.7%, p=0.487) and mismatched (32.4% vs. 41.7%, p=0.215). ATG decreased the cumulative incidence of grade 3-4 acute GVHD (10% vs. 40.2%, p=0.068) and severe chronic GVHD (21.2% vs. 52.2%, p=0.037). The use of ATG (HR=0.248, p=0.029) was related with favorable OS for partially-matched group. However, the favorable effect of ATG was not observed in well-matched and mismatched groups.
Conclusion
ATG effectively improved the survival rate for partially-matched group in unrelated donor transplantation. However, the positive effect of ATG was not observed in well-matched and mismatched groups.

No conflict of interest to disclose

Monday 31 October **1530-1630**
HSANZ Symposium 6: Hot Topics in Stem Cell Transplantation (Joint with APBMT/BMTSANZ) ***Auditorium B***

Stem Cell Transplantation for Severe Aplastic Anemia

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Aplastic anemia is a disease of high prevalence in Asia. A recent epidemiology study in Thailand shows that the incidence of aplastic anemia is at least 2-3 folds higher than that in Europe and the USA. However, the etiology of the disease is largely unknown; only less than 5 percent are due to drugs (sulfonamide, thiazide and mebendazole). Other risk factors include benzene and agricultural pesticide exposures (organophosphate, DDT and carbamate). Novel risk factors are exposures to ducks and geese, use of animal fertilizers and non-bottled water drinking.
Management of aplastic anemia comprises specific treatments with immunosuppressive agents and hematopoietic stem cell transplantation as well as symptomatic and supportive treatments. In patients with severe aplastic anemia, immunosuppressive therapy is the treatment of choice in elderly patients and in those without HLA-matched sibling. Hematopoietic stem cell transplantation is indicated in younger patients.
Updated results from EBMT show that the overall survival in the patients aged from 40-50 years appears to be not different from those aged under 40 years. The standard conditioning is high dose cyclophosphamide of 50 mg/kg/day for 4 days and GVHD prophylaxis is short course of methotrexate and cyclosporine A. The addition of antithymocyte globulin in the conditioning regimen is beneficial in those who had received multiple blood transfusions. Fludarabine in combination with cyclophosphamide and antithymocyte globulin yields a better outcome in patients aged over 30 years. Bone marrow is the recommended source of stem cell, while the use of peripheral blood stem cell is associated with the higher incidence of chronic GVHD.
During the period from June 1988 - December 2010, 32 patients (males=13 and females=19) underwent HLA-matched sibling transplantations at Chulabhorn Bone Marrow Transplant Center, Siriraj Hospital, with median age of 33 years (17-51 years). Thirty patients (93.8%) were severe aplastic anemia and two patients (6.3%) were very severe aplastic anemia. Median time to transplantation was 177 days (42-2,012 days). Median number of blood transfusions prior to transplantation was 39 units (3-310 units). Most patients (90.6%) received high dose cyclophosphamide 50 mg/kg/day for four days as a conditioning regimen. Other conditioning regimens included cyclophosphamide/ antithymocyte globulin in two patients (high transfusion=1 and second transplant=1) and fludarabine/cyclophosphamide/antithymocyte globulin in one patient who underwent second transplantation. We used peripheral blood stem cell in 25 patients (78.1%) and bone marrow stem cell in 32 patients (21.9%). All patients received cyclosporin A and methotrexate for graft versus host disease prophylaxis regimen. Median time to neutrophil and platelet engraftment was 10 days (8-19 days) and 14 days (10-21 days) for peripheral blood stem cell transplantation, 19 days (17-26 days) and 22 days (15-27 days) for bone marrow transplantation, respectively. Graft rejection was found in two patients (6.25%). Acute graft versus host disease (GVHD) was evident in 4 patients (12.5%). Chronic GVHD (40.6%) was found more common in peripheral stem cell use (34.4%) than in bone marrow stem cell use (6.2%). We observed a favorable survival outcome after transplantation with 1 year overall survival 94% and 5 year overall survival 87%. Five patients had disease recurrence with median time to relapse 10 months (6-12 months). Second HLA-matched sibling transplantation were performed in two patients, which resulted in curable disease and long term survival.

Monday 31 October **1530-1630**
HSANZ Symposium 6: Hot Topics in Stem Cell Transplantation (Joint with APBMT/BMTSANZ) ***Auditorium B***

Stem Cell Transplantation for Haemoglobinopathies

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Stem Cell Transplantation [SCT] today offers an alternative to life-long transfusion and chelation for patients with thalassemia major. However the procedure is associated with a significant risk of infection, regimen related toxicity, graft versus host disease and relapse particularly in those patient who because of inadequate treatment and poor chelation have developed hepatomegaly and hepatic fibrosis: patients with all three risk factors are Lucarelli Class III, one or two risk factor Class II and no risk factors Class I. Data from Pesaro in Italy show that there is a 90% chance of disease free survival post transplant in patients who are in Class I, 85% in Class II and only 65% in Class III.

The decision that a family with a child with thalassaemia has to make if there is a histocompatible sibling who can serve as the donor is whether they should continue transfusion chelation with its low present risk but significant late morbidity or have a transplant with its immediate risk, but high probability of good quality life without the burden of lifelong transfusion and chelation.

In developing countries the economic advantage of a bone marrow transplant for thalassemia is compelling because many families can manage a one-time investment of US$15,000 but a lifelong expenditure of US$4,000 a year is a difficult proposition. Sibling cord blood transplant may be associated with a higher risk of rejection so some centers wait till the baby is two years old and combine marrow with the harvested cord blood cells. The decision whether to transplant a child with thalassemia intermedia or sickle cell anaemia is more difficult since the phenotype is so variable. Details of transplant conditioning, toxicity and outcome will be discussed. Post transplant care is vital including chelation and venesection to bring down excessive stored iron.

HAA 2011
HSANZ ANZSBT ASTH

Monday 31 October **1530-1630**
ANZSBT Symposium 7: Current Issues in Transfusion ***Auditorium A***

Blood Groups and Disease

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In 1919, Hirszfeld and Hirszfeld observed the distribution of ABO groups varied according to the geographic origin of the individuals tested. Their work raised a question as to the cause and significance of this variation. In the intervening years theories promulgated to explain the variation have ranged from relatively harmless nonsense (ABO group correlates with personality; individuals with different ABO groups require different diets) to provision of a pseudoscientific crutch to validate the evil policies of Hitler's regime.

Recent work using DNA markers to elucidate the migration patterns of human populations around the world has done much to clarify our understanding of blood group variation between peoples. It seems clear for example, that the indigenous peoples of the Americas are group O because of a founder effect. Similarly, the occurrence of Haemolytic Disease of the Newborn in Europe and Central Asia likely results from interbreeding between two migrating populations one group D positive and the other D negative.

Nonetheless, there are persuasive arguments for selection of certain blood groups in response to infectious diseases. Group O may well have first emerged in Africa in response to malaria. The Fy(a-b-) phenotype certainly did. In addition, there is very good evidence linking ABO and/or Secretor status with susceptibility to diseases caused by *Helicobacter pylori*, noroviruses and cholera.

Monday 31 October **1530-1630**
ANZSBT Symposium 7: Current Issues in Transfusion ***Auditorium A***

Age of Red Cells

Speaker to be confirmed at time of going to print

Monday 31 October **1530-1630**
ASTH Symposium 6: Malaria - Parasite vs Physician ***Bayside 204***

Coagulopathy in Malaria

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Blood coagulation activation is frequently found in patients with malaria. Clinically apparent bleeding or disseminated intravascular coagulation (DIC) is associated with very severe disease and a high mortality. The protein C, protein S, and antithrombin levels were found to be low in *P. falciparum*, and were normal in *P. vivax* infection. Plasma levels of plasminogen activator inhibitor-1 were high in cases of *P. falciparum* infection whereas tissue plasminogen activator levels were low. Elevated plasma levels of soluble adhesion molecules (sICAM-1, sVCAM-1, sE-selectin), von Willebrand factor (vWF) and vWF propeptide, thrombomodulin, endothelial microparticles have been reported in *P. falciparum*-infected patients. Recently, it has been demonstrated that severe *P. falciparum* infection is associated with acute endothelial cell (EC) activation, abnormal circulating ultralarge vWF multimers, and a significant reduction in plasma ADAMTS13 function. This may result in intravascular platelet aggregation, thrombocytopenia, and microvascular disease. It has also been shown that *P. falciparum*-parasitized red blood cells induce tissue factor (TF) expression in microvascular ECs *in vitro*. The cornerstone of the treatment of coagulopathy in malaria is the use of effective anti-malarial agents. DIC with spontaneous systemic bleeding should be treated with screened blood products. Study in Thailand has shown that for patients who presented with parasitemia > 30% and severe systemic complications such as acute renal failure and ARDS, the survival rate in the group who received the exchange transfusion was superior. The use of heparin is generally restricted to patients with DIC and extensive deposition of fibrin, as occurs with purpura fulminans or acral ischemia. The use of activated protein C has been reported in patients with multi-organ failure resulting from severe *P. falciparum* malaria. The finding that TF is potentially a crucial mediator of malaria pathogenesis suggests that therapeutics targeting TF and/or ECs may provide a new adjunct therapy for severe *P. falciparum* malaria.

Monday 31 October **1530-1630**
ASTH Symposium 6: Malaria - Parasite vs Physician ***Bayside 204***

Brain Microvascular Endothelium: Both a Target and an Active Player in Cerebral Malaria Pathogenesis

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Vascular Immunology Unit, Department of Pathology, Sydney Medical School, The University of Sydney, Camperdown, Australia, and La Jolla Infectious Disease Institute, San Diego, CA, USA

Cerebral malaria, a severe complication of P. falciparum infection, is characterised by pleomorphic endothelial cell (EC) alterations, systemic activation of circulating cells, adhesion of parasitised erythrocytes, leucocytes and platelets to vessel walls and subsequent blockade of post-capillary venules, notably in the brain [1]. While studying various aspects of EC changes in relation to tissue damage, we showed that infected erythrocytes, together with host cells, lead to profound endothelial alterations alterations[1]. In turn, EC can trigger immunopathological changes. We identified miRNAs able to regulate EC that are modulated in cerebral but not in uncomplicated malaria [2]. In the *P. berghei* ANKA model, MRI studies revealed that intravascular accumulation of infected erythrocytes and host cells is associated with brain oedema. This oedema, close to that reported in paediatric malaria patients, can lead to compression of cerebral arteries[3]. *In vivo* in mice and *in vitro* using human cells, platelets were shown to potentiate cytokine effects on EC and, more recently, platelet-derived microparticles dramatic ally enhanced falciparum-infected erythrocytes and altered EC functions[4]. We are investigating the part that vascular cells and their derived microparticles play in this pathogenesis. In African as well as South-East Asian patients, we found elevated plasma levels of microparticles, from various cellular origins, specifically in patients suffering from cerebral malaria[5-7]. Human brain EC, notably upon cytokine stimulation, are able to pick up *P. falciparum* antigens and to develop transmigratory-like cups[8]. Data will be presented indicating that EC, both from mouse and human origin, can act as antigen presenting cells. Altogether these findings provide new pathogenic mechanisms and may present new avenues for therapeutic intervention.

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Monday 31 October **1530-1630**
Nurses Symposium 5: How Do I .... ***Bayside 102***

How Do I Read and Understand Statistics?

Judith Fethney
Sydney Nursing School, University of Sydney, NSW, Australia

Statistics can be viewed as language; it is one way of analysing and communicating research results in a succinct way. However, many clinicians feel underconfident (if not completely nauseous) when it comes to reading and interpreting research results when they are presented in statistical language. This can mean that clinicians skip over statistical results, and therefore miss out on being fully able to critically appraise research that may be of interest or relevance to their practice.
Understanding statistical results does not have to be painful or boring. With statistics, interesting stories can be explored and told. Like any language, it can be learned – every clinician has the potential to become statistically literate, and being statistically literate can strengthen your own practice. You do not have to be a mathematical expert to achieve this literacy.

Statistics are less scary when you know what the symbols, abbreviations and numbers mean. Using data from actual publications, this workshop will explore some of the more common types of statistics reported in the literature, including the type of statistical test, why that test was chosen as appropriate and what the associated symbols and numbers mean. There will be a focus on being critical so that you can draw your own conclusions from data. By the end of the workshop, participants will know the difference between t- tests, ANOVAs, Chi Square and regression, what types of data are suitable for those tests and feel more informed about interpreting means and standard deviations, confidence intervals, odds ratios, *p* values and effect sizes.

Monday 31 October **1530-1630**
Nurses Symposium 5: How Do I .... ***Bayside 103***

How Do I Get Started, Embrace the Challenges and Enjoy the Process: Cancer Support Groups? A Workshop for Haematology Nurses

Kim Pearce, John Friedsam
Cancer Council NSW

Cancer Council NSW commissioned research into the effectiveness of support groups (Kirsten, Ussher, Butow, Wain, et al 2003). It showed support groups improve patients' psychological well being and quality of life. The research highlighted effective group leadership as a key factor, in ensuring the maintenance and continuation of cancer support groups. Cancer Council implemented the recommendations from the research, which identified the needs of leaders to be; training, clear guidelines and support strategies.

Working with existing, new and potential leaders in the development and maintenance of support groups has been a key focus area for the Cancer Council. Drawing on the research and experience of working with over 300 support groups in NSW, this one hour workshop will explore: the competencies of a group leader; the importance of screening participants of a group; how to plan the purpose of a group; the importance of establishing a group agreement or rules; and how to facilitate group discussion.

These vital components of a support group will introduce and empower the haematology nurse to the mechanisms involved in setting up a group in their community or for those already leading groups the opportunity to reflect on your current practice. The aim being to ensure you have established a safe place where people impacted by cancer can come together to: feel the benefit of peer support; develop friendships while sharing ideas and thoughts; obtain information from health professionals; learn coping skills in a non-judgemental and caring atmosphere.

The workshop will highlight the pathways to and necessity of accessing further training and support. Participants will: receive the latest publication, "A Guide to Setting Up and Maintaining A Support Group"; receive handouts on recent Australian support group research into face to face and telephone support groups; explore the importance of debriefing and supervision for the self care of you as leaders of groups.

Monday 31 October **1530-1630**
Nurses Symposium 5: How Do I .... ***Bayside 104***

How Do I Transform My Niggling Question Into a Study and Then Share It With the World?

Moira Stephens
Sydney Nursing School, University of Sydney, Sydney, NSW
School of Nursing, Midwifery & Indigenous Health, University of Wollongong, NSW

Why. What. How ….. What if ……? Niggling questions such as these about practice, treatment or experiences often pop into our minds and conversations. Sometimes we follow them up and investigate by undertaking an audit or study and sometimes we don't.

This session will introduce and discuss how we can take these questions forward and develop them into research questions. It will discuss ideas about methodology and methods in research and how we can start to answer many questions and complexities that are evident in health care. In short – this session will provide a quick guide to research from developing a question, finding an answer, through to letting the world know about it!

No conflict of interest to disclose

Monday 31 October **1530-1630**
Nurses Symposium 5: How Do I .... ***Bayside 105***

How Do I Unglue My Feet and Feel Able To Dance On Stage During My Conference Presentation?

Mary Chiarella
Sydney Nursing School, University of Sydney, NSW, Australia

A brief exposé of inside secrets on public speaking - from someone who has been there and done it many, many times.

Monday 31 October **1730-1830**
HSANZ Masterclass #1 ***Bayside 102***

Addressing the Molecular Heterogeneity of Diffuse Large B-Cell Lymphoma (DLBCL) with Novel Therapies

Kieron Dunleavy
National Cancer Institute, Bethesda, Maryland, USA

Gene expression profiling has revealed that diffuse large B-cell lymphoma (DLBCL) – the most common type of non-Hodgkin lymphoma (NHL) – is a molecularly heterogeneous entity and can be divided into three subtypes: germinal center B-cell-like (GCB), activated B-cell-like (ABC) and primary mediastinal B-cell (PMBL) DLBCL. All of these subtypes derive from B-lymphocytes at different stages of differentiation and are characterised by distinct molecular pathogeneses. Clinically, these subtypes have different outcomes and cure rates with standard treatment approaches and the ABC subtype is associated with a significantly inferior outcome. ABC DLBCL, for example, shows high expression of target genes of the NF-κB/Rel family of transcription factors, and may benefit from strategies such as NF-κB inhibition and these are being pursued in several clinical trials. In GCB DLBCL, several studies raise the hypothesis that inhibition of the Bcl-6 transcription factor may be therapeutically important and this is currently under investigation. PMBL, like its biological cousin nodular sclerosis Hodgkin lymphoma, may benefit from dose intensity approaches and inhibition of the janus kinases. Overall, understanding and elucidating the molecular heterogeneity of DLBCL is important in terms of developing novel therapies and investigating approaches to improve the curability of these diseases.

Monday 31 October **1730-1830**
HSANZ Masterclass #2 ***Bayside 103***

How I Treat Patients Who Mobilize Hematopoietic Stem Cells Poorly

Luen Bik To[1,2,] Jean-Pierre Levesque[3,4,] Kirsten E Herbert[5]
[1]Haematology, IMVS/SA Pathology and Royal Adelaide Hospital, Adelaide, South Australia, Australia
[2]School of Medicine, University of Adelaide, Adelaide, South Australia, Australia
[3]Mater Medical Research Institute, South Brisbane, Queensland, Australia
[4]School of Medicine, University of Queensland, Brisbane, Queensland, Australia
[5]Division of Cancer Medicine, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Transplantation using 2-5 x 10^6 mobilized $CD34^+$cells/kg BW lowers transplant costs and mortality. Mobilization is most commonly performed with recombinant human granulocyte colony stimulating factor (G-CSF) with or without chemotherapy but a proportion of patients/donors fails to mobilize sufficient cells. Bone marrow (BM) disease, prior treatment and age are factors influencing mobilization but genetics also contributes. Poor mobilization affects patient outcome and increases resource utilization. Mobilization may fail because of the changes affecting the hematopoietic stem/progenitor cell (HSC)/BM niche integrity and chemotaxis. Until recently increasing GCSF dose and adding SCF have been used in poor mobilizers with limited success. However plerixafor through its rapid direct blockage of the CXCR4/CXCL12 chemotaxis pathway and synergy with G-CSF and chemotherapy has become a new and important agent for mobilization. It efficacy in upfront and failed mobilizers are well established. To maximize HSC harvest in poor mobilizers the clinician needs to optimize current mobilization protocols and to integrate novel agents like plerixafor. These include when to mobilize in relation to chemotherapy, how to schedule and perform apheresis, how to identify poor mobilizers and what are the criteria for pre-emptive and immediate salvage use of plerixafor.

References

1) 1997 Blood
2) 2011 Internal Medicine Journal
3) 2011 Blood

Monday 31 October **1730-1830**
HSANZ Masterclass #3 ***Bayside 104***

How I Approach the Patient with Myelofibrosis

Ruben Mesa

Division of Hematology & Medical Oncology, Mayo Clinic, Scottsdale, AZ , USA

The Masterclass will focus on the current diagnostic and prognostic information used in managing patients with primary and post polycythemia vera/post essential thormbocythemia vera myelofibrosis. It will discuss the thought process, and data, between choosing between individualized treatment approaches which currently exists including allogeneic stem cell transplant. JAK2 inhibition, and the potential alteration of management of MF patients, will be discussed. Finally, challenging scenarios in managing MF patients will be discussed in this interactive session.

Monday 31 October **1730-1830**
HSANZ Masterclass #4: ***Bayside 105***

Problems in Iron Chelation - How to Tailor Therapy to the Individual Patient

John Porter
Department of Haematology, University College London, UK

The tailoring of chelation to an individual requires two distinctive but complementary approaches. The first is a scientific understanding of iron overload, its monitoring and its treatments. The second is an understanding of the particular issues impacting on an individual's ability to adhere to the challenges of lifelong therapy. Effective chelation requires the implementation of a regime that balances iron intake with iron excretion. The iron intake rate is easily derived from a record of an individual's transfusion history over a measured time. Evidence based guidelines of the doses of deferasirox (DFX) or desferrioxamine (DFO) required to maintain iron balance, based on the transfusional iron-loading rates are available *(Cohen, Glimm, Porter (2008) Blood, 111, 583-587).* If a dangerous excess of body iron has already developed, (as evidenced by unacceptably high serum ferritin or liver iron concentration (LIC) values), or there is excess myocardial iron (a low myocardial T2*), treatment intensification is required. Patients and carers need to understand that, because the majority of body iron stores are *not* available for chelation at any moment, simple dose escalation without consideration of the duration of treatment exposure may be ineffective and also increase drug toxicity. The long half-life of DFX usually allows dose escalation with efficient removal of excess liver or myocardial iron. DFO and DFP have short plasma half-lives but by alternating DFO with DFP, more efficient removal of body iron and myocardial iron can be achieved than with mono-therapy of either agent. In the presence of myocardial dysfunction, intensive chelation with 24h DFO is indicated with the possible addition of DFP. Long-term chelation treatment will not work unless taken without significant interruptions. Success in achieving uninterrupted therapy requires an environment where continuity of care is provided with both expert practical and psychological support *(Evangeli, Mughal, Porter (2010) Hemoglobin, 34, 305-21).*

Monday 31 October **1730-1830**
ANZSBT Masterclass 5 ***Bayside 201***

Effects of Platelet (Plt) Dose on Transfusion Outcomes

Sherrill J Slichter
Puget Sound Blood Center and University of Washington School of Medicine; Seattle, Washington, USA

Effects of Plt Dose on Post-Transfusion Plt Responses
At plt counts <100,000/µl, there is a direct relationship between plt counts and plt survivals. Therefore, as expected, in three studies that gave increasing plt doses to the same or different patients, higher doses produced greater plt increments and longer transfusion intervals.

Effects of Plt Dose on Hemostasis
Four trials randomly assigned patients to receive all their plt transfusions at a given dose. Three studies enrolled only 101 to 109 patients and were too small to allow many conclusions. However, none showed a significant increase in WHO ≥ Grade 2 bleeding between the arms. In the fourth study, median dose (MD) (2.2 x 10^{11} plts/m^2 BSA), low dose (LD) at half the MD, and high dose (HD) at twice the MD transfusions were randomly assigned to 1,272 patients. MD was considered equivalent to the current standard dose of six pooled plt concentrates or one apheresis collection. Twenty-five percent of the enrolled patients received chemotherapy for acute leukemia, 34% had an autologous stem cell transplant (SCT), and 41% an allogeneic SCT. The frequency of WHO ≥ Grade 2 bleeding was 70% overall but was 79% for allogeneic SCT, 73% for chemotherapy, and 57% for autologous SCT patients ($p<0.001$ compared to the other groups), but there were no bleeding differences related to plt dose. The total number of plts transfused was higher with increasing doses, while transfusion frequency decreased. LD was the most cost-effective transfusion strategy as long as plt costs were based on dose transfused.

Conclusions
Plt doses between 1.1 to 2.2 x 10^{11} plts/m^2 BSA have no effect on bleeding rates. Patients' treatment does affect bleeding rates independent of dose; i.e., allogeneic SCT > chemotherapy > autologous SCT. Low doses decrease plts transfused but increase transfusion frequency.

Monday 31 October **1730-1830**
ANZSBT Masterclass 6 ***Bayside 202***

Strategies for Donor Screening for TRALI

Hitoshi Okazaki
Japanese Red Cross, Tokyo, Japan

TRALI is one of the most serious complications of blood transfusion. A substantial number of TRALI cases has been caused by donor HLA and/or HNA antibodies generated in alloimmunized, i.e., parous females. Considering this mechanism, TRALI mitigation strategies such as the use of male-only plasma have been implemented in many blood centres or countries, which successfully reduced the number of TRALI cases caused by fresh frozen plasma.
This strategy may be applied to other plasma-rich products, such as platelet concentrates, if the supply of these products is abundant. In most countries and blood centres, this may not always be the case. In Japan, approximately 25 % of apheresis platelet donors are females, and if we exclude females from donation, there will be a shortage of these products. The strategies related to donor recruitment can be implemented if public consensus on female exclusion is successfully established. However, asking female donors about pregnancy history is a relatively sensitive issue. Asking personal questions can be replaced by testing for the presence of the HLA antibody, that is, donors are notified that an HLA antibody test is required for apheresis donation.
There are some disadvantages to the HLA antibody test for TRALI mitigation. This test does not eliminate donors who have leukocyte-activating antibodies other than HLA antibodies. Moreover, if the cut-off is set at a very low level, most of the relatively safe donors may be eliminated. At this moment, the cut-off level is decided considering the balance between the percentages of donor loss and secure supply. We have recently shown the relationship between the strength of the HLA antibody and TRALI development in a retrospective observational study. This will allow us to set the cut-off level on the basis of scientific evidence.

Monday 31 October **1730.1830**
ANZSBT Masterclass 7 ***Bayside 203***

Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic Uremic Syndrome (HUS): Patients' Stories

James N George
Departments of Medicine and Biostatistics & Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

Observations from the 415 patients in the Oklahoma TTP-HUS Registry emphasize the difficulty of diagnosis, the diversity of the TTP and HUS syndromes, and the importance of long-term follow-up. The diagnosis of TTP or HUS is difficult because the spectrum of severity is great and because multiple disorders may present with microangiopathic hemolytic anemia and thrombocytopenia. Patients with severe ADAMTS13 deficiency (activity <10%) may have minimal symptoms; the thrombocytopenia and anemia are often initially attributed to primary immune thrombocytopenia (ITP) with anemia due to blood loss. Although platelet transfusions are commonly considered to be dangerous, they are commonly given as part of initial management before the diagnosis of TTP is considered; we have observed no evidence of harm. In 31 patients the presenting clinical features were subsequently attributed to a systemic infection caused by 17 different organisms. In 10 patients the presenting clinical features were subsequently attributed to 8 different systemic malignancies. Six patients with a systemic infection or malignancy had severe ADAMTS13 deficiency; 4 had a demonstrable inhibitor. Therefore severe ADAMTS13 deficiency is not specific for TTP. Multiple drugs have been reported to cause acute, presumably immune-mediated TTP-HUS, but drug-dependent antibodies have only been documented in quinine-induced TTP-HUS and repeated episodes of TTP-HUS with repeated drug exposure have only been reported for quinine. Among 52 Registry patients with a suspected drug-induced etiology, quinine was the suspected drug in 25 patients; 2 had multiple episodes of quinine-induced TTP-HUS before the etiology was recognized. Long-term follow-up of all patients is critical. Most patients who recover from quinine-induced TTP-HUS have chronic kidney disease. For patients with severe ADAMTS13 deficiency, there is risk of relapse, a possible increased risk for other autoimmune disorders, the common occurrence of minor cognitive abnormalities, but no evidence of persistent kidney abnormalities.

Monday 31 October **1730-1830**
ASTH Masterclass 8 ***Bayside 204***

Hypercoagulability in Thalassemia

Pantep Angchaisuksiri
Division of Hematology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Thalassemia is a hereditary hemolytic anemia characterized by impaired globin chain synthesis. Standards of care for thalassemic patients have improved in recent years resulting in increasing average life expectancy. As a consequence, additional previously undescribed complications are now being recognized. The presence of a higher than normal incidence of thromboembolic events and the existence of prothrombotic hemostatic abnormalities, particularly in splenectomized patients, have led to the recognition of the existence of a chronic hypercoagulable state in thalassemic patients. Thrombosis at both venous and arterial sites has been reported. In Thailand, almost all show thrombosis at the pulmonary site. Splenectomized thalassemic patients have an increased amount of circulating phosphatidylserine exposing red blood cells (RBCs) which can facilitate the coagulation process. A small amount of generated thrombin can sensitize platelets which are known to be persistently and markedly increased after splenectomy. Increased activation of both coagulation and platelets has been shown to occur in these patients. Resulting widespread and chronic thrombosis in small pulmonary arteries can result in thrombotic pulmonary arteriopathy leading to pulmonary arterial hypertension and hypoxemia. Chronic inflammatory state and increased amount of poorly deformed nucleated RBCs after splenectomy could facilitate both the vascular endothelial cells-RBCs interaction and coagulation process in the microcirculation. Other contributing factors in the hypercoagulable state are decreased plasma levels of natural coagulation inhibitors (protein C, protein S, antithrombin) and decreased clearance of activated clotting factors in asplenic state. Suggested strategies for the prevention of thrombotic complications in patients with thalassemia are: avoid splenectomy and transfuse RBCs as necessary according to hemoglobin values with adequate iron chelation, use long-term aspirin to inhibit platelet aggregation, control high platelet counts with hydroxyurea, consider antioxidants to reduce excessive oxidative stress to the RBCs, give peri-operative thromboprophylaxis in patients undergoing major surgery. These approaches should be evaluated in the controlled clinical trials.

Monday 31 October **1730-1830**
Nurses Masterclass 9 ***Bayside Terrace***

Nurse Prescribing and Transfusion

Elizabeth Pirie
Scottish National Blood Transfusion Service, Edinburgh, Scotland

A collaborative project between the Scottish National Blood Transfusion Service (SNBTS) and NHS Blood and Transplant (NHSBT) explored the feasibility of nurses and midwives 'prescribing' blood components, and identified that there were no legal barriers to this role development. As good governance is central to all advanced practice a governance Framework document was developed to support practitioners who wished to undertake this role, and is available at *www.transfusionguidelines.org.uk*.
In Scotland there is support for the implementation of this initiative from the Chief Nursing Officer and Nurse Directors of NHS Scotland Boards, where service need is identified. A Blood Authorisation working group has been established which includes representation from the Scottish Government, NHS Quality Improvement Scotland, NHS National Education Scotland, SNBTS, clinicians and educators from the Boards. The aim of the group is to support and encourage a consistent and national approach to nurse prescribing of blood components.
To date the clinical areas that have shown interest are specialist areas such as neonatology, haematology, and intensive care. The nurses within these areas are experienced, senior nurses working at advance practice level. This project has shown that there is a service gap for patients who require blood transfusion support, and by using the untapped knowledge and expertise of these experienced practitioners there is the potential to achieve high quality person-centred, clinically effective and safe healthcare.

Tuesday 1 November **0830-1000**
HSANZ Symposium 7: Novel Agents ***Auditorium B***

Oral Arsenic Trioxide in the Management of Acute Promyelocytic Leukaemia

Yok-Lam Kwong
Department of Medicine, Queen Mary Hospital, Hong Kong

Arsenic trioxide (As_2O_3) has been used medicinally for centuries. It was first used in the treatment of chronic myelogenous leukemia in the late nineteenth century. In the 1970s, As_2O_3 was found to be highly efficacious in the treatment of acute promyelocytic leukaemia (APL) in China. This observation was confirmed worldwide about a decade later. As_2O_3 is now a standard and the most efficacious medication for the treatment of relapsed APL. It is also being used in the frontline treatment and consolidation of remission in newly diagnosed APL.

As_2O_3 is given intravenously. An important problem is QTc prolongation, which may lead to ventricular arrhythmias if other co-morbidities are present. The problem is related to a rapid increase in blood arsenic level during intravenous infusion, as QTc is directly proportional to the arsenic level. Furthermore, intravenous As_2O_3 is inconvenient, limiting its use as a maintenance medication.

An oral formulation of As_2O_3 has been developed in our unit for APL treatment. Pharmacokinetic studies have shown that oral-As_2O_3 is very well absorbed, achieving about 90–95% bioavailability as compared with an equal dose of intravenous As_2O_3. However, because of slow intestinal absorption, a much lower peak blood arsenic level is reached. Therefore, QTc prolongation is not a problem with oral-As_2O_3, rendering the medication very safe for home administration. Efficacy studies have shown that oral-As_2O_3 achieves the same complete remission (CR) rates as intravenous As_2O_3 in relapsed APL.

Oral-As_2O_3 makes maintenance arsenic treatment for APL realistic. In a study of 76 APL patients, who attained CR1 after induction with all trans retinoic acid (ATRA), daunorubicin and cytarabine, and received consolidation with daunorubicin and cytarabine; maintenance treatment with ATRA, As_2O_3 and ascorbic acid for 2 years led to three-year leukaemia-free-survival, event-free-survival and overall-survival of 87.7%, 83.7%, and 90.6% respectively. Conventional risk factors including high white cell count and low platelet count at presentation, observed previously with chemotherapy as consolidation and maintenance therapy, were not significant when As_2O_3 was incorporated as part of the maintenance treatment.

The efficacy of As_2O_3 in maintaining APL CR2 or beyond has also been tested. With maintenance employing ATRA, As_2O_3 and ascorbic acid in APL of ≥ CR2, the 3-year and 5-year overall-survivals were 79% and 66%. The 3-year and 5-year actuarial event-free-survivals were 70% and 64%. Therefore, a proportion of APL patients ≥ CR2 can clearly achieve durable remission with As_2O_3-based treatment. These results show that hematopoietic stem cell transplantation (HSCT) is not always needed for APL in ≥ CR2, which is very different from other AMLs, where CR2 is regarded as not durable unless consolidated by HSCT. Since the advent of oral-As_2O_3, allogeneic HSCT for APL is no longer performed in our unit.

Our observations have shown that oral-As_2O_3 is safe and effective as a maintenance therapy for APL in remission. Its role in the induction and consolidation of newly diagnosed APL will need to be critically addressed in future studies.

HAA 2011 HSANZ ANZSBT ASTH

Tuesday 1 November **0830-1000**
HSANZ Symposium 7: Novel Agents ***Auditorium B***

JAK2 Inhibitors for Myeloproliferative Neoplasms: Impact Six Years After JAK2-V617F Discovery

Ruben A Mesa
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The watershed discovery of the JAK2-V617F mutation in 2005, and the high prevalence of this mutation in the Philadelphia Chromosome negative myeloproliferative neoplasms (MPNs), has led to a renaissance in the science and therapy of these disorders. Inhibitors of JAK2 entered clinical trials in 2007 and ushered in an unprecedented era of trials with agents specifically designed for MPNs. Currently 10 different JAK2 inhibitors have been tested in myelofibrosis (MF) with varying degrees of specificity for the JAK2 kinase. The first of this group, ruxolitinib, recently reported data from 2 large phase III trials demonstrating that JAK2 inhibition was superior to placebo (USA and Australia Trial – Comfort 1) or best available therapy (EU Trial – Comfort 2) for reducing massive splenomegaly and improving MF associated symptoms. Additional agents with significant experience include TG101348, CYT387 (which a possible additional benefit in anemia has been described), and SB1518 in which minimal myelosuppression has been observed (a not infrequent issue with the others). In MF these agents have shown near universal ability to decrease pathologic splenomegaly, and improve disease associated symptoms. However, ability of these agents to significantly impact disease associated cytopenias, JAK2 allele burden, or bone marrow histologic features remains unclear. JAK2 inhibition in polycythemia vera (PV) and essential thrombocythemia (ET) for this class of agents appear promising to reduce myeloproliferations, symptoms, and perhaps prevent thrombohemorrhagic events. Alternative agents (with alternative targets), used either alone, or in combination might perhaps further augment the spectrum of efficacy and therapeutic options for MPNs.

Tuesday 1 November **0830-1000**
HSANZ Symposium 7: Novel Agents ***Auditorium B***

Best Practice in the Management of Myelodysplastic Syndromes

Ghulam Mufti

Abstract not received at the time of going to print

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 1 ***Auditorium A***

O078 0830

Development of a Model for Understanding Clinical Demand for Red Blood Cell Transfusion

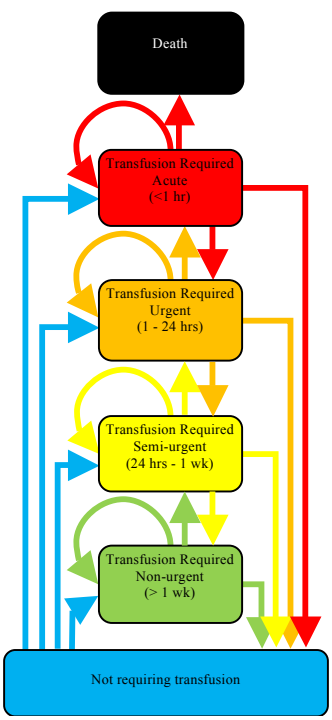
L Phillips[1], Z McQuilten[1,4], F Hong[4], N Aoki[1], C Stevenson[2], R Brown[4], A Cheng[3], R Hall[3], E. Wood[4]
[1] Transfusion Outcomes Research Collaborative, 2 Epidemiological Modelling Unit 3 Infectious Disease Unit, Department of Epidemiology and Preventive Medicine, Monash University, Vic [4] The Australian Red Cross Blood Service, Melbourne, Vic

Background
A major disaster, such as an influenza pandemic, has the potential to adversely impact sufficiency of blood supply through the interaction between the event itself, blood supply and clinical demand. Factors influencing clinical demand in such an event are poorly understood. In order to meet the challenges of such a major event, a greater understanding of the factors involved and their interaction is required.

Aim
To develop a model of clinical demand for red blood cells (RBC) to inform contingency planning.

Method
Data were extracted and analysed from a variety of sources, including published reports of RBC use and urgency. Additional data were collected from the medical records of 240 patients who received RBC issued as part of the BloodHound study at three major tertiary hospitals in Melbourne to assess the specific indication and urgency of transfusion and likely outcomes in the event of RBC non-availability.

Results
RBC use was categorized into 14 disease categories and 4 urgency levels. For each of the resultant 56 disease/urgency states, estimates were made of likely patient outcomes if RBC were not available. This information was used to develop the model of clinical demand for transfusion. When supply of RBC meets or exceeds demand, the model of transfusion is simple. Where RBC supply is insufficient the model is more complex, with possible outcomes ranging from no transfusion required, to escalation of urgency and possible death (see Figure).



Conclusion
Availability of clinical demand models for RBC will allow projection of the impact of disasters (such as pandemics) on demand, modelling of effects of different strategies of restriction/triage of RBC and will facilitate preparedness for future emergency events.

This research was supported by the Australian Red Cross Blood Service

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 1 ***Auditorium A***

O079 0845

Casting the Net on the Incidence of Critical Bleeding: Massive Transfusion Event Identification Using Multiple Definitions

Amanda Zatta[1], Biswadev Mitra[1,2], David Roxby[3], Romi Sinha[3], Susan Whitehead[2], Zoe McQuilten[1,4], Scott Dunkley[5], Erica Wood[4], Louise Phillips[1]

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Background

Clinical management of critical bleeding (CB) patients requiring a massive transfusion (MT) is based on clinical criteria rather than a definition of MT. However definitions of MT are used as selection criteria for studies that inform guidelines and clinical practice in these patients. A commonly used definition of ≥10 units of red cells (RBC) in 24 hours excludes patients that die early. An alternate definition of ≥5 units of RBC in 4h has gained credibility, however it is unclear what CB events may be 'missed' by using this acute definition.

Aim

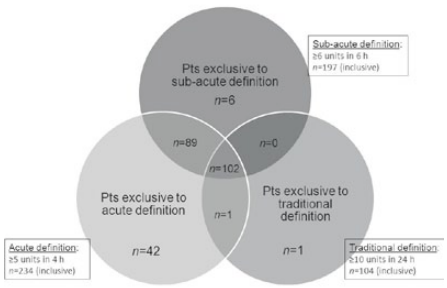
To examine completeness of capture of CB events included according to three different definitions of MT.

Methods

Ethics approval was obtained to review data on all patients receiving RBC transfusions in 2010 at the Alfred Hospital (Vic) and Flinders Medical Centre (SA). MT patients were identified using three definitions: ≥10 units RBC in 24 hours (10/24), ≥6 units RBC in 6h (6/6) and ≥5 units RBC in 4h (5/4).

Results

241 patients met at least one MT definition, of which 102 (42%) were included by all definitions (see figure). The most inclusive definition was 5/4 (234 patients, 97%) followed by 6/6 (197, 82%) and 10/24 (104, 43%). Only one patient in 10/24 was excluded from 5/4 but 137 patients in 5/4 were excluded from 10/24.



Conclusions

An acute definition of MT includes a group of patients that have been under-represented in current literature through exclusion by current definitions of MT. These patients may be likely to benefit from improvements in transfusion practice in CB events. The Transfusion Outcomes Research Collaborative has recently established a Massive Transfusion Registry that will utilise this more inclusive definition to maximise understanding of the treatment of critically bleeding patients.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 1 ***Auditorium A***

O080 0900

Day 28 and Day 42 Stored PRBC Significantly Reduce Recipient Cell Proliferation in a Whole Blood Transfusion Model

Kelly M Rooks[1], Luke Samson[1], Lacey Johnson[2], Robert L Flower[1], Melinda M Dean[1]
1 Research and Development Division, Australian Red Cross Blood Service, Brisbane, QLD, Australia. 2Research and Development Division, Australian Red Cross Blood Service Sydney, NSW, Australia

Background
The mechanisms involved in transfusion related immunoregulation and poor outcomes in some patients post transfusion remain largely unknown. An *in vitro* whole blood model of transfusion was established to investigate recipient cell-proliferative responses.
Methods
Fresh whole blood was cultured with ABO compatible leukodepleted packed red blood cells (PRBC) at 25% blood replacement volume for 6 hours. LPS was added in parallel to mimic infection. Leukocyte proliferation was assessed on recipient PBMCs isolated after 6hr exposure in the transfusion model. PBMC were then stimulated (±CD3/CD28, ± PHA-M) and proliferation assessed on D4 (tetrazolium conversion). PRBC were assessed through the duration of storage against the same recipient at Day (D)2, D14, D28 and D42. Changes in proliferation were calculated by comparison to the same recipient without components. Results are presented as a ratio to non-transfused controls. P values were calculated by comparing recipient cell proliferation in the 25% PRBC transfusion.
Results

PRBC	Mean Fold Change	Range	P value	No. of bags
D2	2.27	0.87 - 5.50	N/A	10
D14	0.61	0.14 - 0.93	0.7909	14
D28	0.37	0.15 - 0.52	0.0003	6
D42	0.52	0.39 - 0.64	0.0019	6

Recipient leukocyte proliferation was significantly decreased in the presence of D28 and D42- PRBC. The addition of bacterial component LPS and specific cell stimulatory reagents (CD3/CD28 or PHA-M) did not overcome the reduced cell proliferative response in the presence of D28 and D42-PRBC.
Conclusion
Stored blood components **(**D28 and D42-PRBC) significantly reduced recipient leukocyte proliferation, which was also seen in co-culture with LPS or specific proliferation inducing agents. These data support previous studies of blood component modulation of isolated T-lymphocyte proliferation. The complexity of the transfusion context was reflected in the whole blood approach for initial stimulation. A significant reduction in leukocyte proliferation may contribute to patient outcomes, such as increased risk of infection and longer hospital stay, following blood transfusion.
No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 1 ***Auditorium A***

O081 0915

Can Final Year Medical Students Safely Collect a Sample of Blood for Crossmatching?

Claire Dendle, Jennifer Conn, Chris Hogan, Erica Wood
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Background
Haemovigilance programs aim to capture and analyse adverse events related to transfusion. Many of these events are process-related, particularly in the areas of patient identification and pre-transfusion sample labelling, and haemovigilance reports continue to highlight problems in hospital systems and practice, inter-professional communication and error prevention. Concurrently, there is growing recognition of the importance of promoting the principles of quality and safety in medical school education and these are increasingly being included as curriculum topics.

Aim
The aim of this study was to assess the skills of medical students in patient identification and pre-transfusion sample labelling – key steps in the transfusion safety chain.

Methods
We analysed the performance of a cohort of 90 final year medical students in a hybrid Objective, Structured Clinical Examination designed to assess collection and labelling of a crossmatch sample. All had recently completed a workshop in blood transfusion skills as part of a vertically-integrated curriculum in haematology.

Results
Only 18% students correctly confirmed patient identity by checking both name and wristband. Fifty-three per cent of students gained fewer than 50% of the marks available for labelling the crossmatch sample tube, and many students did not accurately complete one or more elements of the pathology request form. In contrast, most students scored highly with respect to venepuncture skills.

Discussion
Students performed well in the technical aspects of collecting a sample for crossmatch but not in attention to detail and accuracy with respect to identity checking, despite recently participating in a workshop on transfusion skills. These findings reflect the need for integrated approaches to promoting transfusion quality and safety in medical education. This important aspect of practice, including generic issues around patient identification, is currently under-emphasised in medical school curricula.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 1 ***Auditorium A***

O082 0930

Survival After Massive ABO-Incompatible Blood Product Transfusion Following Multiple Trauma, A Case Report

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Department of Haematology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, NSW ,Australia.

We report the case of a 40 year old man who sustained head trauma, chest trauma with haemothorax, multiple fractures of the skull, facial bones, spine, pelvis, and limbs as a consequence of a high speed motor vehicle accident. Initial crossmatching determined his blood group to be A positive. The injuries required surgical intervention, and he required transfusion support according to the institutional massive transfusion protocol. In total he received 14 units of packed red cells (4 un-crossmatched O positive units, and 10 A positive units), 10 units of A positive FFP, 8 units of A positive cryoprecipitate, and two A positve pooled platelet concentrates. He then received standard care in the intensive care unit.
Six days after transfusion he developed features of haemolysis, and repeat testing indicated that his blood group was in fact O positive. DNA testing of the initial crossmatch specimen, and of the patient, confirmed that the initial specimen was not collected from the patient. Thus, the patient had been massively transfused with ABO incompatible blood products.
The patient underwent emergency exchange transfusion with 10 units of O positive packed red cells, and the patient recovered completely from his haemolysis and avoided major complications from the transfusion of ABO incompatible blood.

No conflict of interest to disclose.

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 1 ***Auditorium A***

O083 0945

Improving Safety of Blood Administration at Alfred Health

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Haematology Unit, Alfred Pathology Service, The Alfred Hospital, Prahran, Victoria, Australia

Aim
Transfusion safety is of great importance throughout the world. The final patient identification check at the bedside is the last opportunity to avoid potentially fatal transfusion reactions. After a number of incidents where ABO incompatible blood was transfused due to bedside errors in patient identification, we decided to perform an observational audit of the checking process in our institution. This subsequently led to an education program followed by introduction of a pilot project using proven technology to assist nursing staff in safely administering blood components.

Method
An audit tool was produced based on that used by Turner C.L. et al[1] that listed all the components of the checking procedure. In order to comply, both staff needed to complete each component individually and in the presence of the other staff member.

Results
60 transfusion episodes were assessed across a range of wards. Thirty were prior to any interventions and thirty were post implementation of a mandatory on-line education tool, highlighting the administration process. While there was some improvement post implementation of the education program, it was minimal. In order to reduce or eliminate the risk of ABO incompatible transfusion, a trial of an electronic tool to perform the bedside check was undertaken. This system uses a handheld computer which assists the user to positively identify the patient using a 2D barcode on the patient wrist band, and then ensuring the blood product is compatible with this patient by using a 2D barcode on the compatibility label. Preliminary results show significant improvement consistent with published data.

Conclusion
Our audit of blood administration at various stages of implementation of firstly an education program, followed by introduction of a patient identification system using 2D barcode technology, demonstrates improvement in patient safety.

References
1. Turner CL, Casbard AC and Murphy MF Barcode technology: its role in increasing the safety of blood transfusion. Transfusion 2003;43(9):1200-9.

No conflict of interest to disclose.

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 2 ***Bayside 104***

O084 0830

Perioperative Patient Blood Management Guidelines – the Likely Need for System Changes

Chris Hogan, Paul Hyland, Jen Roberts
National Blood Authority, Canberra, ACT, Australia
And on behalf of the Clinical Reference Group: Larry McNicol, Zsolt Balogh, Shannon Farmer, Craig French, Russell Gruen, Richard Seigne, Daryl Teague, Amanda Thompson, Philip Truskett, John Vinen

Background
Currently, a suite of clinical scenario based Patient Blood Management Guidelines (PBMGs) are being developed to replace the 2001 NHMRC/ASBT Appropriate Blood Use Guidelines, which were product based. These PBMGs are being developed under the auspices of the NHMRC by the National Blood Authority, the Australian and New Zealand Society of Blood Transfusion and a range of relevant medical and other experts. The second module of this suite focuses on Perioperative practice.

Methods
A major systematic literature review was undertaken. The PICO (Patient, Intervention, Comparison, Outcome) schema was used in this review. NHMRC defined processes were used to form an evidence matrix for each question. Following this, formal Evidence Statements were developed for each research question. Arising from these, formal Recommendations and Practice Points were crafted.

Results
Specific Recommendations and Practice Points were developed in a number of areas of Perioperative practice, including: Establishing multidisciplinary multimodal perioperative PBM programs, pre-operative anaemia assessments and interventions, iron therapy and the use of erythropoietin stimulating agents, the timing of cessation of platelet antagonists, haemodilution, thromboelastography, the use of fibrinolytic inhibitors, post-operative cell salvage, transfusion triggers and use of rFVIIa.

Conclusion
There are likely significant logistic and resource implications to the full implementation of the recommendations in these new Perioperative Patient Blood Management Guidelines. These Guidelines are scheduled for release in October 2011.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 2 ***Bayside 104***

O085 0845

Establishment of an Electronic Patient Blood Management (PBM) Data System for Western Australia (WA)

S Aqif Mukhtar[1], Michael Leahy[2,3], James Semmens[1], Julie Tovey[2], Valentina Jewlachaow[2], Audrey Koay[4], Kevin Trentino[4], Simon Towler[4]
1 Centre for Population Health Research, Curtin Health Innovation Research Institute, Curtin University. 2 Department of Haematology, Fremantle Hospital, Western Australia. 3 School of Medicine and Pharmacology, The University of Western Australia. 4 Western Australia Department of Health, Perth, WA

Aim
To develop an electronic PBM database to assemble data on current patterns of blood use.
Method
System feasibility, analysis and design were done by looking at the reporting requirements of the PBM programme in relation to the available data sources. Existing databases were found to already collect much of the required information and are also sufficiently refined to enable interrogation.
A single patient identifier was used to link Patient Administration System (PAS) with Laboratory Information System (LIS). The data linkage was done by linking LIS with PAS records where blood transfusion or lab result date/time fell between admission and discharge date/time. Duplicates due to overlapping admissions or statistical discharges were then flagged and removed. The two datasets were consolidated into the PBM database for further processing and interrogation.
Result
We managed to successfully link 33,038 transfusions (99.5% of total transfusions) with 194,142 patient admissions in pilot hospital during 01/11/2007 to 31/12/2010. During that period, 4,681 patients (6% of total patients) were transfused with an average of 2.8 RBC units per patient admission. Haematology (22%), General Medicine (20%) and Orthopaedics (14%) were the top users of blood.
Results comparing 1st half of 2010 (1H-2010) with 1st half of 2009 (1H-2009) showed that during 1H-2010 the RBC usage decreased by 5% despite 8% increase in patient activity. Also, the proportion of single unit use increased from 18% to 26% during 1H-2010 ($P<0.001$). Compared with 1H-2009, 435 RBC units were saved during 1H-2010. This reflects a cost saving of $609,606 p.a.
Conclusion
We have created a working data linkage system between patient admissions, transfusions and lab results which identifies key users of blood and target patient groups. This project has measured the impact of the PBM program at pilot hospital and provided an impetus for the WA wide patient blood management system.
No conflict of Interest to disclose

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 2 ***Bayside 104***

O086 0900

First Do No Harm: Ensuring the Good of the Patient is the Highest Priority

Rachel Donegan
New Zealand Blood Service, Auckland, New Zealand

What happens when a therapy thought to be safe demonstrates possible harm to our patients? Blood has always been associated with life and vitality, however many publications associate transfusion with increased mortality, increased post-operative infection and longer hospital stays.

A one week externship in "Patient Blood Management and Bloodless Medicine and Surgery" at Englewood Hospital and Medical Centre, New Jersey, USA, made possible thanks to the CSL Biotherapies Travel Award 2010 identified strategies to minimise patient exposure to allogeneic blood.

My experience identified critical processes that inform health professionals of blood management and utilisation, reduce blood wastage and aid anaemia management. The practice of Patient Blood Management at Englewood involves medical and surgical techniques as well as technology and behavioural strategies to decrease blood loss and enhance a patient's own blood supply. An entire healthcare team of physicians, nurses, pathologists, pharmacists, dietitians and support staff work together to ensure optimal haemoglobin levels. Time spent with medical and nursing staff in outpatient clinics, ICUs,,theatres and wards demonstrated the staff commitment to Blood Management. All medical and nursing staff at Englewood receive ongoing education in Bloodless Medicine.

I attended the Blood Utilisation Committee meeting with representatives from departments throughout the hospital. Discussion included

- Blood and blood products use review
- Perioperative and autologous blood collection service report
- Review of appropriateness of all inpatient transfusions over the last 3 months.
- Management of transfusion-dependent patients.

At Englewood, hospital policy dictates that the clinician ordering blood must consent the patient. This ensures the standard of consent is met and current risk/benefit information is supplied.

My experience has identified the need to reinforce a restrictive transfusion strategy to reduce inappropriate transfusions. My hope is to integrate these strategies into patient management at Auckland City Hospital and adapt them to the New Zealand environment.

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 2 ***Bayside 104***

O087 0915

A Survey of the Effectiveness of Autologous Serum Eyedrops (ASED) for Keratoconjunctivitis Sicca and Non-Healing Corneal Ulcers

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The Australian Red Cross Blood Service, Alexandria, NSW, Australia

Aim
The Blood Service in NSW has collected whole blood donations for ASED since 2006. A survey was undertaken of patients referred in 2010 to assess the effectiveness of ASED in relieving ocular symptoms and improving visual-related functioning and quality of life.
Method
Demographic details of NSW patients referred in 2010 were extracted from the Blood Service ASED database. Patients who used ASED were asked to complete a survey based on several dry eye questionnaires (National Eye Institute Visual Functioning Questionnaire-25, Ocular Surface Disease Index, Dry Eye Questionnaire 2001 and Impact of Dry Eye on Everyday Life questionnaire). The survey assessed their health status, ocular symptoms, visual functioning and quality of life pre- and post-ASED.
Result
Most of the 38 patients surveyed rated ASED highly with a median score of 7.5 (0 not effective, 10 extremely effective). Underlying conditions included Sjögren's syndrome, Stevens-Johnson syndrome, graft-versus-host disease post-bone marrow transplant and non-healing corneal ulcers after chemical burns.
ASED were effective in alleviating most of the ocular symptoms, especially pain and dryness. Over 50% experienced an improvement in their visual-related functioning (e.g. reading and driving) as well as quality of life with ASED. Positive comments included "immediate relief" or "instantaneous improvement". 24% had minor side effects, most commonly stinging. 58% were supportive of the use of homologous serum eyedrops.
Conclusion
ASED manufactured by the Blood Service were effective in alleviating ocular symptoms and improving visual-related functioning. They were generally well tolerated. Most patients were supportive of the potential for the Blood Service to manufacture a homologous product.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 2 ***Bayside 104***

O088 0930

Management of Fetomaternal/Neonatal Alloimmune Thrombocytopenia (NAIT): An Evaluation of Current Australian Practice

Zoe McQuilten[1,3], Stephen Cole[4], Mark Davies[5], Rhonda Holdsworth[3], Helen Savoia[4], Ben Saxon[3,6], Bronwyn Williams[5], Erica Wood[2,3], Louise Phillips[1]
1 Transfusion Outcomes Research Collaborative, Department of Epidemiology and Preventive Medicine, & 2 Department of Clinical Haematology, Monash University, Melbourne. 3 Australian Red Cross Blood Service, Melbourne, Vic.
4 Royal Women's Hospital, Melbourne. 5 Royal Brisbane and Women's Hospital, Brisbane, Qld. 6 Women's and Children's Hospital, Adelaide, SA.

Background
Optimal protocols for NAIT investigation and management remain uncertain and there is evidence of substantial variation in practice. The recently established Australian NAIT Registry is open for recruitment at 16 sites nationwide.
Method
Multiple-choice survey (multiple option answers, opportunity for additional comments) sent to clinicians treating NAIT at 21 Australian hospitals to document current management practices.
Results
Responses were received from 13 hospitals. Three have protocols for NAIT management. Regarding antenatal management of high-risk pregnancies (history of antenatal intracranial haemorrhage [ICH]), 80% commence intravenous immunoglobulin (IVIg) before 20 weeks and 10% at 20-24w, with 90% using 1g/kg/week and 10% 2g/kg/week. None routinely perform fetal blood sampling (FBS) before commencing or to monitor response to IVIg. 30% never and 70% sometimes perform FBS for these indications. For women with a history of NAIT without ICH, 20% commence IVIg before 20 weeks and 60% at 20-24w. 20% sometimes and 70% never perform FBS to monitor response. 50% use maternal prednisolone (40% 0.5mg/kg/day, 60% 1mg/kg/day) for high-risk pregnancies or suboptimal response to IVIg. 60% always and 40% sometimes recommend elective caesarean section for suspected/confirmed NAIT. None use fetal platelet count or obstetric indication alone to direct mode of delivery. Regarding management of non-bleeding term infants, 67% use a platelet count of 30 $x10^9$/L and 33% use 10 $x10^9$/L as the treatment threshold. For non-bleeding pre-term infants, 67% use 50 $x10^9$/L and 33% use 30 $x10^9$/L as the treatment threshold. 50% use IVIg for non-bleeding infants and 67% use IVIg if there is evidence of bleeding. 57% routinely perform ultrasound screening for ICH.
Conclusion
The survey confirms substantial variability in current management of NAIT in Australia. The establishment of the national NAIT Registry will aid further study of this rare but important disorder and assist in defining optimal management in the Australian setting. More information: http://www.torc.org.au/nait.html

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ANZSBT Free Communications 2 ***Bayside 104***

O089 0945

A Serological Investigation of Dengue Virus Exposure in Blood Donors During the 2008/2009 Dengue Epidemic in North Queensland

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[2]*James Cook University, Cairns Campus, Cairns, QLD*

Background
The potential for transmission of Dengue virus (DENV) infection poses a risk for safe transfusion of blood when the virus is present, with confirmed reports of transfusion-transmission from Hong Kong and Singapore. The largest outbreak in 50 years occurred in north Queensland during 2008/2009, with more than 1,000 confirmed cases in Cairns and Townsville. During this outbreak, the Australian Red Cross Blood Service implemented the DENV management strategy, which consisted of supplementary questioning for all donors travelling to the region, and restricted fresh component manufacture from at risk donors. This study aimed to determine the prevalence of DENV exposure in the blood donor population during this epidemic.
Methods
Additional plasma samples were collected during the epidemic and tested for anti-DENV IgM with ELISA-based assay kits. Confirmation testing was performed at a reference laboratory when donations were repeat reactive on the ELISA kits.
Results
Six of 5,052 donations collected in Cairns (0.12%: 95% CI: 0.025 – 0.215%) and two of 5,075 donations collected in Townsville (0.04%: 95% CI: 0 – 0.095%) showed serological evidence of recent exposure. Of the six Cairns donations (from five donors) with anti-DENV IgM, all showed serotype-specific reactivity towards DENV-3. Of the two Townsville donations (from two separate donors) confirmed for anti-DENV IgM, one showed serotype-specific reactivity towards DENV-2, whilst the other did not display specific reactivity towards any of the four DENV serotypes. An additional donation from Townsville showing confirmed IgM reactivity against a flavivirus pool, displayed reactivity against Kunjin virus (KUN), another member of the Flaviviridae.
Summary/Conclusions
This study demonstrates evidence of DENV exposure in a self-declared asymptomatic population. Interestingly, 75% of those donations confirmed for anti-DENV IgM demonstrated serotype-specific reactivity towards DENV-3, the most common circulating sero-type during the epidemic. Collectively, this study justifies the use of current DENV management strategy during a DENV outbreak in north Queensland.
No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ASTH Symposium 7: Integrins as Therapeutic Targets ***Bayside 204***

Integrin Signalling and a New Concept in Antithrombotic Therapy

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Integrins are a family of cell adhesion receptors that play important roles not only in cell adhesion to extracellular matrices, but also in cell migration, survival, and proliferation. Blood platelets express members of b1 and b3 families of integrins. In particular, the integrin aIIbb3 (glycoprotein IIb-IIIa) plays a critical role in stable platelet adhesion, platelet aggregation, and thrombus formation. Integrin aIIbb3 typically transmits signals bidirectionally. Intracellular signals induced by various platelet agonists converge into a common “inside-out” signaling process that activates the extracellular ligand binding function of integrin $\alpha_{IIb}\beta_3$. Activated integrin $\alpha_{IIb}\beta_3$ not only mediates platelet adhesion and aggregation, but also triggers so-called “outside-in” signaling, resulting in platelet spreading, granule secretion, stabilization of platelet adhesion, and importantly, amplification of platelet activation, adhesion and aggregation. Thus, agents targeting integrin “outside-in” signaling events may inhibit platelet activation signal amplification without totally abolishing the adhesive function of integrins, which may reduce the risk of bleeding. Recent studies reveal that integrin outside-in signaling requires the interaction between integrin cytoplasmic domains and a member of the heterotrimeric G protein family, Gα13. This interaction mediates activation of Src family kinases and Src-dependent regulation of small GTPases such as RhoA. Agents that interfere with the interaction between integrin β subunits and Ga13 inhibit integrin “outside-in” signals, and thus have the potential to treat thrombosis with reduced risk of bleeding side effect.

Tuesday 1 November **0830-1000**
ASTH Symposium 7: Integrins as Therapeutic Targets ***Bayside 204***

Targeting Integrins in Disease States

Karlheinz Peter
Baker IDI, Heart & Research Institute, Department of Medicine & Immunology, Monash University, Heart Centre, Alfred Hospital, Melbourne, Victoria, Australia

Integrins are integral membrane proteins that mediate cell to cell and cell to matrix interactions, including cell signalling, cell adhesion and aggregation. They are important players in many cellular functions such as haemostasis, immunity, inflammation and tumor biology. Therapeutic targeting of integrins is therefore highly attractive in many diseases. GPIIb/IIIa ($\alpha_{IIb}\beta_3$, CD41/CD61) is the prototype example of both, success as well as failure of therapeutic integrin targeting. Several GPIIb/IIIa inhibitors have been developed, all on the basis of ligand-mimetic inhibitors. These reagents are successfully used as intravenous anti-platelet agents, although with substantial limitations. Unexpectedly, the whole class of oral GPIIb/IIIa inhibitors, which were seen as “superaspirins”, caused an increase in mortality and thus failed badly. The reason for this is most probably the ligand-mimetic effect of these inhibitors causing outside-in signalling and thus paradoxical platelet activation. This clearly exemplifies that ligand-mimetic strategies for integrin inhibition do not take into account that integrins are not only transductors of mechanical force but are also transductors of signals from the cell inside to the outside and vice versa. In several examples of other integrins in addition to GPIIb/IIIa it can be demonstrated that ligand-mimetic inhibition causes cell activation. Finally, alternative strategies of integrin inhibition have been developed, allowing to pursue successful integrin inhibition without side effects. Overall, integrin as therapeutic targets are highly attractive for the potential treatment of many diseases and would certainly cover many areas of medical need. However, the strategy of integrin inhibition is important and may decide on success or failure of these therapeutic strategies in the clinic.

Tuesday 1 November **0830-1000**
ASTH Symposium 7: Integrins as Therapeutic Targets ***Bayside 204***

Glycoprotein IIb/IIIa Antagonists: Current Role and Future Directions

David Brieger
Department of Cardiology, Concord Hospital, Concord, NSW, Australia

There are three intravenously available receptor antagonists abciximab, eptifibatide, and tirofiban. All have been found to be effective in preventing thrombotic complications among patients undergoing percutaneous coronary intervention (PCI), albeit at a cost of increased bleeding. Attention is now focused on the identification of strategies whereby bleeding can be minimised while maintaining antithrombotic potency. These strategies include 1) use of adequate dosing of oral antiplatelet agents in patients undergoing elective PCI with the reservation of the IV agents for those at high thrombotic risk, 2) restriction of the use of these agents among patients with acute coronary syndromes to those undergoing coronary intervention at high thrombotic risk, 3) limited duration of use by delaying therapy until the patients arrive in the catheterisation laboratory. A strategy of identifying the patient at risk of bleeding is also being advocated, with the goal of avoiding these potent agents among these patients. This is proving difficult because factors that predispose to bleeding also mark a greater risk of thrombotic events.
In the short term, use of these drugs may be expanded with the more widespread application of radial access angiography which reduces access related bleeding complications, and patient selection may be further refined through validation and application of more discriminatory bleeding scores.

Tuesday 1 November **0830-1000**
Nurses Free Communications 3 ***Bayside Terrace***

O090

Paper withdrawn

Tuesday 1 November **0830-1000**
Nurses Free Communications 3 ***Bayside Terrace***

O091 0830

Practicalities in the Treatment of Refractory Thrombotic Thrombocytopenic Purpura (TTP) in 2011

Elizabeth Newman[1], David Collins[2], Judith Trotman[1]
1 Concord Repatriation General Hospital, Concord, NSW, Australia. 2 Blood and Marrow Transplant Network, Darlinghurst, NSW, Australia.

Aim
This case study outlines the management challenges addressed by nursing, medical and laboratory staff in treatment of a young woman with refractory TTP.
Background
The patient was transferred from a referral hospital with severe abdominal pain, anaemia, thrombocytopenia and elevated LDH. A clinical diagnosis of TTP was confirmed on a later blood film and the patient was commenced immediately on prednisolone 1mg/kg/d and daily1.5 blood volume plasma exchange (TPE). The exchange fluid was Fresh Frozen Plasma (FFP). Following initial rapid improvement in her clinical and laboratory parameters her condition deteriorated after four days.
Methods/Treatment
Twice daily TPE with Cryoprecipitate Depleted Plasma (CDP) was commenced along with pulse methylprednisolone 500mg/d. Three days later her TPE was temporarily ceased for 36 hours and she commenced on Rituximab (375mg/m^2) weekly for four weeks. TPE was withheld for at least 36 hours after each infusion and gradually reduced in frequency with sustained clinical and laboratory recovery
Results
A number of logistical challenges were addressed by the team, including: vascular access; obtaining large volume replacement fluid for the number of TPEs; and extensive re-rostering of apheresis staff to accommodate the number of collections in addition to simultaneous other routine and emergency apheresis demands. There were also challenges in site preference for specialist blood tests that were required to be sent off -site for testing. The patient had childcare and other issues requiring social work assistance and given the life-threatening nature of her disease, extensive support and education of an internet savvy family was required.
Conclusion
This case history illustrates the logistical challenges that were managed through good communication, negotiation and coordinated teamwork to successfully treat a refractory, and potentially fatal haematologic emergency.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
Nurses Free Communications 3 ***Bayside Terrace***

O092 0845

What Dose of Cyclophosphamide To Use? An analysis of Cyclophosphamide Doses for Stem Cell Mobilisation

Tracy Clarke
Prince of Wales Hospital, Randwick, NSW

Aim

To determine whether;

1. Sufficient haemopoietic progenitor cells are collected by apheresis (HPC-A) for transplant with 2gm/m^2 Cyclophosphamide or whether better results are obtained with 4gm/m^2.
2. There is a higher incidence of febrile neutropenia for patients receiving 4gm/m^2 Cyclophosphamide compared with 2gm/m^2.

Method

A retrospective analysis of patients who received Cyclophosphamide as mobilisation was undertaken. Patients with Multiple Myeloma who received Cyclophosphamide as mobilisation for HPC-A harvest were included.

Data collected for each patient included: Age, gender, Cyclophosphamide dose, CD34 counts, CD34 dose collected, the number of collections required to reach the required dose, the amount of pre treatment received and whether or not they have ever received radiation to the pelvis or sternum. Hospitalisation rate for febrile neutropenia following the Cyclophosphamide administration was also captured.

Result

A total of 32 patients were included in the analysis. The two groups were similar in age, gender and amount of pre treatment. Overall peak peripheral blood CD34 counts were reasonably high and all patients achieved the minimum CD34 collection of 2.0 x 10^6/kg CD34 positive cells required to undergo peripheral blood stem cell transplantation at least once.

Patients who received the higher dose of Cyclophosphamide were more than twice as likely to achieve a CD34 count high enough for 2 transplants with fewer procedures that patients receiving the lower dose of Cyclophosphamide. There was a slight increase in the number of presentations with the higher dose of Cyclophosphamide however numbers were too small to test for statistical significance.

Conclusion

A standard 'one dose fits all' approach may not be appropriate in determining Cyclophosphamide doses for haemopoietic progenitor cell mobilisation for collection via apheresis. The patients' requirement for multiple transplants, the target cell dose for collection and the patients' ability to tolerate cytotoxic agents should be considered when determining doses of Cyclophosphamide.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
Nurses Free Communications 3 ***Bayside Terrace***

O093 0900

An Apheresis Unit's Experience With Plerixafor

Tamla Tait, Prahlad Ho, Carole Smith, Andrew Grigg, Daniela Zantomio
Austin Health, Heidelberg, Victoria, Australia

Background
Plerixafor is an inhibitor of the CXCR chemokine receptor and blocks binding of its cognate ligand SDF-1A (CXCL12) which assists in mobilizing haematopoietic progenitor cells (HPC). It has an important role in (a) subsequent mobilization attempts in patients who fail prior mobilization with G-CSF +/- chemotherapy and (b) salvage of failing mobilizations as predicted by pre-collection CD34 count of <18/uL at time of WCC recovery.

Method
Two patient groups who received plerixafor were analysed (a) those who failed prior collection (CD34 count of <10/uL following standard mobilization regimens) **;** plerixafor (240ug/kg) was commenced on day 4 of G-CSF only mobilizations, in conjunction with 10ug/kg G-CSF.(b) those in whom salvage plerixafor was commenced on day 10-13 in patients receiving chemotherapy and 10ug/kg G-CSF and had CD34 and WCC counts below 18/uL and 20 $x10^9$/L respectively. All patients were collected using the Haemonetics MCS+ single needle cell separator.

Results
Three and two patients in each group received plerixafor. The median CD34 count prior to commencing plerixafor was 10.5/ul (range:2-16), which increased to 21/ul (range: 3-54) after 1-2 doses of plerixafor. Four of the 5 patients successfully mobilized with >$2x10^6$ CD34/kg achieved. In 3 patients, plerixafor use was predicted in advance due to heavy pre-treatment negating the need for weekend collections or re-collection. There were no major side-effects. Median collection efficiency achieved was 57% (range 33-83%)

Conclusion
Plerixafor is effective in stem-cell mobilization in patients who have previously been unsuccessful or heavily pre-treated. This reduces costs of re-mobilisation, further chemotherapy-related side effects and psychological stress. Patients expected to mobilise poorly are now flagged for plerixafor use, which helps with planning the timing their HPC collection.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
Nurses Free Communications 3 ***Bayside Terrace***

O094 0915

Low Density Lipoprotein (LDL) Column Apheresis - Case Study and Implementation

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Background
Homozygous familial hypercholesterolaemia has a high mortality from progressive cardiovascular disease. Reducing LDL may prevent this progression but drug therapy by itself may be insufficient. LDL apheresis has been postulated to effectively reduce LDL levels. We describe a case of a 23 year old Iraqi refugee with homozygous familial hypercholesterolaemia resulting in severe ischaemic heart disease requiring three coronary artery bypass surgeries and aortic valve/root replacement since the age of nine. He also has end-stage fatty liver disease and was awaiting liver transplantation. His LDL was 14 mmol/L despite maximal drug therapy. We commenced LDL apheresis November 2007 after receiving health department funding in April 2008 expanded the service.

Method
Mr A received fortnightly LDL apheresis from November 2007. Our protocol is to process three litres of plasma via the Evaflux 5A column using the COMTEC cell separator. Pre and post LDL lipid profiles are completed and apheresis is performed fortnightly. Procedure time is around three hours. Pre-apheresis LDL was 13.8 mmol/L and post was 5.5 mmol/L. Procedure time was around three hours and performed using the COMTEC cell separator.

Results
With fortnightly apheresis, Mr A maintained median pre LDL levels of 10.1mmol/L (range 14.3-8.5) & median LDL levels post 3.9mmol/L (range 2.8-6.4). His cardiac function remained stable with no worsening of coronary artery disease and exercise tolerance improved. He received a liver transplant in May 2010 and remains well requiring no further apheresis treatment.

Conclusion
LDL apheresis is effective in reducing LDL levels in patients' refractory to maximal drug therapy. Subsequently, we have performed a further 3 LDL apheresis with excellent LDL reductions and minimal complications. In these patients, the median pre-LDL of 5.6mmol/L (range: 3.7-9.2) and median post-LDL of 1.5mmol/L (range: 0.7-3.5). These patients have subsequently demonstrated improvement in xanthomas and no advancement of cardiac disease.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
Nurses Free Communications 3 ***Bayside Terrace***

O095 **0930**

Psychological Experiences of Related Bone Marrow Donors

Deborah Hayes[1], Melissa Oxlad[2], Anna Boynton[3], Melissa Bond[1], Alison Virieux[1], Rino Amato[1]
1 Royal Adelaide Hospital, Adelaide, South Australia. 2 Private Practice, Adelaide, South Australia. 3 Childhood Cancer Association, Adelaide, South Australia

Aim
Identifying related stem cell donors is becoming more challenging due to multicultural diversity compounded by geographical location and complex family dynamics. Literature searches revealed minimal information about the support required by related donors. However, through clinical consultations, a need for psychological assessment and support for related donors was noted. Thus, the current research sought to examine the experience of donation, including the psychological well-being of related donors, the adequacy of information and support received, family dynamics as a consequence of donation, and support needs required to assist donors to make positive health/ lifestyle changes. Additionally, it sought feedback in relation to a new donor support program recently initiated by the Royal Adelaide Hospital.
Method
Thirteen participants (5 Male, 8 female), aged 28 and 67 years (M = 50.1, *SD* = 11.29), took part in individual telephone interviews (female, age = 59). Questions included: donor demographics, donation process, and donor perceptions of phases/stages of donation, family dynamics, donor support, donor lifestyle/health behaviour changes, and perceptions of the new related-donor support program.
Results
Donors reported feeling empowered and honoured to donate and would advise others to do it. Pre-donation was difficult due to the unknown as to if/when the process would occur, and anxiety about falling ill prior to donation. Many donors reported a great sense of loneliness, and several reported a decline in their own physical health after donation. A positive attitude from the hospital staff was seen as important and donors requested more information about different donation options, and whether changing their lifestyle/health behaviours would have impacted the donation process or outcome. The relationship between the donor and recipient did not appear to alter significantly, although some donors reported that their relationship became slightly closer. There was unanimous backing for the new support program.
Conclusion
Pre-donation information sessions and donor support programs are vital with participants suggesting that psychological services should be compulsory for all donors, even if they don't necessarily believe they may benefit. Post-donation psychological and medical follow up for donors was highlighted as imperative. Greater support for interstate and rural donors is required.

No conflict of interest to disclose

Tuesday 1 November **0830-1000**
ISCTA Symposium 1: Ex vivo Production of Cells for the Clinic ***Bayside Gallery B***

Isolation of Placental Mesenchymal Stromal Cells for the Clinic

Kerry Atkinson
Mater Health Services, Brisbane, Australia.

Mesenchymal stem cells (MSC) are multipotent cells that can be derived from many different organs and tissues, including term placenta – a readily available source. MSCs have been demonstrated to play a role in tissue repair and regeneration in both preclinical and clinical studies. They also have significant immune suppressive properties. Their biology, their use in preclinical models of disease, the manufacturing of MSCs for clinical trials and our initial clinical trials will be described. MSCs can also be used effectively as vehicles for gene delivery. Since tissue matching between MSC donor and recipient does not appear to be required, MSCs may be the first cell type able to be used as an “off-the-shelf” therapeutic product. Complementing the use of MSCs for regenerative medicine are the organ-specific progeny of induced pluripotent stem cells and our attempts to generate human cardiomyocytes and human enterocytes will also be described.

HAA 2011 HSANZ ANZSBT ASTH

Tuesday 1 November **0830-1000**
ISCTA Symposium 1: Ex vivo Production of Cells for the Clinic ***Bayside Gallery B***

Ex-Vivo Expanded Cord Blood With Mesenchymal Stromal Cells Promotes Recovery From Cytopenia After Cord Blood Transplant

Chitra Hosing, Marcos de Lima, Richard Champlin, Elizabeth J Shpall
The University of Texas M. D. Anderson Department of Stem Cell Transplantation and Cellular Therapy, Houston, Texas, USA

Umbilical cord blood (CB) is used increasingly to restore hematopoiesis in transplant patients lacking matched donors. A major disadvantage of CB is the low cell dose resulting in delays in engraftment. Transplantation of ex-vivo-expanded CB progenitors can provide more rapid hematopoietic reconstitution and reduced frequency of graft failure. Mesenchymal stromal cells (MSC) create a microenvironment that promotes expansion and fosters the differentiation of hematopoietic cells. We designed a clinical trial to test the clinical efficacy of this strategy. The primary goal of this trial was to evaluate the feasibility of transplanting ex-vivo expanded CB MNC, in patients with hematologic malignancies.

Methods

Patients were required to have two CB units matched in at least 4/6 HLA antigens, with a minimum dose of $1x10^7$ total nucleated cell (TNC)/Kg per CB unit. For the trial we used CB MNC with either third party haploidentical family member marrow derived MSCs (N=8) or off-the-shelf mesenchymal progenitor cells (MPCs) from Angioblast® (N=24). Thirty-two patients with advanced hematological malignancies were enrolled. Preparative regimen was fludarabine, melphalan, thiotepa and anti-thymocyte globulin (n=32), =/- rituximab (N=4). Graft versus host (GVHD) prophylaxis was with tacrolimus and mycophenolate mofetil. Median weight was 75.2 Kg (range, 15-118) and median age was 35.3 years (2.8-62 years). Donor-recipient HLA matching was 6 of 6 in 5%, 5 of 6 in 28% and 4 of 6 in 67% of the cases, respectively. One vial of Angioblast® MPCs was thawed and expanded to confluence in 4 days (n=24). The CB unit with the lowest TNC dose was then thawed, divided into 10 fractions, and each placed into 1 flask containing the confluent layers of MSCs in expansion media with a cocktail of growth factors. After 7 days, the non-adherent cells were removed from each flask, placed into ten one-liter Teflon-coated culture bags (American Fluoroseal) and cultured for an additional 7 days, while media/growth factors were added to the flasks to culture the remaining adherent layer during that time period. On day 14 the cells from the bags and the flasks were combined, washed and infused along with an unmanipulated CB unit.

Results

The median expansion was 14-fold (range 1-30) for TNC and 40-fold (range 4-140) for the CD34+. Median time to neutrophil and platelet engraftment was 15 days (range 9-42) and 40 days (range 13-62) respectively. There were no toxicities attributable to the expanded cells. Thirty-one (97%) and 26 (81%) of all patients engrafted neutrophils and platelets, respectively. The non-relapse mortality was 19% at 100 days. Median donor(s) chimerism was 100% in the mononuclear, T lymphocyte and myeloid cell populations. After a median follow up of 9 months, the actuarial 1- year survival was 40%.

Conclusion

MSC-CB expansion is feasible and leads to fast engraftment of neutrophils and platelets.

Tuesday 1 November **0830-1000**
ISCTA Symposium 1: Ex vivo Production of Cells for the Clinic ***Bayside Gallery B***

Clinical Translation of Cord Blood Expansion Technologies

David Haylock
Materials, Science and Engineering, CSIRO, Clayton, Vic, Australia

Despite the increased use of umbilical cord blood for transplantation, early haemopoietic reconstitution remains as a major concern and perhaps a limitation for this procedure, especially for adult patients. Two approaches have been used to address this problem, including transplantation with multiple cord blood units and ex vivo expansion of units to produce cells that contribute to early phase haemopoietic recovery. This presentation will discuss recent advances in these two areas and highlight the benefits and limitations of reported clinical studies. Studies conducted in our laboratory support the recent data describing the potential use of the small molecule, stemregenin-1 (SR1) for ex vivo expansion of cord blood stem cells. The possible use of SR1 alone or in combination with other small molecule or peptide mimics for haemopoietic stem cell expansion will be discussed.

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Tuesday 1 November **1030-1130**
HSANZ Free Communications 5: Molecular Genetics of Haematopoiesis ***Bayside 103***

O096 1030

Exploring the Control of Erythropoiesis by Next Generation ChIP-seq and mRNA-seq Technologies

Michael R Tallack[1], Tom Whitington[1], Evgeny Glazov[2], Marcel Dinger[1], Lei Sun[1], Timothy L Bailey[1], Andrew C Perkins[1,3]
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[2]*Diamantina Institute, The University of Queensland*
[3]*The Princess Alexandra Hospital, Brisbane*

The production of healthy red blood cells (erythrocytes), known as erythropoiesis, is a process tightly controlled by a suite of unique extracellular signals, cell-niche interactions, and lineage restricted transcription factors. The transcription factor KLF1 (formerly known as EKLF, erythroid Krüppel-like factor) is expressed only in erythroid cells and their precursors and regulates all aspects of erythrocyte development and biology. Loss of *Klf1* in mice leads to death *in utero* due to severe anemia, caused by defects in hemoglobin production, the integrity of the cytoskeleton and membrane, and iron metabolism. Recent human mutations in *KLF1* have been discovered and result in altered expression of blood group antigens, persistence of fetal hemoglobin, and congenital dyserythropoietic anemia (CDA). In particular, the pathology of CDA, which results in distortion to the size, shape and hemoglobin content of erythrocytes, accurately reflects the roles of KLF1 in erythrocyte biology that have been described in mice.
We have taken advantage of the advances in DNA sequencing technology (or "next-generation" DNA sequencing) to comprehensively characterize the functions of Klf1 in erythropoiesis. We have performed chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) to determine all of the *in vivo* binding sites for endogenous Klf1 in mouse erythroid precursors. This has revealed new insights into the mechanism of Klf1 gene activation, co-operation with other transcription factors, and previously unappreciated erythroid genes. We have also characterized the erythroid transcriptome in the presence ($Klf1^{+/+}$) and absence ($Klf1^{-/-}$) of Klf1 by performing mRNA-seq. This has provided a more comprehensive set of Klf1 target genes than was possible using traditional microarray technology and uncovered the existence of previously undescribed transcript variants, in particular alternative promoters that are erythroid specific and Klf1 dependent. We have discovered new roles for KLF1 in nuclear condensation, apoptosis and enucleation and expanded our understanding of how KLF1 controls the cell cycle. Our studies illustrate for the first time the full repertoire of KLF1-dependent events that underpin erythroid cell development and homeostasis, and shed light on how distruptions to these events result in human pathologies affecting erythroid cells.
There are no conflicts of interest to declare

Tuesday 1 November **1030-1130**
HSANZ Free Communications 5: Molecular Genetics of Haematopoiesis ***Bayside 103***

O097 1045

Phosphodiesterase-2A (*Pde2a*) Regulates Haematopoietic Stem Cell (HSC) Turnover and Erythropoiesis

Peter Papathanasiou[1], Robert Tunningley[1], Graham Magor[2], Belinda Whittle[1], Adam Hamilton[1], Simon Cridland[2], Andrew Perkins[2]
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2 Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

Chemical mutagenesis of mice combined with advances in hematopoietic stem cell reagents and genome resources can efficiently recover recessive mutations and identify genes essential for generation and proliferation of definitive hematopoietic stem cells and/or their progeny. We used FACS to quantify rare haematopoietic stem cell and progenitor cell subsets in the fetal liver of ENU-mutagenized mice bred to homozygosity. We used a SNP based whole genome scan coupled with exon capture and next generation sequencing of the genetic interval of interest to rapidly identify the causative mutation in two mouse strains (called *Kamu* and *Mulkirri*), that have a similar recessive phenotype characterized by a five fold increase in stem cells and anemia. The strains harbour different mis-sense mutations in the *pde2a* gene, a dual cAMP and cGMP phosphatase. The mutations in exon 23 and exon 26 lead to mis-sense mutations in residues known to be involved in dimerization and function of the phosphatase domain, which demonstrate the power of ENU to find functionally important residues in proteins. The stem cell phenotype will be presented in detail. Pde2a is a novel regulator of stem cell turnover which could be targeted by specific drugs to enhance stem cell function or turnover *in vitro* or *in vivo*.

There are no conflicts of interest to declare

Tuesday 1 November **1030-1130**
HSANZ Free Communications 5: Molecular Genetics of Haematopoiesis ***Bayside 103***

O098 1100

Enhanced Megakaryocytopoiesis and Thrombopoiesis by Mouse Megakaryocytic Progenitor Cells Over-expressing the Transcription Factor GATA-1

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Aim
Delayed platelet recovery after bone marrow transplantation and/or high-dose chemotherapy is often a serious complication. This condition occurs mainly due to insufficient number of megakaryocytic progenitor cells. We plan to explore the possibility of a cellular approach to minimize/prevent thrombocytopenia after transplantation with genetically modified megakaryocytic progenitor cells. Therefore, the purpose of this study is to over-express the transcription factor GATA-1 in mouse hematopoietic stem/progenitor cells to enhance megakaryocyte development and platelet production *in vitro* and *in vivo*.
Methods
The GATA-1 gene was transferred into mouse bone marrow cells via retro-viral transduction. Over-expression of GATA-1 was confirmed by Western blotting. To evaluate megakaryocyte maturation and platelet production, assays for megakaryocytic progenitor cell production, megakaryocytic marker expression, polyploidisation, proplatelet formation and platelet production *in vitro* and *in vivo* were performed.
Results
We found that over-expression of GATA-1 in mouse haematopoietic stem cells increased thrombopoietin responsive megakaryocytic progenitor cells, while interleukin-3 responsive megakaryocytic progenitor cells remained unchanged. The over-expression of GATA-1 in megakaryocytic progenitor cells increased megakaryocytic marker positive cells and decreased myeloid marker positive cells proportionately. The polyploidy pattern showed higher ploidy classes in mature megakaryocytes over-expressing GATA-1. More importantly, the significant increase in proplatelet and platelet production observed *in vitro* upon GATA-1 over-expression was recapitulated *in vivo* using a mouse transplantation model.
Conclusion
Our results suggest that over-expression of GATA-1 in primary hematopoietic cells significantly enhances all aspects of megakaryocyte maturation, including platelet production. Thus, genetically modified megakaryocytic progenitor cells may help reduce the period/severity of thrombocytopenia after stem cell transplant and/or high-dose chemotherapy.

No conflict of interest to disclose

Tuesday 1 November **1030-1130**
HSANZ Free Communications 5: Molecular Genetics of Haematopoiesis ***Bayside 103***

O099 1115

A Recessive Screen for Genes Regulating Hematopoietic Stem Cells

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3 Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia
4 Department of Pathology, Mater Hospital, Brisbane, QLD, Australia

Identification of genes that regulate the development, self-renewal and differentiation of stem cells is of vital importance for understanding normal organogenesis and cancer; such knowledge also underpins regenerative medicine. Here we demonstrate that chemical (ENU) mutagenesis of mice combined with advances in hematopoietic stem cell reagents and genome resources can efficiently recover recessive mutations and identify genes essential for generation and proliferation of definitive hematopoietic stem cells and/or their progeny. We employed high-throughput FACS to analyze nine subsets of blood stem cells, progenitor cells, circulating red cells and platelets in >1,300 mouse embryos at embryonic day (E) 14.5. From 45 pedigrees we recovered six strains (given Australian Aboriginal names) with defects in definitive hematopoiesis including stem cell numbers. We demonstrate rapid identification of a novel mutation in the c-Myb transcription factor (E308G) that results in failure to bind the transcriptional co-activator, p300/CBP. Recombinant mutant MYB protein fails to transactivate MYB target genes in reporter assays. Homozygous *Booreana* mice have increased stem cells, no B cells, disordered erythropoiesis, thrombocythemia and late onset myelofibrosis. This provides proof-of-principal that high throughput recessive genetic screens in mice can uncover point mutations that are relevant to human blood diseases.

No conflicts of interest to declare

Tuesday 1 November **1030-1130**
HSANZ Free Communications 6: CLL and Amyloidosis ***Auditorium B***

O100 1030

Identification of Genetic Susceptibility Loci in Familial Chronic Lymphocytic Leukaemia

Stephen Fuller[1], Andrew Timms[2], Christopher Hahn[3,4], Milena Babic[3], Kristen Skarratt[1], Graeme Suthers[5,6], Richard D'Andrea[3,4,7], James Wiley[8], Marshall Horwitz[2], Hamish Scott[3,4]
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Aims
We hypothesised chronic lymphocytic leukaemia (CLL) susceptibility genes can be identified in familial cases using a combination of genetic linkage and genomic analyses. We aimed to perform genetic linkage studies on multiple-case families to identify candidate loci and whole genome copy number and sequence analyses to identify variants that segregate with CLL.
Methods
Linkage studies were performed on families HJB (11 CLL and 6 monoclonal B lymphocytosis/MBL) and MPU (6 CLL and 1 ALL), using SNP microarray analysis. Genome-wide mutation analysis was performed on DNA from affected pedigree members using whole genome sequencing (WGS), whole exome sequencing (WES), whole transcriptome sequencing (WTS) and copy number variation analysis.
Results
Genome-wide linkage analysis of both families identified a common candidate locus between 14q24.1 and 14q31.2. A lymphoid cell line from an affected MPU member showed loss-of-heterozygosity at this locus. WGS and WTS of 1 affected member from MPU identified potentially pathogenic variants, which when compared to lymphoblast cell lines from 3 affecteds and 1 control revealed a Phe>Leu variant in a gene at 14q31.1. WES of 2 CLL affecteds from HJB identified an Arg>His variation in the same gene. The Phe>Leu and Arg>His variants were not detected in 916 and 653 normal controls, respectively. While Phe>Leu segregates with CLL and MBL in MPU, Arg>His segregates with disease only in one branch of HJB. We have screened an additional 44 multiplex CLL families and not found mutations in this gene.
Conclusion
A novel genetic variant, which segregated with CLL/MBL cases, was identified in the 14q candidate linkage region of a family predisposed to CLL. An alternative variant in the same gene partially segregated in a second family. Further screening is necessary to ascertain whether this gene contributes to leukaemogenesis and whether other genes/variants are required in these families for predisposition to CLL.
No conflict of interest to disclose

Tuesday 1 November **1030-1130**
HSANZ Free Communications 6: CLL and Amyloidosis ***Auditorium B***

O101 1045

A Phase II Study of Risk-Adapted Intravenous Melphalan in Patients with AL Amyloidosis

Peter Mollee[1], Campbell Tiley[2], Ilona Cunningham[3], John Moore[4], Miles Prince[5], Paul Cannell[6], Steve Gibbons[7], Jill Tate[8], Sanjoy Paul[9], Devinder Gill[1]
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Aims
High-dose IV melphalan is standard autograft (ASCT) conditioning in patients with AL. In patients ineligible for ASCT, intermediate-dose IV melphalan (IDM) may provide advantages over oral dosing where the already variable intestinal absorption is complicated by gastrointestinal amyloid deposition. We aimed to assess a risk-adapted strategy to IV melphalan dosing in patients with AL.
Methods
The ALLG MM8 study was the first prospective clinical trial in AL amyloidosis in Australia and New Zealand. Transplant candidates (minimal cardiac disease including BNP<300ng/L, age <65, ECOG≤2, ≤2 organs involved) received melphalan at 140 or 200mg/m^2 IV. All other patients received melphalan 20mg/m^2 IV d1 and dexamethasone 40mg PO d1-4 every 4 weeks for 3-6 cycles.
Results
21 patients enrolled with median age 61yrs. Organ involvement was cardiac 48%, renal 81%, liver 14%, neurologic 43%. 6, 10 and 5 were low, intermediate and high cardiac biomarker risk, respectively. 7 underwent ASCT and 14 IDM.
The trial closed early due to excessive myelotoxicity in the IDM arm. In this cohort, grade 3/4 neutropenia and thrombocytopenia during the 1st cycle occurred in 54% and 23%, with 15% having neutropenic fever. First cycle severe neutropenia was not predicted by age, cardiac or renal function but was significantly more common with lower body surface area (p=0.01). 8 IDM patients died before the 6 month response assessment, 2 achieved a 50% reduction in baseline involved FLC and 4 failed to respond. All 7 patients receiving ASCT are alive at a median of 33 months.
Conclusions
IV melphalan at 20mg/m^2 is excessively myelotoxic for patients with AL. BNP<300ng/L may identify patients suitable for ASCT.
No conflict of interest to disclose. This study was supported by the Leukaemia Foundation of Queensland and Amgen Australia.

HAA 2011 HSANZ ANZSBT ASTH

Tuesday 1 November **1030-1130**
HSANZ Free Communications 6: CLL and Amyloidosis ***Auditorium B***

O102 1100

A Cohort Analysis of Patients from Australian Amyloidosis Clinics

Fiona Kwok[1], Ming-Wei Lin[1], Graeme Stewart[1], David Booth[1], Nikky Isbel[2], Dariusz Korczyk[2], Sally Mapp[2], Patricia Renaut[2], John Taper[1], Amanda Johnston[1], Richard Phoon[1], Liza Thomas[1], Ragwha Sharma[1], Bartlomiej Getta[1], Steve Vucic[1], Peter Mollee[2], David Gottlieb[1]
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Aim
To describe the characteristics and management of patients from Australia's two amyloidosis clinics.
Method
A retrospective cohort analysis of the Westmead (WAC) and Princess Alexandra Hospital Amyloidosis clinics (PAAC) was performed. These clinics were established in August 2007 and April 2009 respectively as centres for provision of accurate diagnosis and advice on management of patients with amyloidosis.
Result
126 patients (WAC n=83, PAAC n=43) were available for analysis. The median follow-up time from diagnosis was 15 months. There were 69 patients with AL (AL) amyloidosis, 17 with localised amyloidosis (LA), 13 with familial amyloidosis (FA), 12 with senile systemic amyloidosis (SSA), and 11 with AA amyloidosis (AA). 3 remain indeterminate. For AL amyloidosis the median age of diagnosis was 62 years. 60 had primary AL, 6 had multiple myeloma and 3 a lymphoproliferative neoplasm. The most common serum clonal protein isotype was free lambda light chain (39%). An average of 2 organs was involved (range 1-7), most commonly kidney (76% of patients), heart (59%) and nerves (37%). Cyclophosphamide, dexamethasone and thalidomide was the preferred induction therapy. 6 patients underwent autologous stem cell transplant. LA most often affected the respiratory tract (n=9), bladder, skin and GIT (all n=2). The 13 FA cases belonged to 10 families. 3 were index cases. Mutations affected the TTR (n=7), lysozyme (n=2) and fibrinogen (n=1) gene regions. All SSA cases had cardiac involvement, 4 had multiorgan disease. Diflunisal has been prescribed for 1 inherited TTR case and 2 SSA cases. All AA cases had renal amyloidosis, 4 had multiorgan disease. The majority had autoimmune disease (n=8) although no inflammatory disease could be identified in 2.
Conclusion
This cohort of amyloidosis clinic patients reflects the recognised epidemiology of amyloidosis. A multidisciplinary approach to diagnosis and management is necessary for this multisystem disease group of varying aetiologies.
No conflict of interest to disclose

Tuesday 1 November **1030-1130**
HSANZ Free Communications 6: CLL and Amyloidosis ***Auditorium B***

O103 1115

ZAP-70 Expression Measured by Quantitative PCR (QPCR) as a Prognostic Marker in Chronic Lymphocytic Leukaemia (CLL)

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Aim
ZAP-70 expression provides important prognostic information in CLL, however, routine flow cytometric assessment has been limited by difficulties in method standardisation. We aimed to test whether ZAP-70 expression measured by quantitative PCR was superior to flow cytometric assessment.
Methods
We determined ZAP-70 expression by QPCR on CD19 selected peripheral blood B-cells in 95 patients with CLL and compared this to ZAP-70 measured by flow cytometry. Utility as a predictor of IgVH mutation status and clinical outcome was compared along with other standard prognostic markers.
Results
95 patients were analysed: median age 62years, 62% male, Binet stage at diagnosis (A 84%, B 11%, C 5%). 46% progressed to require treatment at a median time to first treatment of 7.6 years. Median overall survival has not been reached at a median follow-up of 5 years. 72% were ZAP-70 positive (defined as >20%) by flow cytometry, 32% were CD38 positive (defined as >30%) and 41% had unmutated IgVH status. Median ZAP-70 by QPCR was 3,437 per 10,000 copies of ABL (range, 163 to 30,452). ZAP-70 by flow cytometry and QPCR were significantly correlated (Spearman's correlation coefficient 0.52, p=0.002). Positive ZAP-70 by flow cytometry had a sensitivity of 100% and specificity of 56% for an unmutated IgVH status. Using a cut-off of 5000, ZAP-70 by QPCR had a sensitivity of 91% and specificity of 88% for an unmutated IgVH status.
Correcting for Binet stage, a shorter time to first treatment was predicted by higher ZAP-70 by QPCR (p=0.001), CD38 positivity (p=0.004) and unmutated IgVH status (p=0.003), but not ZAP-70 positivity by flow cytometry (p=0.09).
Conclusion
ZAP-70 expression determined by QPCR provides better prediction of IgVH mutation status and time to first treatment in patients with CLL than does ZAP-70 expression as measured by flow cytometry.
No conflict of interest to disclose. This research was supported by a grant from the Leukaemia Foundation of Australia

Tuesday 1 November **1030-1130**
HSANZ Free Communications 7: Non-Malignant Haematology ***Bayside 104***

O104 1030

Development of a Patient Blood Management Program at Fremantle Hospital

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Background
A Patient Blood Management Program (PBMP) was started at Fremantle Hospital in 2009 as a pilot for a WA Health Department Initiative. The aims were to improve patient outcomes, optimise the use of red cells and "fresh" blood products and reduce costs.
Methods
A State Health Research Advisory Council grant provided funding for the construction of a database on blood product use providing feedback for clinical and audit purposes. The Health Department of WA funded a PBM Clinical Nurse Consultant, who along with the Transfusion Medicine and Patient Blood Management Committees, has a core management role in program development.
Strategies employed were based on the 3 pillars of Patient Blood Management:
1 Optimising erythropoiesis
2 Reducing blood loss and bleeding
3 Improving the patient's tolerance of anaemia
Implementation
a) Frequent blood sampling may lead to the development of anaemia, therefore small volume collection tubes were introduced to ICU and Haematology.
b) A "one unit" Red Cell Transfusion policy was adopted for non bleeding patients for whom a transfusion was requested with reassessment of symptoms before further red cells are considered.
c) Optimising preoperative clinic assessment utilising HemoCue® to rapidly identify, investigate and treat anaemic patients with IV iron prior to surgery.
d) Rapid identification and targeted treatment of intraoperative coagulopathy utilising whole blood sample based rotational thromboelastography (ROTEM ®) with "real-time" results demonstrated in Theatre.
e) PBM Education to Medical staff with feed back to Departments on blood product use.
Conclusion
PBMP has led to changes in blood transfusion practice with a reduction in overall red cell use, despite an increase in hospital activity. Targeting of coagulopathies has led to an increase in cryoprecipitate use with reduced FFP and platelet consumption. A comprehensive PBMP can help reduce the increasing overall demand for blood and blood products.

No conflict of interest to disclose

Tuesday 1 November **1030-1130**
HSANZ Free Communications 7: Non-Malignant Haematology ***Bayside 104***

O105 1045

The Health of Adults Living with a Clinically Significant Haemoglobinopathy in NSW, Australia. The NSW Haemoglobinopathy Project

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Aim
To comprehensively review the health needs of patients living with clinically significant haemoglobinopathies (Thalassaemia and Sickle Cell Disease (SCD)) in NSW, Australia.
Methods
Sixty three of 121 adults (representing approximately 80-90% of adult patients with transfusion-requiring haemoglobinopathies in NSW) completed an in-house disease specific health needs assessment survey and a health-related quality of life assessment (SF36v2).
Results
Adult patients with clinically significant haemoglobinopathies in NSW come from more than 8 world regions; with those with SCD being more likely to be born outside of Australia than subjects with Thalassaemia ($p<0.001$, LR 20.64) as well as more likely to have been refugees (26% vs 2%). The population contained socially disadvantaged subjects with 13 subjects (20.6%) having annual incomes less than the Australian poverty line. Complications of Thalassaemia were comparable to previous reports from elsewhere in the world although our subjects had a high rate of secondary amenorrhea (>12 months = 27%), and surgical splenectomy (55.6%).Use of Hydroxyurea for management of SCD was less than expected with only 46.6% of subjects having used it at some point. Lack of universal access to MRI guided chelation, recognised internationally as best practice was evident, although 65.5% had been able to access this either via enrolment in a clinical trial, or through self-funding.
Conclusions
Patients with SCD and Thalassaemia experience considerable morbidity and mortality and require complex, multidisciplinary care. This study revealed both variance with international best practice and variance between specialist units. The results of this research may provide the impetus for the development of clinical and research networks for haemoglobin disorders to enable the uniform delivery and assessment of health services benchmarked against international standards.

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Tuesday 1 November **1030-1130**
HSANZ Free Communications 7: Non-Malignant Haematology ***Bayside 104***

O106 1100

A Multi-Centre, Single-Arm, Open-Label Study Evaluating the Safety and Efficacy of Fixed Dose Rituximab in Patients with Refractory, Relapsing or Chronic Idiopathic Thrombocytopenic Purpura (R-ITP1000 Study)

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Background
Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterised by low platelet count and mucocutaneous bleeding. Post-acute presentation, 25-30% adult patients develop chronic ITP; up to 30% of chronic patients become refractory to corticosteroids requiring additional therapy. B-cells play an important pathophysiological role in autoimmune disease. Rituximab depletes CD20+ B-cells and a dosing regimen based on lymphoma therapy (375 mg/m^2 weekly x 4) has shown efficacy (~38% Overall Response Rate-ORR) in adults with chronic and relapsing ITP; here we explored an abbreviated rituximab schedule, consistent with the approved rheumatoid arthritis dosing.

Aim
The primary objective was to determine ORR, at week 8, among adults (≥ 18 years), diagnosed according to the ASH guidelines, with chronic or relapsing ITP (platelet count > 10x10^9/L and ≤ 50x10^9/L) who are receiving rituximab 1000 mg intravenous (IV) on days 1 and 15.

Method
ORR was defined as the proportion of patients achieving a complete response (platelet count > 150x10^9/L) or partial response (> 50x10^9/L) at week 8 with 2 consecutive measurements confirmed at least 2 weeks apart. Simon's 2-stage design was used to determine if the ORR is more likely to be ≤ 38% or ≥ 50%. At least 50/108 responders (46.3%) were required to conclude, with 95% confidence and 80% power, that the ORR was likely to be ≥ 50%.

Results
Of 124 recruited patients 2 did not receive study medication and were excluded from analysis, as were 14 patients with no platelet count ≤ 50x10^9/L within 7 days of first rituximab dose. At week 8, the confirmed ORR was 43.5% (47/108 patients). Treatment was well tolerated with no additional safety signals.

Conclusion
The ORR is comparable with published studies using a more frequent rituximab schedule. Further studies are warranted to investigate whether the same response can be achieved with single or lower dosing, or if longer, more intense dosing might improve ORR.

The R-ITP (1000) study was sponsored by Roche products, Australia. All authors fulfill the ICMJE authorship requirements and have reviewed and approved the abstract for submission. Medical Writing assistance was provided by Dr Joseline Ojaimi from Roche.

Tuesday 1 November **1030-1130**
HSANZ Free Communications 7: Non-Malignant Haematology ***Bayside 104***

O107 1115

Identification of Novel and Differentially Expressed Isoforms of Human α2 and α1 Globin Genes

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Aim
Traditionally, the transcription initiation site of the alpha globin genes has been thought to lie at position -37 relative to the translation initiation codon. We were interested to note a recent Genebank entry describing an isoform of Alpha 2 globin initiating at a position 66 bp 5' to the translation initiation codon. The aim of this study was to investigate the occurrence of these two isoforms for the alpha1 and alpha2 genes and to document the relative expression of each.
Methods
Total RNAs were purified from the peripheral blood of 15 healthy volunteers. These were analysed using quantitative-cDNA-PCR protocols developed in the laboratory, with primers designed to enable distinction between α2 and α1-globin transcripts, and to amplify the two proposed isoforms of each. The PCR products were validated by sequencing.
Results
We observed two distinct PCR products for each of the globin genes analysed. Subsequent DNA sequencing of 11 individual PCR products revealed that both α2 and α1-globin transcripts are present in both a long and a short isoform, initiating at positions -66 and -37 respectively. The shorter (-37) isoform is expressed approximately 10,000-100,000 times more strongly than the longer isoform, indicating differential expression within the healthy control population.
Conclusion
This study confirms the presence of two isoforms for both the alpha1 and alpha2 globin genes. The functional significance of the longer isoforms is unknown, but they are expressed in significantly lower quantities than the shorter isoform, possibly reflecting inefficient transcription initiation form this secondary site. The impact of globin gene alterations on the relative expression of the different isoforms may be of interest.

No conflict of interest to disclose

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Tuesday 1 November **1030-1130**
HSANZ Free Communications 8: Biology & Treatment of Lymphoma & Myeloma
Bayside 105

O108 1030

Tissue Microarray in Patients with DLBCL Receiving R-CHOP Chemo-immunotherapy Shows Survival Benefit for Coexpression of LMO2/BCL6

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Aim
Diffuse Large B-cell Lymphoma (DLBCL) is a heterogenous disease. The international prognostic index (IPI) is applicable following 'R-CHOP' chemo-immunotherapy, but variable outcomes in patients with identical IPI scores is still common. IPI does not permit identification of patients who based on biology, might benefit from alternative treatment strategies. Numerous markers either alone or in combination have been proposed.To our knowledge this is one of the first immunohistochemical studies to compare the Hans, Choi and Tally algorithms in R-CHOP treated patients. In addition it is one of the first studies to specifically look at outcome related to dual positivity for LMO2/BCL6.
Method
90 sequential cases were identified in whom formulin fixed parrafin embedded (FFPE) tissue was available. Patients were selected based only on availability of tissue for analysis. 75 patients received R-CHOP like therapy. Patients were identified from a prospective clinical database which includes IPI, treatment and outcome details, and was complete in >95% cases. An extensive tissue microarray (TMA) was performed. Overall and event free survival (OS and EFS) were tested against IPI and TMA markers.
Results
BCL-6 positive patients had improved outcome (EFS p=0.036, OS p=0.037), however a combination of the germinal centre (GC) markers LMO2 and BCL6 positivity further enhanced the prognostic value (EFS p=0.016, OS p=0.003). Even within GCB classified patients, the prescence of dual posivity for LMO2/BCL6 conferred improved outcome. Neither Hans, Choi or Tally algorithms predicted for any difference in survival between GC and post-GC subtypes.
Conclusions
BCL6 positive patients had improved OS and EFS in our cohort confirming the benefit of R-CHOP in this subgroup. In addition we have shown for the first time that dual positivity for LMO2/BCL6 on immunohistochemistry correlated with an extremely good EFS and OS when treated with R-CHOP chemo-immunotherapy.
No conflict of interest to disclose

Tuesday 1 November **1030-1130**
HSANZ Free Communications 8: Biology & Treatment of Lymphoma & Myeloma
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O109 1045

Diagnostic Utility of CD200 Expression by Flow Cytometry in the Differentiation of CD5+ Chronic Lymphoproliferative Disorders

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Aim
To evaluate the clinical utility of CD200 expression by flow cytometry in differentiating between chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL).
Method
The expression of CD200 (BD Pharmingen) was studied prospectively in 56 patients (33M:23F) with a median age of 58 years (range 44-96 years) referred to Eastern Health Pathology over a period of 18 months. All cases had CD5+ B-cell lymphocytosis. Four colour direct immunophenotyping was performed on FC 500 cytometer, equipped with CXP 2.1 software. The lymphocytes were identified using CD45vsSSC gating strategy. The expression of more than 20% of lymphocytes referenced to an internal negative control was considered positive. The diagnosis of CLL vs MCL was made based on clinical data and the 5-point Matutes scoring system**.** Sensitivity and specificity were calculated using GraphPad Prism™.
Result
All 5 MCL patients had low Matutes score (≤2), absent CD23 and CD200 expression. Fifty CLL patients had high Matutes score (≥4), and one had a score of 3. CD23 and CD 200 expression were absent in 9 and 4 CLL patients respectively. The respective best estimates for sensitivity and specificity of absent CD200 expression for MCL were 100% (95% CI: 46%-100%), and 92% (95% CI: 80%-97%) compared to a specificity of 82% (95% CI: 69%-91%) for absent CD23 expression.
Conclusion
CD200 is a more specific marker than CD23 in the diagnosis of MCL. In variance with other studies, 4 patients with CLL had absent CD200 expression. The clinical utility of CD200 appears to be limited to patients with intermediate Matutes score and with MCL-pattern of CD23 antigen expression. Future studies will correlate with genetic results.

No conflict of interest to disclose.

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HSANZ Free Communications 8: Biology & Treatment of Lymphoma & Myeloma
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O110 1100

Dendritic Cell Biology in Multiple Myeloma

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Aim
This project sought to identify abnormalities in the recently defined dendritic cell (DC) subset populations (the three $CD11c^+$ myeloid ($CD1c^+$, $CD16^+$ and $CD141^+$) as well as $CD11c^-$ plasmacytoid (pDC, $CD11c^-$, $CD123^+$) DC subsets (Blood 2010;116:e74-e80) in patients with multiple myeloma (MM), smouldering myeloma (SM) and monoclonal gammopathy of unknown significance (MGUS), with a view to determining the optimal DC subsets to target for therapeutic immune interventions in MM.
Method
Peripheral blood (PB) mononuclear cells were obtained from patients with MGUS (6), SM (6) or MM (22), recruited from the haematology clinic and age matched normal donors (10). Bone marrow (BM) samples from MM (5) and SM (4) patients were also analysed for the recently defined DC subsets within the $Lineage^-$ $HLA\text{-}DR^+$ cells by flow cytometry. Absolute numbers of PB DC and the proportions of BM DC were calculated.
Results
Both the $CD1c^+$ and $CD141^+$ myeloid DCs were significantly reduced in the PB of MM patients, but not in SM or MGUS, whilst the $CD16^+$ DC numbers were not significantly altered. Reduction of both mDC subsets was seen only in patients with stage II or stage III disease ($p<0.001$) as classified by the International Staging System. This was accompanied by significantly reduced numbers of pDC, in both stage I ($p<0.01$) and stage II ($p<0.001$) MM but not MGUS or SM patients. Despite a reduction of some DC subsets in the circulation, BM DC subset analysis revealed, marked enrichment of the pDC component in the BM of MM patients (49.2% SEM 7.7 respectively). Interestingly, the minor $CD141^+$ DC subset was also enriched in the BM of MM patients from a mean percent of mDC of 3.1% (SEM 0.7) in PB to 13.9% (SEM 5.8) in BM.
Conclusion
DC subset distribution is abnormal in MM. Reduction in circulating pDC, $CD1c^+$ and $CD141^+$ DC subsets is paralleled by proportional increases in the BM of MM and SM patients. The increase in BM $CD141^+$ DC, which are known to be efficient at cross presenting necrotic cell antigens and stimulating cytotoxic T cell responses may reflect a tumor immune response. These data emphasise that they may be an attractive subset to target for MM immune therapy.
No conflict of interest to disclose.

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Bayside 105

O111 1115

Optimal Cyclophosphamide Dosing for Peripheral Blood Stem Cell (PBSC) Mobilisation in Myeloma (MM): Getting the Best Bang for our Buck

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Background
Autologous stem cell transplantation remains the standard treatment for patients with MM. The most common regimen used to mobilise PBSC is Cyclophosphamide (CY) and G-CSF. CY dosing ranges from 1.5g/m2 to 5g/m2 in Australian centres. The optimal CY dose together with G-CSF for PBSC mobilisation remains poorly defined.
Aim
To compare outcomes using CY 2g/m2 (CY-2) versus CY 3g/m2 (CY-3) single dose on Day 1 together with G-CSF 10mcg/kg from Day 3 to mobilise PBSC in MM pts.
Patients and Methods
Between 6/03-6/11, 110 pts underwent PBSC mobilization for MM, 61 pts received CY-2, 49 pts received CY-3. Induction therapy varied, reflecting changes to induction therapy over time. No pts received lenalinomide as induction.
Results
CY-2 and CY-3 had high rates of successful PBSC mobilisation and collection (97% vs 96%). There was no difference in the total CD34 collected ($6.5x10^6$/kg vs $7.33x10^6$/kg, P=0.243), number of collections required to achieve CD34≥$2x10^6$/kg (1 vs 1, P=0.585) or successful harvest for two transplants following single apheresis (70.5% vs 75.5%, p=0.668). CY-2 pts were managed as outpatients while CY-3 pts required overnight inpatient stay. Days of G-CSF use were significantly shorter in CY-2 vs CY-3 (8 days vs 10 days, p<0.005). Time to neutrophil and platelet engraftment was not significantly different between groups. Disease free survival was significantly improved in the CY-2 group with no difference in overall survival between groups.
Conclusion
The combination of cyclophosphamide and G-CSF is an effective regimen for PBSC mobilisation in MM. CY-2 and CY-3 are equally efficacious regimens in PBSC mobilization, but CY-2 offers the additional benefits of outpatient administration and less G-CSF use which offer significant cost reduction and less impact on patient quality of life.
No conflict of interest to disclose

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Tuesday 1 November **1030-1130**
ASTH Free Communications 2: Haemostasis ***Bayside 204***

O112 1030

Effect of FEIBA on Platelet Aggregation and Activation in Severe Haemophilia Patients with Inhibitors

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Aim
Factor eight inhibitory bypassing agent (FEIBA) is used as a therapeutic option in haemophilia patients who have developed inhibitors. The measurement of thrombin generation has been applied to monitor the efficacy of FEIBA. However a major concern about the clinical use of FEIBA is whether or not an increase in thrombin activity causes subsequent platelet activation.
Method
We evaluate the effects of FEIBA on platelet and leucocyte activity, a concomitant measurement of thrombin generation was also made. Initially an *in vitro* study was conducted to evaluate the effects of FEIBA on platelet and leucocyte activity by using peripheral blood from normal volunteers. The measurement was made using flow cytometry. We then performed an *ex vivo* study looking at the effect of FEIBA on the above parameters in 2 haemophiliacs with high titre inhibitors. A parallel study was also carried out *ex vivo* to evaluate thrombin generation using a thrombinoscope.
Result
FEIBA did not cause platelet and leucocyte activation in either the *in vitro* or *ex vivo* studies but showed a predictable increase in thrombin generation.
Conclusion
Our study is the first to address the effect of FEIBA on platelet and leucocyte function. We found no evidence of 'systemic' platelet activation. The findings suggest that whilst FEIBA improves global haemostasis, platelet activation is likely to be contained to the site of injury and systemic platelet activation, a previously feared consequence of FEIBA infusion that that may have contributed to thrombotic risk is absent.

Scott Dunkley has received funding for research and speaker fees from BAXTER and NovoNordisk

Tuesday 1 November **1030-1130**
ASTH Free Communications 2: Haemostasis **Bayside 204**

O113 1045

The Impact of Indium-Labelled Autologous Platelet Sequestration Studies (ILAPS) in the Evaluation of Patients with Immune Thrombocytopenia (ITP) Prior to Splenectomy: An Australian Institutional Experience

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Introduction
The role of splenectomy in the contemporary management of ITP is uncertain, due to the lack of predictors of response to splenectomy, concerns regarding early and late complications of the procedure as well as the emergence of alternative treatment options such as thrombopoeitin receptor agonists. Promising international data on the use of ILAPS as a predictor of response to splenectomy prompted us to introduce ILAPS in all patients at Geelong Hospital being considered for splenectomy.

Aim
To assess the validity of the ILAPS study in predicting response to splenectomy in ITP patients within an Australian setting.

Method
We performed a retrospective study on 29 patients with primary or secondary ITP undergoing ILAPS studies at Geelong Hospital between 2003 -2011 of whom 4 were excluded from analysis. ILAPS results were expressed as an “R”value (spleen/ liver uptake ratio as a means of quantitating the pattern of sequestration in either the liver or spleen) for each patient and compared to clinical outcome post splenectomy; as assessed by platelet count and need for medical treatment post splenectomy.

Result
Of the 25 patients assessed, 12 proceeded to splenectomy while 13 patients continued to receive medical therapy. Patients with a predominant splenic uptake were more likely to remain in complete remission at 12 months post splenectomy than patients with a predominant hepatic or mixed uptake.

Conclusion
In line with international data, our study confirms that patients with pure or predominant splenic uptake have a superior outcome following splenectomy despite low pre-study platelet counts and recent use of intravenous immunoglobulin. We continue to use ILAPS study results to guide clinical decision making. We suggest a negative study be considered a medical contraindication to splenectomy as an eligibility criterion for PBS-funded romiplostim.

No conflict of interest to disclose.

Tuesday 1 November **1030-1130**
ASTH Free Communications 2: Haemostasis **Bayside 204**

O114 1100

Practical Usage of Recombinant Activated Factor VII in Patients with Haemophilia A or B With Inhibitors

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Aim
The development of inhibitors in haemophilia is the most serious complication of haemophilia, and management of bleeds in these patients is complex; recombinant activated Factor VII (rFVIIa; NovoSeven® RT) is an established therapy for treating bleeding in patients with haemophilia A or B complicated by inhibitors. Our aim was to develop a consensus-based guide on the practical usage of rFVIIa in patients with haemophilia with inhibitors.
Methods
An expert group of 9 haematologists from Australia and New Zealand developed consensus-based practice points on the usage of rFVIIa, based on their experience and supported by published clinical, safety and pharmacoeconomic data.
Results
Practice points were developed for the following thirteen topics that were identified as being key to the practical usage of rFVIIa in patients with inhibitors: management of acute bleeds; prophylaxis; surgical prophylaxis; control of breakthrough bleeding during surgery; use in paediatrics and elderly; intracranial haemorrhage; immune tolerance induction; refractory bleeds; monitoring of rFVIIa therapy; concomitant use of antifibrinolytics; and optimal dosing and dosing intervals. The access to home therapy with rFVIIa is important in allowing patients to administer treatment at the earliest opportunity in bleed management. A starting dose of rFVIIa in adults of 90–120 µg/kg was recommended in most settings. Due to the effect of age on the pharmacokinetics of rFVIIa, a starting dose of 90–180 µg/kg was recommended in paediatric patients. In the management of acute bleeds, 2 hourly dosing was recommended until the bleed is controlled. If required, concomitant medication with antifibrinolytics was deemed appropriate.
Conclusions
The consensus-based practice points will provide practical guidance for all clinicians on the usage of rFVIIa involved in the management of individuals with haemophilia complicated by inhibitors.
This project was supported by Novo Nordisk. The company had no role in drafting the recommendations or preparing the abstract.

Tuesday 1 November **1030-1130**
ASTH Free Communications 2: Haemostasis **Bayside 204**

O115 1115

Australian Bleeding Disorders Registry – A Collaborative Approach

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Aim
The Australian Bleeding Disorders Registry (ABDR) was first established in 1988. The aims of the ABDR were to provide a clinical tool for improved management and national demographics for People with Bleeding Disorders (PWBD).
Method
Through collaboration with clinicians, patients and governments, the redeveloped Registry was deployed in 2008 to provide, for the first time in Australia, a national view on the size, nature and complexity of the clinical treatment requirements of PWBD. A Steering Committee was established comprising individuals from the Australian Haemophilia Centre Directors' Organisation (AHCDO), the National Blood Authority (NBA) and the Haemophilia Foundation of Australia (HFA). This Committee ensured extensive consultation with every speciality involved in the care of patients including AHCDO, the Australian Haemophilia Nurses' Group, the Australia New Zealand Haemophilia Social Workers/Counsellors' Group and the Australian and New Zealand Physiotherapy Haemophilia Group.
Data base managers (DBM), funded by the NBA, are responsible for data input and maintenance. DBMs meet regularly to create and maintain data dictionaries, trouble shoot, produce reports for the Haemophilia Treatment Centre and provide training for the newly appointed DBMs.
Result
The ABDR Annual Report 2009-10, released early in 2011, represented the first analysis of the ABDR data since the redevelopment in 2008. This allowed comparison of data input by jurisdiction and product. The results were tempered by some data quality issues such as incomplete records and some product use not recorded. Also, inconsistent definitions appear to be used for particular fields and others require further development to ensure a 'standardised' collection such as a list of values for 'purpose of treatment' for clinical consistency.
Conclusion
The release of the ABDR Annual Report provides valuable insight into supply challenges and bleeding disorder demographics in Australia. However, it is clear that further development in data quality and consistency is required.
No conflict of interest to disclose

Tuesday 1 November **1030-1130**
Nurses Free Communications 4 Treatment and Follow-up ***Bayside Terrace***

O116 1030

Nursing Challenges: Treatment of Haematological Malignancy for the Pregnant Patient. Three Case Studies

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Aim
One of the most challenging situations for haematology physicians and nurses is the new diagnosis of malignancy in a pregnant female. The type of malignancy, gestational time of the pregnancy and the general health of the mother, will all be crucial guides for the haematologist in the decision to treat. Once the decision is made the treatment must involve the consultation of a large multidisciplinary team to ensure the health of both mother and baby.
Method
This discussion outlines the recent experience in Ward C5a at Westmead Hospital of three pregnant women who required antineoplastic treatment during their pregnancy. Each woman had different challenges and was treated by a different physician, but with careful planning the Clinical Nurse Consultant was able to coordinate the multidisciplinary care required for the patient.
Result
Each case required close communication with obstetric staff to coordinate the care delivery, including the education of haematology nurses about the additional observations required for optimal patient care. Foetal development was monitored regularly, and the effects of the chemotherapy were closely observed. The three examples will be presented as case studies with similarities drawn between them and differences explained. All three cases resulted in a healthy delivery, and successful treatment of the mother. With careful management pregnancy does not need to preclude the mother from the effective treatment of a haematological malignancy.

Tuesday 1 November **1030-1130**
Nurses Free Communications 4 Treatment and Follow-up ***Bayside Terrace***

O117 1045

Development of a Nurse-Led Survivorship Care Intervention for Long Term Survivors of Hodgkin Lymphoma

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Background
As the numbers of survivors of Hodgkin Lymphoma (HL) accumulate it is increasingly important that they normalize their lives and incorporate healthy behaviours into their lifestyles in order to achieve optimal health outcomes and minimise the risk of late adverse effects from their prior curative chemo/radiotherapy.
Aim
In order to meet increasing patient need within the late effects (LE) clinic an innovative model of nurse-led care was developed to enhance HL survivors' awareness of individual health risks, the benefits of adopting healthy lifestyle behaviours and to reduce psychosocial distress.
Method
In the nurse-led consultations information is presented to survivors in an education package directed specifically at their individual health needs informed by their prior therapies. Screening for emotional distress is undertaken using the LE supportive care needs screening tool. Each survivor receives an individualized survivorship care plan.
Results
Data collection is commencing and is currently being collected using two validated tools: the General Health Index and the Health Promoting Lifestyle Profile II. They measure whether receiving a health promoting intervention from a specialist nurse consultant demonstrates an improvement in knowledge of, and motivation to adopt, healthy behaviours.
Conclusion
This innovative nurse-led model of survivorship follow-up is based on best available evidence. As such, this initiative demonstrates an evolution in thinking about the development of nurse-led follow-up and may offer a useful model for the development of other nurse-led models of cancer survivorship care in the future.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Tuesday 1 November **1030-1130**
Nurses Free Communications 4 Treatment and Follow-up ***Bayside Terrace***

O118 1115

Mothers of Children with Haemophilia: An Exploration of Their Experiences

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Aim
There continues to be poor treatment adherence in children and adolescents with haemophilia, despite obvious treatment benefits. Parents are responsible for administering treatment up until, and frequently throughout, adolescence. Therefore, they play a fundamental role in treatment adherence. Paediatric haemophilia literature has, until now, been predominantly quantitative and focussed largely on the quality of life of patients and parents. The few studies that have investigated parents' experiences of having a child with hemophilia have been conducted predominantly in the United Kingdom. We aimed to qualitatively explore experiences of parents with children who have severe hemophilia A, in the hope of identifying influences on treatment adherence and nonadherence.
Method
Seven biological mothers, of children (aged between 2 and 16 years) with severe hemophilia A on primary prophylaxis, were interviewed. The data were analyzed using interpretative phenomenological analysis (IPA). IPA involves detailed examination of participants' lived experience. Behaviour is, in part, influenced by an individual's beliefs and experience. As such, undertaking IPA will help to identify possible influences on parents' behaviour and thus their approach to their child(ren)'s treatment.
Result
Six main themes were extracted: parental responsibility to protect, acceptance, appreciation, self-efficacy, this is dangerous and others don't get it, and treatment importance versus practicality.
Conclusion
Poor adherence in children with hemophilia is a significant medical problem. Possible interventions for improving adherence include: increasing parents' acceptance, appreciation, self-efficacy, and time management; and challenging their perception of danger and trustworthiness of others. Further research is required in order to assess whether improvements in any of these areas can result in improvements in adherence behaviours in children with hemophilia.

No conflict of interest to disclose

Tuesday 1 November **1030-1130**
Nurses Free Communications 4 Treatment and Follow-up ***Bayside Terrace***

O119 1115

Exploring the Place of Death of Patients with a Haematological Malignancy

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Aim
A literature review to explore the place of death of patients with a haematological malignancy
Method
A literature search was conducted on CINAHL and Medline databases using the key words palliative care and haematology with no exclusion dates.
Results
A study comparing the experience of carers with loved ones dying in a hospice versus the acute care setting demonstrated end of life cares were of a poorer quality in the acute setting. A literature review examining the place of death of haematology patients identified that they were more than twice as likely to die in hospital compared to other cancers. One study found that haematology patients were more likely to die while receiving curative treatment and were less likely to be referred to palliative care services compared to solid tumours. Several studies revealed that haematology patients need more support and education to facilitate a home death. Two case studies demonstrated treatment options including blood product support within the home environment in creating satisfying dying experiences. In a prospective mixed method study, heath care professionals reported they felt place of death had a significant effect on the bereavement process. However, a case study on concurrent palliative care and curative care in a bone marrow transplant patient led to a positive dying experience despite dying in intensive care. Results from a qualitative study identified barriers that prohibit open communication as patients transition from curative to palliative treatment; leading to missed opportunity for patients in making informed choices. Two articles discussed caregiver burden associated with death in the hospital setting and identified the need for palliative care throughout the disease trajectory of haematological malignancies and bone marrow transplantation.
Conclusion
A review of the literature identifying retrospective, qualitative, mixed method and case studies supports the notion that patients with a haematological malignancy predominantly die in the curative system with inappropriate end of life cares and lack of support for carers. It was revealed that positive death experiences can occur in the curative system and are closely linked with honest disclosure of information and patient autonomy.
No conflict of interest to disclose

Tuesday 1 November **1030-1130**
ISCTA Symposium 2: Manipulation of Alloreactivity ***Bayside Gallery B***

Role of NK Alloreactivity in GVH and GVL Reactivity

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1 Department for Haematology, St George's Hospital and Medical School, London, UK
2 Blood Services Group, Health Sciences Authority, Singapore

Natural killer (NK) cells are powerful effectors of the innate immune system. Their spontaneous cytolytic action is orchestrated through an array of activating/inhibitory receptors and effective against a broad range of malignancies. Nevertheless, mixed clinical benefits were reported from traditional autologous immunotherapies involving short-term interleukin (IL)-2-activated lymphokine-activated-killer (LAK) cells or high-dose IL-2 infusions for endogenous NK cell activation. Obstacles included insufficient cytotoxic potential of LAK cells and severe side effects induced by systemic high-dose IL-2 treatments. Approaches involving alloreactive NK cells show greater promise in enhancing graft vs leukaemia (GvL) effects while not inducing significant graft versus host disease (GvHD). Haploidentical haematopoietic cell transplants (HCT) exploiting increased NK cell alloreactivity as a consequence of haplo-mismatched inhibitory killer immunoglobulin-like receptors (KIRs) have resulted in impressive disease control of refractory acute myeloid leukaemias. This has spurred renewed interest in expanding allogenenic NK cell populations. Recent significant advances in cell selection/culture/expansion technologies and greater understanding of NK cell biology cell activation modes allow more effective manipulation of NK cells. Our laboratory studied different Good Manufacturing Practices compatible culture conditions for generation of LAK-NK effectors with superior potency including the novel cytokine IL-15. We found that LAK-NK cell stimulation was particularly effective with the combination of IL-15 and CellGro® SCGM. A translational trial is being developed. At the same time, we have explored T cells with NK-like activity in a phase I/II clinical trials. These cytokine induced killer cells have proven to be safe both in the autologous and allogeneic setting for patients with haematological malignancies and have been expanded under GMP conditions. To date, more than 25 patients have been treated and GvHD has not been an issue with these allogeneic cells.

Tuesday 1 November **1030-1130**
ISCTA Symposium 2: Manipulation of Alloreactivity ***Bayside Gallery B***

The Role of Mesenchymal Stromal Cells in the Management of Acute Graft-versus-Host Disease

Ian D Lewis
Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, SA, Australia

Mesenchymal Stromal Cells (MSCs) are a non-homogeneous population of plastic-adherent cells which were initially isolated from post-natal bone marrow. They have the capacity to differentiate to multiple mesodermal lineages including bone, cartilage and adipose tissue. In stringent culture conditions, MSCs can also be induced to differentiate into different cell types of endoderm and neuroectoderm lineages. To date, no specific marker identifies MSCs, although a number of cell surface antigens have been described which enrich for MSCs. Mesenchymal stromal cells possess a number of properties which has generated a great deal of interest in utilising them in a diverse number of cellular therapeutic applications. A large body of data indicates that MSCs possess immunomodulatory properties. Mesenchymal stromal cells are immunosuppressive, interacting with T lymphocytes, antigen presenting cells, B lymphocytes, and natural killer cells. In addition, they are immunogenic allowing transplantation across allogeneic barriers. These immunomodulatory properties have seen infusion of MSCs for the treatment of steroid refractory graft-versus-host disease (SR-GVHD), a life threatening complication of haemopoietic cell transplantation, with promising results. A number of studies have been published with contrasting results. To determine if MSC infusion represents an advance in the therapy of SR-GVHD requires further well designed randomised studies. Improved understanding of the mechanisms of MSC action and homing may lead to development of optimal culture conditions to produce an MSC product tailored for the treatment of GVHD.

Tuesday 1 November **1030-1100**
Lab Haematology 3 ***Bayside 202/203***

The Major Morphological Differences Between the Malaria Species

Robyn Wells
Core Haematology, Pathology Queensland Central Laboratory, Brisbane, Queensland, Australia

Aim
To identify and describe the features and characteristics of each of the five malaria species that infect humans.
Method
Each of the five species will be discussed and the main differentiating features and characteristics will be examined. Many features are common to all the species eg multiply infected red cells, but some occur more frequently in a species than others eg band forms and heavy pigmentation in *P. malariae,* red cell enlargement and Schuffners dots in *P. vivax* and *P. ovale*, accolé forms in *P. falciparum*. There are some characteristics are unique to a species and it is these exclusive characteristics that enable the morphological distinction between them. Examples of these are Maurer's clefts in *P. falciparum* and fimbriated red cells in *P. ovale.*
Conclusion
By the end of the talk, the participants will have refreshed and consolidated their knowledge of the characteristic features of the five species of malaria and gained confidence in the speciation of malarial parasites from both thick and thin films.

Tuesday 1 November **1030-1130**
Lab Haematology 3 ***Bayside 202/203***

The Role of the Laboratory in the Diagnosis and Management of Haemoglobin Disorders in Pregnancy

Jill Finlayson
Department of Haematology, PathWest Laboratory Medicine, QEII Medical Centre, Perth, WA, Australia and School of Pathology and Laboratory Medicine, Perth, University of Western Australia, WA, Australia

The haemoglobinopathies comprise a heterogeneous group of disorders, broadly categorised into two main groups – the thalassaemias and the haemoglobin variants. Within these groups, syndromes of major clinical significance include Haemoglobin Barts hydrops fetalis, beta thalassaemia major and sickle cell disease.
In the context of pregnancy, laboratory testing is important in identifying couples at risk for these severe syndromes. The laboratory should have standardised methodology to screen for beta thalassaemia trait and sickle cell disease, with a suitable turnaround time to facilitate further management such as partner testing and genetic counselling where required. The diagnosis of alpha thalassaemia trait requires DNA analysis to identify carriers of the alpha zero (α^0) allele who are at risk for Haemoglobin Barts Hydrops fetalis.
In population groups where HbS is prevalent, neonatal screening to identify infants at risk for sickle cell disease is an important consideration, in order to improve outcomes through early treatment and care.
Prenatal diagnosis may be offered to couples at risk. Chorionic villus sampling or amniocentesis are both invasive tests and carry a risk of miscarriage of 0.5 – 1%. The potential presence of maternal cell contamination (MCC) in chorionic villus or amniotic fluid samples poses a serious preanalytical risk for prenatal misdiagnosis. Methods to exclude MCC should include a range of informative markers to avoid false negative results.
Non-invasive testing for fetal DNA in maternal plasma has been investigated, particularly for beta thalassaemia, where the aim is to exclude beta thalassaemia major in the fetus. Provided that the assay has been optimized to have a high sensitivity for the detection of circulating fetal DNA, a negative signal for the paternal mutation indicates that no further prenatal testing is needed for a particular case.
Preimplantation genetic diagnosis (PGD) is considered as an alternative for prenatal diagnosis. It offers the advantage that it avoids the need for pregnancy termination and is an adjunct to assisted reproductive technology, and requires in vitro fertilization (IVF) to obtain embryos for evaluation.

Tuesday 1 November **1130-1230**
ANZSBT: Ruth Sanger Oration ***Auditorium B***

Clinical Transfusion Practice in the "-omics" Era

Wendy N Erber
Pathology and Laboratory Medicine, University of Western Australia, Crawley, WA, Australia

The human genome project along with improved technology for the assessment of cellular components and biological processes has resulted in improved understanding of human disease. Accompanying the scientific developments has been the introduction of a new vocabulary. Such terms as genomics, transcriptomics, proteomics and lipidomics, for example, are now commonplace in medical science. We are now in the era of the "-omics". What impact have the "-omics" had on clinical transfusion practice? This presentation will focus on some recent work on the genomics and transcriptomics of platelets and human disease to demonstrate the clinical relevance of the "-omics" in understanding disease. Mention will be made of genomics, in relation to blood donor and recipient assessment, and proteomics as applied in collection centres in ensuring quality of the blood supply. In spite of these examples of the applicability of the new sciences to transfusion, the day-to-day impact on clinical transfusion practice remains small. Whilst the "-omics" have expanded our scientific knowledge base, the improvements in clinical transfusion practice are largely being patient driven. The major drivers to optimising clinical blood transfusion practice have been changes in models of care, developments in surgical techniques, advances in patient blood management and patient expectation. Although we are in the scientific "-omics" era, clinical transfusion practice remains an art form and must remain patient-focussed.

Tuesday 1 November **1330-1500**
HSANZ Symposium 8: Lymphoma ***Auditorium B***

Treatment of B Cell Lymphoma – The Experience of the Japanese Oncology Group

Tomomitsu Hotta
National Hospital Organization (NHO) Nagoya Medical Center, Nagoya, Aichi, Japan

Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for approximately 30 percent of patients with NHL. CHOP has remained a standard chemotherapeutic regimen for aggressive NHL. However CHOP only cures 30% - 50% of patients. The lymphoma study group of Japan Clinical Oncology Group (JCOG) conducted a randomized phase III trial (JCOG 9809) to compare the PFS as primary endpoint between CHOP-14 and CHOP-21. The planned interim analysis of 286 patients revealed that CHOP-14 was unlikely to be superior to CHOP-21 and the trial was terminated early. The final results of this study with 7-year follow-up showed no substantial difference was observed PFS and overall survival (OS) between CHOP-14 and CHOP-21. Rituximab(R)–CHOP is well recognized as a standard treatment for advanced DLBCL and indolent B-cell NHL. Optimal conjunction of rituximab and chemotherapy, however, remains to be elucidated. JCOG has recently published the results of phase II/III study of R-CHOP-21 compared to R-CHOP-14 in indolent B-cell NHL (JCOG0203) to determine whether patients with indolent B-cell NHL would have long-term benefits from dose–dense immunochemotherapy. Three hundred patients were enrolled. At the median follow-up of 5.2 years, there was no significant difference in PFS between both arms: median was 3.7 (R-CHOP-21) v 4.7 (R-CHIP-14) years, 57% v 58% at 3 years, and 41% v 43% at 6 years (HR, 0.92; 95% CI, 0.68 to 1.25; one-sided P= .30) . The median OS time was not reached in either arm. The R-CHOP dose-dense strategy failed to improve PFS in patients with untreated indolent B-cell lymphoma. Further improvement of front-line treatment or investigations on post-remission therapy following R-CHOP should be explored. JCOG is conducting a phase II/III trial (JCOG0601) to determine whether the interval shortening of rituximab with CHOP-21 is superior to standard R-CHOP in advanced DLBCL.

Tuesday 1 November **13301500**
HSANZ Symposium 8: Lymphoma ***Auditorium B***

Thymic B-Cell Lymphomas: Novel Biological Insights and Shifting Treatment Paradigms

Kieron Dunleavy
National Cancer Institute, Bethesda, Maryland, USA

Over the past few years, an overlap in biologic and clinical features has been identified between mediastinal classical Hodgkin lymphoma (CHL) and primary mediastinal large B-cell lymphoma (PMBL). While PMBL is a subtype of diffuse large B-cell lymphoma (DLBCL), its molecular profile more closely resembles that of CHL and indeed, they share approximately a third of their genes. Further strengthening this relationship is the identification of lymphomas with clinical and morphologic features transitional between CHL and PMBL, known as 'grey zone lymphomas' (GZL). How GZLs differ molecularly from PMBL and CHL is not well understood but a recent large-scale methylation analysis demonstrated that GZLs have a unique epigenetic signature, different from other thymic B-cell lymphomas. There is much controversy regarding the clinical management of these lymphomas. Mediastinal CHL and PMBL have typically been treated with approaches that combine chemotherapy and mediastinal radiotherapy but the latter has been associated with a high risk of secondary cancers and ischaemic heart disease. The optimal therapy for mediastinal GZL has not been defined. Novel approaches that obviate the need for mediastinal radiotherapy in PMBL and GZL are being investigated and some have demonstrated very high efficacy. Novel agents like janus kinase inhibitors are also being investigated in these diseases.

Tuesday 1 November **1330-1500**
HSANZ Symposium 8: Lymphoma ***Auditorium B***

Salvage Options in Relapsed Diffuse Large B-cell Lymphoma

Mark Hertzberg
Department of Haematology, Westmead Hospital, NSW, Australia

For patients with diffuse large B-cell lymphoma (DLBCL) relapsing after first-line chemotherapy the standard of care includes salvage chemotherapy followed by high dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT). The recent large international CORAL study confirmed that there was no measurable difference in response rates, mobilisation rates, or progression-free and overall survival (OS) between RICE and R-DHAP salvage regimens. For patients relapsing prior to the addition of Rituximab to first-line therapy, the study confirmed results of the original PARMA study in which salvage therapy resulted in a 3-year event-free survival (EFS) of 40 to 50%. Furthermore, it identified the group that carries the worst outcomes as those patients who have had prior rituximab and relapse within 12 months of initial diagnosis. For these patients novel therapies are required. In addition, maintenance rituximab following ASCT did not provide a benefit in terms of improvements in either EFS or OS.

Not all relapsing patients can undergo HDCT and ASCT for reasons of age and/or co-morbidities. Again for those patients relapsing within 12 months of Rituximab-containing CHOP-like therapy the outlook is poor. For these patients there is no standard of care, while regimens such as R-DHAP are associated with substantial toxicity and poor long-term disease control. Hence, innovative salvage approaches have been used in this circumstance and include regimens incorporating Gemcitabine, oxaliplatin, ofatumomab, as well as Bendamustine, Bortezomib, and Obatoclax (ABT-263). For non-transplant eligible patients, we have undertaken a study using a modified R-ICE regimen in which the carboplatin is omitted and the ifosfamide and etoposide are delivered at 80% of the dose (R-IE). In a study of 30 relapsed/refractory patients, R-IE is characterised by an overall response rate of more than 70%, with a favourable toxicity profile, and is an acceptable salvage regimen for patients who are not eligible for HDCT.

Tuesday 1 November **1330-1500**
ANZSBT/ASTH Combined Symposium: Patient Blood Management Guidelines: Critical Bleeding/Massive Transfusion ***Auditorium A***

Massive Bleeding Algorithms: Experience of a German Level I Trauma Center

Klaus Görlinger[1], Daniel Dirkmann[1], Sven Lendemans[2], Björn Hußmann[2], Christian Waydhas[2], Karl Piepenbrink[1], Alexander Hanke[3]
[1] *Department of Anesthesiology and Intensive Care Medicine, University Hospital Essen, Germany*
[2] *Department of Trauma Surgery, University Hospital Essen, Germany*
[3] *Department of Anesthesiology and Intensive Care Medicine, Medical School Hannover, Germany*

Introduction
Ratio-based transfusion strategies for management of severe bleeding in trauma patients such as the 1:1:1-concept can be performed without coagulation monitoring but may be associated with unnecessary blood transfusion. However, allogeneic blood transfusion is associated with increased morbidity and mortality. In order to reduce transfusion requirements, we developed and implemented an algorithm for coagulation management in trauma patients based on point-of-care thromboelastometry (ROTEM®) combined with calculated goal-directed therapy with fibrinogen and prothrombin complex concentrate (PCC).
Methods
In a retrospective cohort study including 24,842 patients we analyzed the pre-ICU transfusion requirements before and after algorithm implementation.
Results
From 2002 to 2010 yearly transfusion of packed red blood cells (PRBC) (1332 vs. 888 units; -33%), fresh frozen plasma (FFP) (1221 vs. 261 units; -79%), and platelet concentrates (82 vs. 29 units; -65%) decreased. At the same time the number of operations in trauma surgery increased from 2594 to 2996 per year by 15%. In 2010 the incidence of massive transfusion (≥10 units PRBC before admission at ICU) was 0.6% (18 / 2996 patients) and the incidence of overall FFP transfusion was 1.2% (36 / 2996 patients). In 2009 overall 651 patients were treated in the emergency room, including 263 trauma patients and 163 multiple trauma patients with an ISS > 16.
Conclusions
The implementation of an transfusion and coagulation management algorithm based on point-of-care thromboelastometry (ROTEM®) combined with calculated goal-directed therapy with fibrinogen and prothrombin complex concentrate (PCC) was associated with decreased transfusion requirements for PRBC, FFP, and platelet concentrates in trauma surgery. This effect was most pronounced for the reduction of FFP and platelet transfusion requirements. Furthermore, this may be important for the reduction of FFP and platelet transfusion-related morbidity such as nosocomial infections, sepsis, transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and multiple organ failure (MOF).

Tuesday 1 November **1330-1500**
ANZSBT/ASTH Combined Symposium: Patient Blood Management Guidelines: Critical Bleeding/Massive Transfusion ***Auditorium A***

Development of the Critical Bleeding Guidelines

Craig French

Abstract not received at time of going to print

Tuesday 1 November **1330-1500**
ANZSBT/ASTH Combined Symposium: Patient Blood Management Guidelines: Critical Bleeding/Massive Transfusion ***Auditorium A***

Single Centre Experience of Six Years of Massive Transfusion Protocol

Michael Seldon
Hunter Area Pathology Service, Pathology North, NSW, Australia

The history of MTP in the institution will be outlined. Discussion will centre around the original protocol and how and why that has been modified over the time, and the issues with introducing such a protocol into a major surgical centre.

Newcastle is the major trauma referral centre for northern NSW, population of approximately 1 million, which is largely a country area. The majority of major trauma comes from high speed motor vehicle accidents, with the accidents often happening at night and often hundreds of kilometres from John Hunter Hospital, the trauma centre (JHH has 600 beds). Victims may be trapped for a considerable length of time before retrieval. This combines to create coagulation unfriendly situations.

Tuesday 1 November **1330-1500**
Nurses Free Communications 5: Education and Care ***Bayside Terrace***

O120 1330

A Change for the Better: Enhancing CVAD Practice Through Education.

Jo-Anne Greaves
Westmead Hospital, Sydney, New South Wales, Australia

The use of central venous access devices (CVADs) in haematology has become commonplace due to the nature of haematological disorders and the requirements of various treatment protocols. Each time a CVAD is accessed or manipulated there is an increased risk of developing a catheter-related blood stream infection (CRBSI), meaning that the safe utilisation of CVADs is important for the well being of the haematology/BMT patient.

During 2008 an increase in the number of reported CRBSIs were noted among the adult haematology patient population at Westmead Hospital.

Due to various organisational factors existing at the time, hospital policies surrounding CVAD care were outdated and not supported by current evidence. The education of registered nurses in the care of CVADs throughout the hospital was fractionated, with no clear educational strategies or guidelines to help address observed inconsistencies in practice. Overall this resulted in a lack of up to date, clinically relevant, evidence-based, policies and educational programs surrounding the use of CVADs.

To meet the needs of both staff and patients in Haematology/BMT, a number of unit-based interventions were formed into a CVAD 'care bundle'. The care bundle incorporated the use of new equipment and policies which were supported by educational strategies such as: the formulation of an updated evidence-based CVAD Learning Package (specific for Cancer Services); re-education of staff through inservicing; use of simulation experiences; assessment checklists to ensure standardisation of assessments; and the integration of regular yearly re-assessment of competency.
These educational components of our CVAD care bundle were aimed at producing a noticeable change in practice amongst ward nursing staff, and helped bring about a dramatic decline in the CRBSI rates amongst the target population.

No conflict of interest to disclose

Tuesday 1 November **1330-1500**
Nurses Free Communications 5: Education and Care ***Bayside Terrace***

O121 1345

The Experience of New Graduate Nurses in a Specialised Area: Haematology

Jennifer Francis
South Island Bone Marrow Transplant Unit, Christchurch Public Hospital, CDHB, Christchurch, New Zealand

Aim
Transitioning from a student to new graduate nurse is both stressful and complex as they socialise into the new role as a novice registered nurse while developing their own knowledge and clinical skills in the area. The aim of this study was to explore the experience of recently graduated nurses in a specialist area like haematology.
Method
A qualitative approach was used to describe the experience of new graduates in a bone marrow transplant ward in New Zealand. Questionnaires were given out to five nurses, including myself, who had worked in the unit since they registered with four main headings: Orientation, Preceptorship, Learning environment and Clinical Skills.
Result
The main themes that have arisen from these questionnaires were: Orientation structure, ongoing haematology study and staff support. There were sufficient supernumerary days, but four nurses found the orientation programme was unstructured and did not utilise those days. They also suggested that they had more haematology related study days to back up knowledge they had gained through clinical practice. Support from staff was variable with most respondents stating that particular senior nurses were against New Graduates working in the area and were not very encouraging and helpful to their learning. The majority of staff were respectful and willing to assist them.
Conclusion
This small study has shown the need for a more structured orientation in the unit, both for New Graduate nurses and possibly nurses who are new to the haematology area. Also highlighted was the resistance from senior staff on the unit to encourage and support New Graduates to develop their practice. I have discussed this with the clinical nurse specialist and we are working on developing an orientation programme for the ward to meet the needs of New Graduates and to help change the culture within the unit.

No conflict of interest to disclose

Tuesday 1 November **1330-1500**
Nurses Free Communications 5: Education and Care ***Bayside Terrace***

O123 **1400**

How Often Should Intravenous Administration Sets for Central Venous Access Devices be Changed?

Kieren Barker [1], Nicole Gavin[1,2]
[1]*Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia*
[2]*Griffith University, Brisbane, Queensland, Australia*

Aim
To assess the evidence on the safety of prolonging IVAS changes beyond 96 hours in patients with central venous access devices.
Methods
A literature search of available randomized controlled trials was undertaken searching the Cochrane Database of Systematic Reviews, The Cochrane Library Issue 3 2010; OVID Medline 1996 to July week 1 2010; CINAHL 1982 to July week 1 2010 and PubMED to July week 1 2010.
Result
Two studies involving 916 patients measured bacteraemia rates. Patients in the >96 hour group were twice as likely to develop a bacteraemia compared to the ≤96 hour group; the OR was 2.0 (95% CI 0.55, 7.32). The result was not statistically significant. The studies were moderately heterogeneous (I^2 = 38%).
Conclusion
There is insufficient evidence to recommend a time-frame for administration set changes. Until further evidence is available, the Cancer Nurses Society of Australia recommendation to change sets every 96 hours should be followed. Further randomized controlled trials should be adequately powered to show statistical differences between groups and include an economic analysis.

No conflict of interest to disclose

HAA 2011
HSANZ ANZSBT ASTH

Tuesday 1 November **1330-1500**
Nurses Free Communications 5: Education and Care ***Bayside Terrace***

O124 1415

Nursing Grand Rounds: An Opportunity to Expand Clinical Knowledge Through a Case Presentation, Peer Analysis and Review

Danielle Johanson[1], Nicole Gavin[12], Erin Downs[1], David Williams[1]
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[2]*Griffith University, Brisbane, Queensland, Australia*

Background
Nursing Grand Rounds (NGR) provided nurses with an opportunity to present a patient case to their peer group. The patient episode of care is examined, patho-physiology is reviewed, the nursing care is evaluated and the task of nursing care is related to the available evidence.

Aim
NGR can enhance nurse-led education and professional development by expanding haematology nurses' clinical knowledge through a case presentation. By promoting reflective practice NGR aim to improve direct patient care to haematology and bone marrow transplant patients by reviewing patient-centred, evidence-based nursing care.

Structure
A patient case of interest is chosen and milestones during their care are identified. A key nursing issue is highlighted and a literature search carried out to find the current evidence. Senior nurses from the in-patient and out-patient units are invited to the NGR to critically reflect upon the patient scenario and provide an opportunity to identify improvements that can be made to patient care.
The NGR are held every second month during the handover period between the early and late shifts and repeated to capture as many staff as possible. Key stakeholders have given their support and past presenters will mentor novice NGR presenters.

Findings
There has been positive feedback from the nursing staff. It is improving communication between the in-patient and the out-patient units, specifically linking pre- and post-admission care of our patients fostering smoother transition of care between the units. It provides an opportunity for continuing professional development.

No conflict of interest to disclose

Tuesday 1 November **1330-1500**
Nurses Free Communications 5: Education and Care ***Bayside Terrace***

O125 1430

CML Patients – 'Give Them a Tablet and They're Fine' – Are They?

Anthony Steele
Leukaemia Foundation of Australia

Background
Many patients complain to the Foundation that their side effects from TKIs are not being adequately addressed by their health professionals, because they have the attitude of "you should just be thankful – you should have seen what people with CML went through before TKIs".
Objective
To explore the main themes of issues faced by people with CML in 2011, and to use this information to improve services to the CML community.
Methods
The Leukaemia Foundation organised a working group of: haematologists, haematology nurses, researchers, Leukaemia & Blood Foundation of New Zealand, and CML patients with the support of Sweeney Research, to develop a national CML survey to explore issues faced by people with CML in the era of TKI's.
The survey consisted of 93 question topics – and took an average of 45 minutes to complete. The surveys were available online, and as a hard copy.
Results
The results of the survey will be presented at this conference, with implications on practice in the care of CML patients, and will identify where more research is needed.
Conclusion
CML is a blood cancer usually treated as a chronic disease with the use of TKIs. As the disease is often treated successfully with these oral medications in the outpatient setting, there is potential for the patient's psychosocial, and physical needs to be underestimated. This survey was designed to better understand these issues and help to promote discussion to improve patient outcomes.

No conflict of interest to disclose

Tuesday 1 November **1330-1500**
Nurses Free Communications 5: Education and Care ***Bayside Terrace***

O126 1445

"People Say I'm Softer When They Hug Me!" - Photopheresis of a Paediatric cGVHD Patient

James Badman[1,2], Melisa Darby[1,2], Annette Favaloro[1,2], Rachel Conyers[3], Frances Ness[1], Jessy Thambiraj[1] ,Joanne Harrison[4], Francoise Mechinaud[5] and Simon Harrison[1,2,3,6]

1. Apheresis Unit, Centre for Blood Cell Therapies, & 3 Haematology Service, Peter MacCallum Cancer Centre, East Melbourne, Australia. 4. Paediatric Respiratory Service & 5 BMT Service, Royal Children's Hospital, Parkville Melbourne. 6. University of Melbourne, Parkville, Melbourne, Australia

Background
The apheresis service at Peter MacCallum Cancer Centre has provided extracorporeal Photopheresis (ECP) treatment for adult patients with cutaneous T-Cell Lymphoma (CTCL) since 2001. Recently we have extended the service to include adult patients with graft versus host disease (GVHD) post allogenate transplantation. Following the introduction of the Therakos™ Cellex™ in our department, which accurately monitors extra corporal volume (ECV), in 2010, we successfully commenced treatment of a paediatric patient with extensive Grade 4 steroid refractory chronic GVHD of skin and lungs.
Aim
To present a case study of ECP treatment for a Paediatric patient with GVHD using the Therakos™ Cellex™ and how these experiences may inform clinicians of precautions for subsequent Paediatric ECP treatments.
Considerations
Smaller total blood volume (TBV) of the child reduced the amount of extracorporeal volume (ECV) available whilst managing haemodynamic stability. An electrolyte monitoring regime was established for the patient's electrolytes and a replacement regime for ca++ in light of Anticoagulant Citrate Dextrose Solution Formula-A (ACDA) administration during treatment. Management considerations and risk minimisation strategies to optimally care for the paediatric patient in an adult clinical environment. Frequency and treatment volumes for treatment.
Result
There has been a clear and progressive improvement in the grade and severity of cGVHD for our first paediatric patient, who has successfully weaned off all steroids after nine months ECP, allowing the healing of osteoporotic vertebral crush fractures, reduction in infectious complications and reversal of Cushingnoid habitus. Nursing staff have successfully transferred core ECP skills from an adult population to the paediatric setting. The nursing team has an improved awareness of monitoring and precautions to be included as routine part of treatment with particular focus on the management of the paediatric patient during all forms of apheresis.
The patient was initially bed bound and can now "scoot to school"
No conflict of interest to disclose

Tuesday 1 November **1330-1500**
ISCTA Symposium 3: Targeted Cellular Immunotherapy ***Bayside Gallery B***

Targeted Cellular Immunotherapy for Cancer

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Although cancer immunotherapy holds great potential for treatment of advanced malignancies, however, further refinement to increase the in vivo efficacies is urgently needed. In this report, we explored three targeted immunotherapy strategies using mouse breast cancer models. First, we evaluated the capacity and mechanisms of genetically modified HER2-specific T-cells to eradicate $HER2^+$ tumors in syngeneic mice. Primary mouse T-cells were modified to target the breast tumor-associated antigen HER2 through retroviral-mediated transfer of a chimeric antigen receptor, termed single-chain antibody scFv-CD28-ζ. The results demonstrated that treatment with scFv-CD28-ζ-modified T-cells was able to lead to long-term, tumor-free survival in mice bearing $HER2^+$ D2F2/E2 breast tumors. These surviving mice developed a host memory response to D2F2/E2 tumor cells, and this host response was able to protect against a rechallenge with $HER2^+$ D2F2/E2 tumor cells and parental $HER2^-$ D2F2 tumor cells. In addition, scFv-CD28-ζ T-cell expression of perforin and interferon-γ were essential for complete antitumor efficacy. Dendritic cells (DC) as most potent antigen-presenting cells in vivo have been confirmed repeatedly to be capable of inducing prominent antitumor immunity in preclinical and clinical trials, enabling them to be a promising candidate for cancer therapy; therefore, in our second study, we investigated the efficacy of *in vitro* genetically modified targeted DC. We modified DC with HER2-specific scFv-CD40 chimeric receptor by adenovirus-mediated gene transfer, which confers them with controllable HER2-specific migration and activation capacity when they administered in vivo. Our results showed that these scFv-CD40-modified DC could efficiently migrate toward $HER2^+$ tumor cells and become activated upon engagement by HER2 antigen on tumor cells followed by homing into the draining lymph node when applied to tumor-bearing mice. Treatment with these targeted DC induced the tumor-specific CTL responses and memory response, resulting in the eradication of established tumor in mice. To further facilitate translational application of DC vaccine, we designed and generated fusion proteins for directly targeting antigen to DC in situ. The targeted fusion protein fused the extracellular domain of human HER or its rat homologue neu to the scFv specific for CD11c ($scFv^{CD11c}$-HER2/neu) in our third study. Vaccination of BALB/c mice with $scFv^{CD11c}$-$HER2_{CpG}$ protected mice from subsequent challenge with HER2-positive, but not HER2-negative, murine breast tumor cells, accompanied by induction of strong HER2-specific T-cell and antibody responses. In a therapeutic setting, injection of $scFv^{CD11c}$-$HER2_{CpG}$ caused rejection of established HER2-positive tumors. Importantly, antitumoral activity of such a fusion protein vaccine could be reproduced in immunotolerant BALB-neuT mice, where $scFv^{CD11c}$-neu_{CpG} vaccination significantly protected against a subsequent challenge with neu-expressing murine breast tumor cells and markedly delayed the onset of spontaneous mammary carcinomas.

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Tuesday 1 November **1330-1500**
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Efficient Cross-presentation of Soluble Exogenous Antigens Introduced into Dendritic Cells

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Presentation of exogenous antigens by MHC class I molecules, known as “cross-presentation”, is considered crucial for the priming of CTLs to control many infectious pathogens as well as tumors. Presentation of soluble antigens is governed by the endocytosis mechanisms that determine the intracellular routing of endocytosed antigens. Most soluble antigens are internalized into lysosomes destined for classical MHC class II-restricted presentation; cross-presentation requires that antigens gain access to the MHC class I processing machinery that resides in the cytoplasm.

To develop dendritic cell (DC)-based cancer vaccines, we aimed to target soluble antigens to a subcellular localization in the cytosol. Proteins released from endosomes into the cytoplasm are degraded by the proteasome, and fragmented antigenic peptides can then be presented via the MHC class I pathway. Here, subcellular targeting was achieved by dendrimer phthalocyanine-encapsulated micelle (DPc/m)-mediated photochemical internalization or by using a weak-based amphiphilic peptide, Endo-Porter™.

The principle of photochemical internalization is the breakdown of the endosomal/lysosomal membranes by photoactivation of photosensitizers (DPc) localized on the micelle membrane. DCs pulsed with soluble antigens in the presence of DPc/m internalize both antigens and DPc/m via endocytosis. DPc/m accumulate in the endosome/lysosome compartments, where they efficiently disrupt the membrane after irradiation, resulting in antigen release from the lysosome into the cytosol. Accordingly, treatment of DCs with DPc/m plus irradiation enhanced presentation of antigens to $CD8^+$T cells.

Soluble antigens were also delivered into cytosol of DCs with Endo-Porter, which is rapidly endocytosed along with antigen. Upon subsequent acidification of the endosome, Endo-Porter becomes protonated and permeabilizes the endosome, enhancing the release of antigens into the cytosol. Subcellular targeting of DCs with Endo-Porter efficiently stimulates antigen-specific $CD8^+$ T cells.

These antigen delivery systems are promising approaches for developing more efficient cancer vaccines.

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Non-Viral Expression of Chimeric Antigen Receptors in Natural Killer Cells

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Natural Killer (NK) cells hold promise for cancer therapy. Anti-tumor activity of NK cells can be enhanced by expression of chimeric antigen receptors that re-direct NK specificity toward antigen-expressing target cells via engagement of cell surface molecules expressed on target cells. We developed a robust, scalable, cGMP & regulatory-compliant, non-viral approach to engineer NK cells by loading messenger RNA encoding chimeric antigen receptors into ex vivo expanded and into freshly isolated NK cells. Loading of mRNA encoding eGFP resulted in ~90% cell viability with ability to robustly control the level and duration of expression of the eGFP protein on NK cells. An mRNA encoding a receptor directed against CD19 (anti-CD19-BB-z) was used for process development and functional characterization of CAR-engineered NK cells. Messenger RNA loaded cells exhibited specific growth rate identical to control (mock transfected or untransfected) NK cells. Both ex vivo expanded and purified unstimulated NK cells resulted in 85% 6% (N=5) and 86% 4% (N=4) recovery of viable transfected cells. CAR-mRNA engineered NK cells demonstrated enhanced cytotoxicity against CD19+ target cells resulting in ?0% lysis of acute lymphoblastic leukemia and B-lineage chronic lymphocytic leukemia cells at effector target ratios in the range of 1:1 to 1:10. The target-specific cytotoxicity for anti-CD19-BB-z mRNA-transfected NK cells was observed as early as 3 hours after transfection, was maintained for up to 3 days, and was detectable even at 7 days. We are currently collaborating with clinical investigators to translate these findings into an IND submission to enable cGMP compliant manufacture of CAR-mRNA loaded ex vivo expanded NK cells for evaluation of safety and anti-tumor activity in cancer patients.

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Tuesday 1 November **1530-1630**
HSANZ Symposium 9: Lymphoma Diagnosis ***Auditorium B***

Common Misdiagnoses in Lymphomas and Avoidance Strategies

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Lymphoma diagnosis is challenging. Reactive lymphoid conditions have to be distinguished from neoplastic lesions. Common pitfalls include autoimmune lymphoproliferative syndrome and IgG4-related sclerosing disease being diagnosed as neoplastic; and ALK-positive anaplastic large-cell lymphoma (ALCL) variants, classical Hodgkin lymphoma (CHL) variants and infarction of lymphomatous lymph nodes being diagnosed as reactive.

Histological similarities lead to problems in diagnosis that may impact on treatment strategies. Lymphomas that may be difficult to be distinguished from each other include CHL *versus* ALK-negative ALCL; lymphoplasmacytic lymphoma *versus* marginal zone lymphoma; plasmablastic lymphoma *versus* anaplastic plasmacytoma; angioimmunoblastic T-cell lymphoma *versus* peripheral T-cell lymphoma not otherwise specified; and subcutaneous panniculitis-like T-cell lymphoma *versus* primary cutaneous $\alpha\beta$ T-cell lymphoma *versus* extranodal natural killer cell / T-cell lymphoma *versus* panniculitis. In these situations, detailed immunophenotypical results must be interpreted with clinical information to enable an accurate diagnosis to be clinched.

Further diagnostic issues arise in lymphomas where features intermediate between two well-defined subtypes are observed. Mediastinal gray-zone lymphomas may show features of both primary mediastinal large B-cell lymphoma and CHL, and are currently classified as “B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma”. Lymphomas with hybrid features of Burkitt lymphoma and diffuse large B-cell lymphoma may be found, and classified as “B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma”. Lymphomas with concomitant *MYC* and *BCL-2* rearrangement (double-hit lymphoma) often belong to this category.

Although immunophenotyping classifies lymphoma accurately into different lymphoid lineages, occasionally cross-lineage antigen expression may be observed. Thus, CD20, a B-cell antigen, may be expressed on non-B-cell lymphomas or absent on *bona fide* B-cell lymphomas. CD3, a T-cell antigen, may be expressed on non-T-cell lymphomas. Even more rarely, pan-lymphoid markers may be expressed on non-lymphoid malignancies. These caveats must be considered in the interpretation of immunophenotyping results.

The demonstration of monoclonal rearrangement of the immunoglobulin gene and T-cell receptor gene is invaluable for lineage assignment in B-cell and T-cell lymphomas. However, cross-lineage gene rearrangement may be occasionally found. Furthermore, apparent clonal immunoglobulin or T-cell receptor gene rearrangements may rarely be observed in non-neoplastic lymphoid lesions. Therefore, close collaboration between the histopathologist, clinician and molecular biologist is needed so that the most appropriate diagnosis is reached.

Tuesday 1 November **1530-1630**
HSANZ Symposium 9: Lymphoma Diagnosis ***Auditorium B***

Human Herpes Virus 4 – Run Silent, Run Deep

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Credited with being the first known human oncogenic virus, Epstein Barr Virus (EBV) was discovered by Anthony Epstein in samples of lymphoma received from Denis Burkitt in Uganda in 1961. Within 15 years an association with nasopharyngeal carcinoma and Hodgkin lymphoma had also been established and its role as the agent of infectious mononucleosis clearly demonstrated.
The list of human diseases in which it is implicated has however increased dramatically in the subsequent 35 years. The current 2008 WHO Classification of Neoplasms of the Haemopoietic System for example identifies over 11 entities which are known to be EBV associated, yet the virus is also linked with several soft tissue tumours and it has been implicated in autoimmune disease including multiple sclerosis. Moreover the 2008 WHO monograph presents a truncated view of the role of EBV in disease, since it describes only the "neoplastic" end of disease spectra, the prodromal syndromes of which may evolve over many years.

EBV is related to other primate lymphocryptoviruses and has evolved in tandem with homo sapiens over several million years to exploit the B-cell arm of host immunity as a strategy for survival. To do so successfully, the virus must subvert the normal signaling pathways of the cells it infects. EB virus gene expression programs, both proliferative and anti-apoptotic, which have evolved for virus survival, can interact with known ontogenetic pathways to promote lymphomagenesis - for which Burkitt and Hodgkin lymphomas provide illustrative paradigms.

The ability of EBV to persist in latent states, the resulting delicate, lifelong balance between virus and host immunity, and the molecular changes that EBV wreaks upon the host cell in order to survive, account for many of the features of EBV related LPD.
A conceptual understanding of these diseases thus presents a cross-disciplinary challenge, encompassing virology and immunology as much as tumour biology and haemato-oncology.

In this presentation, a review of EB viral gene expression programs is used as a conceptual framework for an understanding of EBVLPD, based upon host immunity, viral latency, patient age and disease class. This framework provides a useful road map with which to view and better understand these eclectic diseases, but it also serves to emphasize our surprising lack of knowledge in relation to one of humankind's more ubiquitous parasites.

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Tuesday 1 November **1530-1630**
ANZSBT Symposium 8: Platelets and Dengue ***Auditorium A***

Hemostatic Changes in Dengue Infection

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Dengue infection is the most important mosquito-borne viral disease in tropical areas. It is classified as dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) depending on its severity and presenting features. Although severe hemorrhage remains the major cause of death, the pathogenesis of bleeding is poorly understood. During the febrile phase, von Willebrand factor antigen, tissue factor, and plasminogen activator inhibitor (PAI-1) were significantly higher, while platelet counts and ADAMTS 13 activity were significantly lower in patients with DHF as compared to patients with DF. During the toxic phase, soluble thrombomodulin, tissue plasminogen activator, and PAI-1 were also significantly higher, while platelet counts, ADAMTS 13 activity, and thrombin activatable fibrinolysis inhibitor were significantly lower in patients with DHF as compared to patients with DF. Endothelial cell injury, activation of coagulation cascade with thrombin generation, increased antifibrinolytic factors, and consumption of natural anticoagulants appear to play an important role in the development of hemorrhage and disseminated intravascular coagulation (DIC) in patients with severe DHF. There has been evidence that the hemostatic defect in DHF is due to the direct effects of virus on endothelial cells, or through the activation of immune and inflammatory pathways. The downregulation of the cytoprotective protein C pathway in human endothelial vascular cells during dengue Infection has also been demonstrated. Successful management of severe dengue infection relies on meticulous regulation of parenteral fluids and colloid during the period of increased vascular leakage, together with proactive management of major bleeding should this situation develop. The management of patients with prolonged shock, severe DIC, and bleeding remains a challenging problem. Insights in vascular biology and the interplay between virus, the immune system, endothelium, and the coagulation system in DHF will be crucial for the development of predictive markers and therapeutic interventions for this condition.

Tuesday 1 November **1530-1630**
ANZSBT Symposium 8: Platelets and Dengue ***Auditorium A***

Platelet Transfusion – The Future

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Introduction
Two aspects of platelet (plt) transfusion therapy need improvement; i.e., extend plt storage times and prevent plt alloimmunization.
Extend Plt Storage Time
Post-transfusion plt viability is based on both the collection method and the storage conditions. The U.S. Food and Drug Administration (FDA) requires that autologous plts should have post-storage radiolabeled plt recoveries that are 66% of the donor's fresh recoveries and plt survivals 58% of fresh. Plt concentrates (PRP or BC) can be stored in either plasma or a plt-additive solution (PAS) for 6 days and meet FDA requirements. Haemonetics and Cobe apheresis plts can be stored in plasma for 8 days, Cobe plts in PAS for 7 days, and Haemonetics for 13 days. The difference in PAS results for the apheresis plts may be due to the storage bag.
Prevention of Plt Alloimmunization
The Trial to Reduce Alloimmunization to Platelets (TRAP Trial) demonstrated that filtration-leukoreduction (F-LR) or UV-B irradiation of plts were equally effective in reducing alloimmunization rates. Use of UV-B irradiation was based on studies in our dog plt transfusion model where UV-B prevented alloimmunization in 45% of the recipient dogs *versus* 82% in patients. The better patient results were likely because they were immunosuppressed from chemotherapy *versus* the dogs who had a normal immune system. Recently, we demonstrated that combining F-LR with pathogen reduction of donor plts (Caridian's Mirasol technology) prevented alloimmunization in 14/15 recipient dogs (93%).
Conclusions
Plt storage for 13 days is possible under certain conditions. Plt alloimmunization has been prevented in 93% of dogs using combined F-LR and pathogen-reduction. Because the dog has previously predicted efficacy in patients, we anticipate that this approach will be successful, possibly even in non-immunosuppressed patients.

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Tuesday 1 November **1530-1630**
ASTH Symposium 8: Platelets and Other Microbes ***Bayside 204***

Staphylococcal Exotoxins and Platelet Function

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Staphylococcus aureus (S. aureus) is a common pathogen capable of causing life-threatening infections. Amongst the deadliest consequences of *S. aureus* infections is the disseminated intravascular coagulopathy (DIC) that is associated with both bleeding as well as thrombus formation. We hypothesised that one of the potential pathomechanisms for this systemic effect of *S. aureus* infections is the existence of an exotoxin that is secreted by *S. aureus* and that directly modulates platelet function.

Staphylococcal superantigen-like protein 5 (SSL5) is a potential candidate for such a central pathophysiological role. Indeed, using flow cytometry, immunoprecipitation and surface plasmon resonance we could demonstrate that SSL5 binds to two platelet receptors, GPIbα and GPVI. This binding causes platelet activation, as shown in flow cytometry and immunofluorescence microscopy, platelet adhesion and platelet aggregation. Furthermore, we demonstrate the *in vivo* relevance of the SSL5 effects demonstrating the induction of pulmonary embolism after intravenous injection of SSL5. Finally, with the intention to develop therapeutic strategies to antagonise SSL5 effects, we developed blocking anti-SSL5 antibodies and furthermore identified glycans as direct interaction partners mediating SSL5 binding to GPIbα and GPVI. *In vitro* and *in vivo* testing demonstrated the potential to fully block SSL5 effects both via antibodies as well as specific glycans. For example, pulmonary embolism could be fully prevented by antibodies as well as glycans.

In conclusion, these findings identify SSL5 as an *S. aureus* exotoxin with a potential systemic platelet-activating effect and elucidate the functional interactions between SSL5 and platelets, including the identification of the receptors that mediate SSL5 binding and platelet activation/ aggregation. Furthermore, we successfully tested an antibody as well as a glycan-based therapeutic approach both *in vitro* and *in vivo*.

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ASTH Symposium 8: Platelets and Other Microbes ***Bayside 204***

Platelet-Bacteria Interactions

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Platelets can interact with bacteria through three distinct mechanisms. One type of interaction is a direct binding of a bacterial protein to a platelet receptor. An example of this is the binding of *Streptococcus sanguinis* and *Streptococcus gordonii* to platelet GPIbα. The second type of interaction is the binding of a plasma protein to the bacteria that in turn binds to a platelet receptor. Thus, *Helicobacter pylori* binds von Willebrand factor which in turn binds to GPIbα and *Staphylococcus aureus* binds fibrinogen which in turn binds to GPIIb/IIIa. The third mechanism is for secreted toxins to activate the platelet directly such as *Escherichia coli* lipopolysaccharide binding to Toll-like receptor 4. These interactions either act to mediate platelet adhesion, trigger platelet activation or both. Unlike aggregation that is induced by soluble agonists such as ADP, bacteria-induced aggregation has a significant delay prior to aggregation. In many cases multiple interactions are required for an aggregation response to occur. In particular, many bacteria bind antibody that subsequently binds to platelet FcγRIIa receptor and this interaction is required for activation to take place. Thus, *S. aureus* Clf A binds both fibrinogen and anti-Clf A antibodies. The fibrinogen subsequently binds to GPIIb/IIIa while the antibody binds to FcγRIIa. This dual signal is responsible for triggering platelet activation. Thus, platelet aggregation in response to bacteria is a complex process and is both species and strain specific.

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Tuesday 1 November **1530-1630**
Nurses Symposium 6: Looking Forward ***Bayside Terrace***

Moving Forward – Blood Transfusion Practice in Europe

Elizabeth Pirie
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Blood is an essential adjunct to modern clinical practice however the unnecessary transfusion of a blood component exposes patients to unnecessary risk and wastes the donor's gift. Over the last decade there have been several countries that have experienced prolonged, socially damaging and costly consequences of patients being harmed by transfusion. In the UK the Serious Hazards of Transfusion (SHOT) reporting scheme has demonstrated that there have been 151 deaths where blood transfusion has been the main or subsidiary cause. The blood supply has also been steadily declining and research has shown there are significant differences in blood use all over Europe.

The collection, processing, testing and distribution of blood is legally controlled in the European Union (EU) by blood directives but this control does not extend to the clinical transfusion process. This area, which can be considered as optimal blood use, is a matter of professional direction and not controlled by law. As part of the 2006 EU Public Health Programme, the Scottish National Blood Transfusion Service (SNBTS) led a three year project to promote the optimal use of blood, based on experience of a quality improvement programme that has been operating in Scotland. Twenty project partners representing 16 EU member states met to develop a Manual, which provides practical guidance for those seeking to improve the safety of the clinical transfusion process and the effectiveness of the use of blood components. The EU Optimal Blood Use Manual was launched in 2010 and is now available in 8 languages, English, French, German, Greek, Italian, Polish, Portugese and Spanish at at *http://www.optimalblooduse.eu/*

This project has been a successful first step in increasing awareness of the importance of improving clinical blood transfusion practice in Europe. Going forward there is enthusiasm from the project participants to continue to share information and best practice, and build a network of transfusion professionals.

Tuesday 1 November **1530-1630**
Nurses Symposium 6: Looking Forward ***Bayside Terrace***

Looking Forward - BM Transplantation in the Asia Pacific Region

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Since the report of the first successful bone marrow transplant (BMT) several decades ago, BMT has rapidly become a well established curative therapy for a range of diseases. In spite of the successes, it remains a high cost and high risk procedure that is out of reach for many in developing countries. The Asia Pacific Blood and Marrow Transplantation Group (APBMT) registry was established 5 years ago and in this short period of time, the registry has provided invaluable transplant activity data on over 95,000 BM transplants in the Asia Pacific region. The 4th APBMT transplant activity survey (APBMT annual report December 2010) shows 10,393 transplants were performed in 2008. The annual number of transplants recorded has doubled in eight years. Several countries including India and China have recorded an annual rise of over 30%. This report has likely underestimated the number of transplants in Asia pacific as only 15 countries in this region reported to the APBMT registry. A recent survey published by the Worldwide Network for Blood and Marrow Transplantation in 2010 (Gratwohl A et al JAMA 2010) shows that transplant rates were expectedly higher in countries with higher gross national incomes. In Asia Pacific, the number transplants per 10 million population in 2006 ranged from 0.6 to 448 compared to 5.7 to 792 in Europe. However, transplantation for haemoglobinopathy and allogeneic BMT were reported to be higher in Asia Pacific region as compared to Europe and America. The median number of transplants in our region in 2006 was 184 compared to 269 in Europe. The reported absolute number of transplants was 7096 in Asia Pacific, 17,875 and 24, 216 in USA and Europe respectively. The relevance of these comparisons is that as the economy of our region continues to improve, it is expected that transplants in Asia will outnumber those in Europe and America in the near future as over half of the world population resides in Asia Pacific region. This will translate to potential for collaboration among countries within our region. This provides us unprecedented and exciting opportunities to promote clinical excellence and to improve BMT outcomes via our active participation in scientific and educational exchanges, registry and APBMT society activities.

Tuesday 1 November **1530-1630**
Nurses Symposium 6: Looking Forward ***Bayside Terrace***

Looking Forward - Clinical Trials in Australia/NZ

Ian Kerridge

Abstract not received at time of going to print

Tuesday 1 November **1530-1630**
ISCTA Free Communications 1: Fundamental and Translational Cell Therapy Research
Bayside Gallery B

O127 1530

Therapeutic Targeting of GSK3β in Stem Cell Transplantation

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During the last few years, the glycogen synthase kinase-3β (GSK3β) was identified as a regulator of many components of stem cell biology, leukaemogenesis and immune responses. The broad array of biological actions affected by GSK3β is attributable to the remarkable number of substrates regulated by GSK3β. Lithium that effectively inhibits GSK3β, is already clinically approved and effective in treatment of neurological diseases. In addition, several new selective inhibitors of GSK3β have been developed in the last few years although only one GSK3 inhibitor, NP12, has advanced to trials in human patients. We and others have shown that several therapeutic strategies targeting GSK3β can be beneficial in stem cell transplantation: (i) pharmaceutical inhibition of GSK3β used to enhance stem cell engraftment and post-transplant reconstitution; (ii) for immunomodulation to prevent Graft-versus-Host Disease (GVHD) (iii) pharmaceutical inhibition of GSK3β to induce direct anticancer effects. We and others have shown that GSK3β inhibitors act through a number of additive or synergizing mechanisms that target normal and leukaemia stem cells, T cells and the surrounding microenvironment in the bone marrow. GSK3β inhibition activates Wnt in stem cells and bone marrow niche and suppresses NfkB in activated T cells and leukaemia blasts. Thus targeting GSK3β may represent an opportunity to achieve combined effects with earlier post-transplant reconstitution, immunomodulation, or direct anticancer effects. GSK3β inhibition appears feasible and potentially useful for clinical application in patients receiving allogeneic hematopoietic stem cell transplants for hematologic malignancies and other blood disorders. The extent of GSK3β inhibition required for clinical efficacy as well as tolerance to this level of inhibition will be addressed in the near future. With improved knowledge of their pharmacokinetics and safety profile in humans, GSK3β inhibitors could represent a valuable supportive clinical tool for treatment of GVHD, reducing leukaemia relapse and improving haematopoietic engraftment.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Tuesday 1 November **1530-1630**
ISCTA Free Communications 1: Fundamental and Translational Cell Therapy Research
Bayside Gallery B

O128 1545

Characterisation of ELF2 in Haemopoietic Development Using a Murine Bone Marrow Reconstitution Model

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Introduction and Aim
ELF2 (E74-like factor 2), a member of the ETS family of transcription factors, has been reported to regulate genes important in B cell development, cell cycle progression, and angiogenesis. ELF2 also interacts with RUNX1 and LMO2, which are important master regulators of haemopoietic development. Two conserved ELF2 isoforms, ELF2A and ELF2B arise from alternative promoter usage and have previously been shown to exert opposing effects on target gene expression; ELF2A transactivates while ELF2B represses gene expression. How either isoform might influence haematopoietic development is poorly understood. Here, we aim to examine ELF2 function in haematopoietic cell lines and in a murine bone marrow reconstitution model.
Method
ELF2 isoform expression was examined by quantitative PCR (qPCR) analysis and western blot analysis in a range of myeloid and lymphoid cell lines. In the murine bone marrow reconstitution model, mouse bone marrow cells over-expressing specific isoforms of ELF2 with GFP, or GFP alone as the control group, were used to reconstitute sub-lethally irradiated *Rag1*$^{-/-}$ mice (n=9 per group). Flow cytometric analysis was used to phenotype cells of both myeloid and lymphoid lineages in the chimeric mice.
Results
By qPCR and western blot analysis, ELF2 isoforms were found differentially expressed within each cell line and the expression profiles varied between the cell lines investigated. Preliminary examinations of peripheral blood in chimeric mice revealed an increase of approximately 2-fold in the percentage of granulocytes and B lymphocytes compared to the control group. Futhermore, the ratio of CD4:CD8 T lymphocytes was found significantly decreased compared to the control group ($p<0.05$).
Conclusion
Our preliminary data indicate a balance in ELF2 isoform expression may be important in the regulation of haematopoietic development in both the myeloid and lymphoid lineages.
No conflict of interest to disclose

Tuesday 1 November **1530-1630**
ISCTA Free Communications 1: Fundamental and Translational Cell Therapy Research
Bayside Gallery B

O129 1600

Evaluation of a Sensitive Cumulative Flow Cytometric Assay for Measurement of Long-Term Expanded LAK Cytotoxicity

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Aim
Novel cancer immunotherapies involving LAK-NK cells aim for large scale production of highly cytotoxic clinical-grade effectors in long-term *ex vivo* cultures. However, robust non-radioactive methods for routine cytotoxicity assessment are scarce. Here we compared side-by-side a cumulative, non-radioactive, sensitive flow cytometric cytotoxicity assay (FCCA) to other established methods, including the traditional radioactive ^{51}Cr-release assay (CRA).
Methods
LAK cells (effectors, E) were cultured for several weeks in serum-enriched AIM V/high-dose-IL-2. For FCCA (Oezdemir, Y. et al., 2003. *Cytometry A*, 56A, 53-60, modified): Tumour targets (T) were labeled with the membrane dye PKH26 for 100% detection. Target lysis was determined comparing Flow-Count™-Fluorosphere-(BC)-adjusted viable target counts - excluding debris, Annexin V- and/or 7AAD-positive targets - at time 0 and time 4hrs.
Results
FCCA exceeded CRA sensitivity markedly at the highest E:T ratio (3:1), with mean K562 lyses of 87.3%, 79.5%, and 33.8% compared to only 63.3%, 61.8%, and 21% lyses at days 14-16, 21-23, and 40-51, respectively (n=5). Interestingly, disregarding early apoptotic events in the FCCA analyses resulted in comparable data. The [^{3}H]TdR JAM assay, which detects target cell death by means of DNA fragmentation, showed only 23%, 13%, and 19% mean K562 lysis at days 15, 24, and 43, compared to 85%, 94%, and 53% mean lysis for the parallel FCCA (n=2). Similarly, assessment of effector degranulation using CD107a-surface (n=3) or intracellular perforin staining (n=3) was insensitive. FCCA's applicability for short term (2hrs/4hrs) and longer term (over-night) cytotoxicity assessment was demonstrated in kinetic studies (n=3). FCCA is suitable for specific detection of tumour target death in heterogeneous healthy cell backgrounds as demonstrated in simulation spiking experiments including PBMC-(insensitive)-K562-(sensitive) mixtures. Furthermore, FCCA revealed differential sensitivity of haematological targets K562, U937, KU812, and MOLT-4 to such LAK effectors (n=3).
Conclusion
FCCA has valuable applicability for routine cytotoxicity measurement of long-term expanded LAK cells.

No conflict of interest to disclose

Tuesday 1 November **1530-1630**
ISCTA Free Communications 1: Fundamental and Translational Cell Therapy Research
Bayside Gallery B

O130 1615

Development of 'Lab-on-a-Chip' Cellular Analysis for Haematology and Tissue Engineering Research

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Aims
Lab-on-a-chip cellular analysis is revolutionising biological research by automating and miniaturising traditional cell culture techniques. Our aims are to develop lab-on-a-chip devices to a) mimic pulsatile flow and embryonic circulation to study the influence of fluid shear on microvasculature and early development of blood from haemogenic endothelium, b) enable parallel live-cell imaging of hundreds of haematopoietic clones and c) develop statistical methods to quantify the rates of lineage commitment, cell division and apoptosis from progenitor pedigree data.
Methods
Both devices were manufactured by a technique called 'soft-lithography' in clean room facilities at the UNSW Nanofabrication facility. Multilayered devices were bonded together using an oxygen plasma. Biocompatibility of microdevices were determined as follows: KG1a cells were grown in microwell devices over 6 days at a rate similar to culture flask growth with imaging of 500-600 wells every 3 minutes; Bovine endothelial cells were seeded onto the microcirculatory device. Statistical methods and software for analysis of granulocyte-macrophage (GM) pedigrees obtained by live cell imaging was performed on data kindly provided by T. Schroeder et al.
Results
We observed the growth of 1,400 KG1a colonies in parallel culture at 3 minute intervals for 6 days of culture. The biocompatibility of the microcirculatory devices was confirmed because endothelial cells were observed to divide and grow to confluence by live cell imaging. Endothelia cells oriented with flow in regions of high shear stress. Over 500 GM pedigrees were analysis, with measurement of the rates of apoptosis, division and unipotent lineage commitment by Kaplan-Meier analysis.
Conclusions
We have confirmed the biocompatibility of both devices. The microwell device has utility in the study of haematopoiesis and immunology. The microcirculatory devices will be applied to the study of vascular biology and haematopoiesis.
No conflict of interest to disclose

Tuesday 1 November **1630-1730**
HSANZ Masterclass 10 ***Bayside 102***

Biology and Treatment of Acute Promyelocytic Leukaemia

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Acute promyelocytic leukaemia (APL) is characterised by chromosomal translocations involving the gene encoding Retinoic Acid Receptor Alpha (*RARA*) at 17q21, fused in the majority of cases with *PML* located at 15q22. Approximately 10% of cases lack the classic t(15;17), but nevertheless harbour the *PML-RARA* fusion, resulting from insertion events or more complex rearrangements. Detection of the *PML-RARA* fusion is clinically important, predicting a favourable response to molecularly-targeted therapies, i.e. all *trans*retinoic acid (ATRA) and arsenic trioxide (ATO), which have transformed management. In 1-2% of APL, *RARA* is fused to an alternative partner gene, including *PLZF* (*ZBTB16*), *NPM1*, *NuMA*, *FIP1L1*, *BCOR*, *PRKAR1A,* and *STAT5b*; ATO sensitivity has not been documented in these molecular variants, which also differ in their response to ATRA. While APL fusion proteins are well established as transcriptional repressors that block myeloid differentiation; molecular mechanisms underlying the formation of translocations such as the t(15;17), which are critical early steps in leukaemogenesis remain poorly understood. To gain further insights we have investigated cases of therapy-related APL, arising as a complication of exposure to drugs targeting topoisomerase II (topoII). We have shown that translocation breakpoints exhibit significant clustering, corresponding to preferential sites of drug-induced topoII DNA cleavage, which are aberrantly repaired to yield the t(15;17). We have also been interested in developing molecular monitoring strategies using standardized real-time quantitative polymerase chain reaction (RQ-PCR) assays to allow the development of more individualized treatment approaches. In particular, in paediatric APL we are evaluating an MRD-directed risk-stratified I-BFM protocol that entails a significant reduction in anthracycline exposure (ICC-01 study). While in adults, the scope to deintensify therapy is being further explored in the NCRI AML17 trial, comparing a curtailed ATRA+anthracycline monochemotherapy protocol with a chemotherapy-free schedule involving ATRA+ATO, with the prospect that the majority of patients may be cured with largely outpatient therapy.

Tuesday 1 November **1630-1730**
HSANZ Masterclass 11

Bayside 103

Novel Agents in Multiple Myeloma: Managing Specific Toxicities

Paul Richardson
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Multiple myeloma (MM) is a hematologic malignancy that results from clonal perforation of plasma cells which produce a monoclonal immunoglobulin typically present in the blood and/or urine. In the United States it is estimated that in 2010 over 20,000 new cases of myeloma will be diagnosed, making it the second most common hematologic malignancy after lymphoma. With the incorporation of novel agents as targeted therapy including proteasome inhibitors and immunomodulatory agents, median survival has significantly improved. Furthermore, with the integration of novel therapies around established standards of care such as autologous stem cell transplant, further improvements in patient outcome have been achieved. Despite these advances, myeloma remains incurable. Moreover, recent studies have supported the notion that continuous therapy and maintenance of treatment are critical to improving patient outcome.

In this context, the management of side effects has become critical. Significant side effects associated with the use of novel agents include thromboembolic disease, myelosuppression, fatigue, gastrointestinal toxicities, and, most importantly, peripheral neuropathy (PN).

PN can be caused by myeloma itself and particularly by certain therapies including Bortezomib, Thalidomide, and conventional agents such as vinca alkaloids and cisplatinum. Clinical evaluation has shown up to 20% of myeloma patients have PN at diagnosis and as many as 75% may experience treatment-emergent PN during therapy. The incidence, symptoms, reversibility, and predisposing factors of treatment emergent PN vary, with PN incidence also affected by dose and schedule of potentially neurotoxic agents as well as base-line patient characteristics. Effective management of treatment-emergent PN is critical to minimizing the incidence and severity of this complication while maintaining therapeutic efficacy. Strategies include earlier and regular monitoring with neurological evaluation as indicated with dose modification, schedule change and treatment discontinuation if needed using evidence-based guidelines.

The optimal sequencing and integration of treatment strategies has become increasingly important as survival has improved. Long-term toxicities have also become an important concern, such as the interaction of traditional genotoxic treatment with immunomodulatory agents which may result in rare but potentially serious complications such as second new primary tumors. Further research, including development of myeloma specific patient focused assessment tools, pharmacogenetic analysis of patient DNA and trials to assess the efficacy of interventions to minimize toxicity are thus all the more important to further improve patient outcome.

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Tuesday 1 November **1630-1730**
HSANZ Masterclass 12 ***Bayside 104***

Flow Cytometry for Minimal Residual Disease

Mary Sartor
Westmead Hospital, Westmead, NSW, Australia

This session will focus on the application of multi-parameter flow cytometry to the detection of minimal residual disease in haematological malignancies including B and T-ALL, AML and CLL. Individual cases will be presented to illustrate techniques of MRD detection with particular emphasis on practical aspects such as panel design, gating strategy, identification of leukaemia associated phenotypes and determination of the level of sensitivity for specific assays. The session is not designed to explore the clinical implications of MRD detection in individual patients but to assist scientists and physicians wishing to establish and interpret MRD assays in their own laboratories or to gain a better understanding of how MRD detection by flow is performed.

Tuesday 1 November **1630-1730**
HSANZ Masterclass 13 ***Bayside 105***

Biology and Genetic of Relapse in Acute and Lymphoblastic Leukaemia

Charles Mullighan

Abstract not received at time of going to print

Tuesday 1 November **1630-1730**
ANZSBT Masterclass 14

Bayside 201

Case Studies - Support of the Alloimmunised Patient

D.J.Anstee
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The sensible application of DNA-based blood typing will contribute to the prevention of alloimmunization in those patient groups (sickle cell disease (SCD), thalassemia) at particular risk of developing alloantibodies. Nevertheless, the advance in patient care offered by DNA typing will not be fully realized if the available donor pool does not contain sufficient blood donors from the ethnic groups commonly afflicted by haemoglobinopathies. This point is readily illustrated by many patients with SCD who have made anti-Fy3.

Despite this advance there will continue to be patients with antibody or antibodies to high frequency antigens which render them virtually untransfusable because compatible donors are so rare. In these cases it is often possible to find compatible blood through the WHO International Panel of Rare Donors or from a sibling. Alternatively, autologous donation may be considered.

Patients with alloantibodies to known or novel high frequency antigens and/or complex antibody mixtures pose particular problems for a Blood Group Reference Laboratory. At the moment the identification of the specificity of these antibodies often involves a long and complex serological investigation and some inspired guesswork combined with targeted DNA analysis. In the future, much of this work will likely be circumvented by the application of rapid DNA sequencing methods which make feasible the sequencing of all blood group genes in such patients.

Tuesday 1 November **1630-1730**
ANZSBT Masterclass 15 ***Bayside 202***

Pandemic Preparedness

Che-Kit Lin
Hong Kong Red Cross Blood Transfusion Service, Hong Kong SAR

On June 11, 2009, the World Health Organization (WHO) declared the Influenza H1N1 virus a pandemic - the first pandemic of the 21st Century. A human influenza pandemic occurs when an influenza virus emerges to which the majority of the human population has little or no effective immunity. As a result it spreads rapidly infecting large numbers of individuals. If the pandemic virus is particularly virulent then as well as causing widespread illness and disruption, it can result in significant complications including large numbers of deaths.
Due to the rapid and extensive mutation of the virus and the current limitations of vaccine technology, we must assume that no effective vaccine will be available in time to mitigate the impact of at least the first wave of the pandemic. Anti-virals can reduce severity of disease and, in theory, can prevent those in close contact from developing the disease. They thus have an important role in prophylaxis, in particular for healthcare workers including blood service staff.
In a pandemic, our service will come under severe pressure to maintain blood supply and safety. With the experience of SARS and the recent H1N1 epidemic, we have developed a plan to prepare for future outbreaks, taking account into consideration the local public health pandemic influenza plans, relevant health and safety guidance and guidance of our blood regulators. In the planning process, we consider it important to collaborate and communicate with our major stakeholders and blood services in neighbouring areas. References have also been taken to the WHO checklist for influenza pandemic preparedness planning. To mitigate risks arising from the pandemic, we need to identify and assess the options available and ensure that all reasonable measures which could reduce the impact of the emergency on safety and sufficiency of blood supply are included in the plan. The plan should be updated and drills carried out regularly.

Tuesday 1 November **1630-1730**
ASTH Masterclass 16 **VENUE**

Managing Perioperative Bleeding With Point-of-care Testing

Klaus Görlinger[1], Dietmar Fries[2], Daniel Dirkmann[1], Christian F Weber[3], Alexander A Hanke[4], Herbert Schöchl[5]
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Introduction
Massive bleeding as well as transfusion of packed red blood cells (PRBC), fresh frozen plasma (FFP), and platelets are associated with increased morbidity, mortality, and costs.
Methods
We analysed the transfusion requirements after implementation of point-of-care (POC) coagulation management algorithms based on early, calculated, goal-directed therapy with fibrinogen concentrate and prothrombin complex concentrate (PCC) in different perioperative settings (trauma surgery, visceral and transplant surgery (VTS), cardiovascular surgery (CVS), and general and surgical intensive care medicine) at three different hospitals (AUVA Trauma Centre Salzburg, University Hospital Innsbruck, and University Hospital Essen) in two different countries (Austria and Germany).
Results
In all institutions implementation of POC coagulation management algorithms was associated with a reduction in transfusion requirements for FFP by about 90% (Salzburg 94%, Innsbruck 88%, and Essen 93%). Furthermore, PRBC transfusion was reduced by 8.4-62%. The incidence of intraoperative massive transfusion (≥ 10 U PRBC) could be more than halved in VTS and CVS (2.56 vs. 0.88%; $p<0.0001$ and 2.50 vs. 1.06%; $p=0.0007$, respectively). Platelet transfusion could be reduced by 21-72% except in CVS where it increased by 115% due to a fivefold increase in patients with dual antiplatelet therapy (2.7 vs. 13.7%; $p<0.0001$).
Conclusions
Implementation of perioperative POC coagulation management algorithms based on early, calculated, goal-directed therapy with fibrinogen concentrate and PCC was associated with a reduction in transfusion requirements for FFP, PRBC, and platelets as well as with a reduced incidence of massive transfusion. Thus, the limited resource blood can be used more efficiently.

Notes:

Wednesday 2 November **0830-1000**
HSANZ Free Communications 9: Acute Leukaemia ***Auditorium B***

O131 0830

Contribution of MicroRNA Dysregulation in the Inhibition of Differentiation In AML

Mark Lutherborrow[1], Catalina Palma[1], Elisa Tonna[1], Adam Bryant[1], Vivek Jayaswal[2], David Agapiou[1], Yee Hwa Yang[2], David Ma[1]
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2 School of Mathematics and Statistics, Centre for Mathematical Biology, University of Sydney, Sydney, NSW, Australia.

Aim
To investigate the contribution of microRNA dysregulation to the inhibition of Acute Myeloid Leukaemia (AML) cell differentiation.
Method
Bone marrow mononuclear cell (MNC) samples from 28 AML patients with a normal karyotype and 8 normal controls were obtained. MicroRNA expression profiling was performed with differentially expressed microRNA expression confirmed by qRT-PCR. Luciferase assays confirmed microRNA targeting of candidate mRNAs.
Results
MicroRNA profiling was unable to accurately delineate different FAB subtypes of AML, however the expression of 12 microRNAs was able to accuracy separate AML M1 and M5 subtypes. The M1 subtype represents a relatively immature cell population when compared to a more monocytic morphology observed in M5 AML blasts. Six candidate microRNAs were selected for further investigation based in their putative targeting of key monocytic transcription factors. Using a cell line model of monocytic differentiation, Vitamin D treatment of HL60 and NB4 cells resulted in a significant decrease in all six candidate microRNAs. Four of these microRNAs were shown to suppress the expression of several key monocytic transcription factors.
Conclusion
MicroRNA profiling of normal karyotype AML identified 12 microRNAs that accurately segregate M1 and M5 subtypes. Cell differentiation and mRNA targeting experiments suggest that over-expression of these microRNAs in M1 blasts contributes to their relatively immature phenotype.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
HSANZ Free Communications 9: Acute Leukaemia ***Auditorium B***

O132 0845

Lenalidomide and Azacitidine Maintenance Therapy for Poor Risk Acute Myeloid Leukaemia: Interim Analysis of a Phase Ib/II Study

Peter Tan[1], Patricia Walker[1], Sharon Avery[1], Sushrut Patil[1], Anthony Schwarer[1], Henry Januszewicz[2], Simon Harrison[2], Wai Khoon Ho[3], Constantine Tam[4], Andrew Spencer[1], Andrew Wei[1]
[1]Department of Clinical Haematology, The Alfred Hospital, Melbourne, Australia. [2]Peter MacCallum Cancer Centre, East Melbourne, Australia. [3]Austin Health, Melbourne, Australia. [4]Department of Haematology, St Vincent's Hospital, Melbourne, Australia

Background
Despite achieving remission after intensive chemotherapy for AML, the relapse risk remains particularly high for patients 1) over 60 years of age 2) with poor risk karyotype 3) who are FLT3-ITD positive or 4) beyond 1st remission. Maintenance therapy is an attractive option, especially when allogeneic stem cell transplantation is not an option. The hypomethylating agent azacitidine and the immunomodulatory drug lenalidomide both have clinical efficacy in MDS/AML. Combining azacitidine with lenalidomide produces a CR rate of 41% in high risk MDS (Sekeres *et al*, Journ of Clin Oncol 2010) and azacitidine has been used as maintenance therapy in AML (Grövdal *et al*, British Journ of Haem 2010). The optimal dosing schedule of azacitidine in combination with lenalidomide post intensive chemotherapy in AML is not known.

Aim
To determine safety and tolerability of escalating doses of azacitidine in combination with lenalidomide as maintenance therapy for high risk AML in CR/CRi after intensive chemotherapy.

Methods
A phase Ib/II open label 3x3 dose escalation study in AML patients with either: age > 60, poor risk karyotype, FLT3-ITD+ or CR>1 in in CR/CRi after intensive chemotherapy. Maintenance therapy was with subcutaneous azacitidine on days 1-5 combined with orally administered lenalidomide on days 5-25 of each 28-day cycle.

Results
Interim analysis of 16 patients (M 8, F 8), median age 65 years (43-73) are reported. Qualifying features included: age >60 (11 patients), poor risk karyotype (1), FLT3-ITD+ (1), CR2 (3). Dose limiting toxicities (DLT) recorded so far: cohort A (azacitidine 50 mg/m^2, 0/3 DLT's), cohort B (azacitidine 50 mg/m^2+lenalidomide 5 mg, 0/3 DLT's), cohort C (azacitidine 60 mg/m^2+lenalidomide 5 mg, 1/6 DLT's; grade 3 ALT elevation in a patient with known hepatitis C), cohort D (azacitidine 60 mg/m^2+lenalidomide 10 mg, 0/4 DLT's). The median number of cycles delivered is 3 (1-10). Grade 3/4 haematologic toxicities were: neutropenia (5), thrombocytopenia (3) and anaemia (1). The maximum tolerated dose (MTD) has yet to be defined. After a median follow-up of 233 days, 6/16 patients have relapsed with a median relapse-free survival (RFS) of 219 days (17-378) and median overall survival (OS) of 443 days (30-478). 3 patients have subsequently received post-study RIC allo-SCT and all are alive and in remission 7, 10 and 13 months post-transplant, respectively.

Conclusion
Azacitidine in combination with lenalidomide is feasible as maintenance therapy after intensive chemotherapy for AML. Dose-escalation is ongoing to determine the MTD.

This research was supported by Celgene

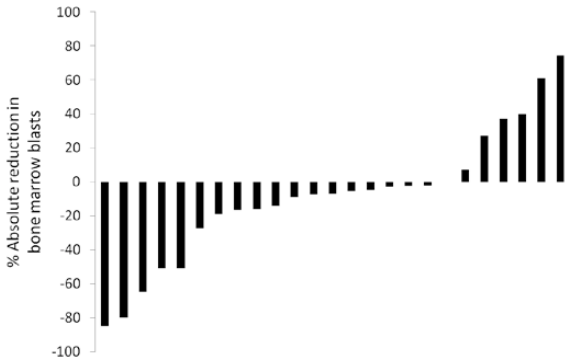
Wednesday 2 November **0830-1000**
HSANZ Free Communications 9: Acute Leukaemia ***Auditorium B***

O133 0900

Phase Ib/II Study of Azacitidine in Combination With the Oral mTOR Inhibitor Everolimus (RAD001) in Relapsed and Refractory AML

Peter Tan[1], John Catalano[2], Patricia Walker[1], Anthony Schwarer[1], Sharon Avery[1], Sushrut Patil[1], Stephen Opat[1], Andrew Spencer[1] and Andrew Wei[1]
[1]Department of Clinical Haematology, The Alfred Hospital, Monash University, Melbourne, Australia. [2] Department of Haematology, Frankston Hospital, Frankston, Australia.

Background
The reported overall response rate (ORR; CR/CRi+PR) for azacitidine in relapsed and refractory AML is 11-19% (**ASH 2009 #1054 and 2044).** Everolimus (RAD001), is an orally bioavailable inhibitor of mammalian Target Of Rapamycin (mTOR).
Aim
To investigate the tolerability and preliminary efficacy of combining everolimus with azacitidine in relapsed and refractory AML.
Methods
Phase Ib/II open label dose escalation study using azacitidine 75 mg/m^2 sc daily on days 1-5 and 8-9 of each 28-day cycle with 2.5, 5 or 10 mg everolimus orally on days 5-21.
Results
This preliminary analysis includes 34 patients (M 21, F 13), median age 64 years (range 17-71) receiving 2.5 mg (n=6), 5 mg (n=12) or 10 mg (n=16) everolimus in combination with azacitidine. 26 (77%) had relapsed (5 after prior alloSCT) and 8 (23%) had primary refractory AML after 1 (n=14), 2 (n=13) or 3 (n=7) previous lines of therapy. 10 (29%) had poor risk cytogenetics. Response in 28 evaluable patients was 39% (2 CR, 9 PR). Absolute bone marrow blast reductions from baseline ranged from 3 to 85% (Figure). At a median follow up of 262 days, median OS is 210 days (194d in primary refractory and 210d in relapsed AML) and median PFS is 178 days. 3/5 patients treated for relapsed AML after allo-SCT had clinical responses and remain alive at 192, 199 and 472 days. Grade 3/4 non-hematologic toxicities are summarized as follows: 2.5 mg everolimus cohort- septicemia (n=1) and mucositis (n=1, dose limiting toxicity; DLT), 5 mg everolimus cohort- septic arthritis (n=1, DLT), 10mg everolimus cohort- rash (n=1). The maximum tolerated dose was not reached. Febrile neutropenia during the first cycle of therapy was reported in 6/34 (18%).
Conclusion
In relapsed and refractory AML, azacitidine in combination with the mTOR inhibitor everolimus was well tolerated and demonstrated substantial clinical activity in this advanced AML population. Further evaluation of this promising combination is ongoing.



This research was supported by Novartis and Celgene.

Wednesday 2 November **0830-1000**
HSANZ Free Communications 9: Acute Leukaemia ***Auditorium B***

O134 0915

Outpatient Management of Elderly Patients with Acute Myeloid Leukaemia Using Low-Dose, Prolonged Administration of Cytarabine and Thioguanine in Combination with Filgrastim: Preliminary Results From a Single-Centre, Retrospective Study

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The incidence of acute myeloid leukaemia (AML) increases with age and outcomes for elderly patients remain poor. Furthermore, intensive induction chemotherapy is often unsuitable for elderly patients and can result in significant periods of inpatient care. This study is a retrospective, single-centre analysis of outcomes in elderly patients with AML managed as outpatients at Royal North Shore Hospital, Sydney, Australia. Between April 2009 and March 2011, 14 patients with either relapsed/refractory AML or *de novo* AML unsuitable for intensive therapy were treated using prolonged, low-dose cytarabine 20mg/m^2 subcutaneously and thioguanine 80mg orally. All patients were targeted for outpatient management with supportive care including filgrastim and prophylactic antibiotics. Patient age ranged from 52 to 89 years (median 75y). All patients had intermediate or poor risk cytogenetics. A morphologic remission according to bone marrow aspirate was obtained in 8 patients (57.1%), with relapse seen in 1 patient at 2.6 months follow-up. Remission was maintained in 7 patients (50%) with follow-up ranging from 4.7 to 26.6 months (median 9.7 months), including 1 patient who was refractory to standard first and second-line induction chemotherapy. Refractoriness to treatment occurred in 5 patients (35.7%). Mortality relating to disease progression occurred in 3 patients (21.4%) and 1 patient died secondary to infection. This study demonstrates that effective management of AML in elderly patients can be achieved in the outpatient setting. The preliminary data suggests surprising efficacy for this strategy, with outcomes comparable to those reported using standard induction chemotherapy but with a potentially favourable toxicity profile.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
HSANZ Free Communications 9: Acute Leukaemia ***Auditorium B***

O135 0930

Use of Temozolomide in Relapsed/Refractory Acute Myeloid Leukaemia, Experience at Two Institutions

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Introduction
Temozolomide is an oral alkylating agent, currently used in the treatment of glioblastoma and metastatic melanoma. The cytotoxic efficacy of temozolomide in myeloid leukaemic blasts has been documented and several small studies have reported varying efficacy in relapsed refractory leukaemia. We describe our experience with temozolomide in patients with relapsed or refractory acute myeloid leukaemia (AML).

Methods
This was a retrospective cohort study of all patients who received temozolomide for AML at The Canberra Hospital and St Vincent's Hospital, Sydney from 2000-2010. Patient characteristics, prior treatment and response was collated from the patient's medical records. The response to temozolomide and patient outcomes were documented.

Results
7 patients received temozolomide for relapsed/refractory AML at two centres. There were 4 male and 3 female patients with age range from 27 to 70 years (median 61). They had received a median of 2 prior therapies. 3/7 patients responded to temozolomide including 1 partial remission (>50% reduction in marrow blasts) and 2 cytogenetic complete remissions. In two patients, response to temozolomide enabled them to proceed to allogeneic transplantation; one of these patients died of graft vs host disease 2 months after treatment with temozolomide, the other patient died due to relapsed AML 7 months after treatment with temozolomide. In the other patient who was not transplanted the initial response lasted for 4 months. Toxicity was mainly haematologic. There was no unexpected serious toxicity.

Conclusion
Temozolomide demonstrated clinically meaningful activity in this heavily pre-treated cohort of patients with relapsed/refractory AML. In two patients with dramatic clinical response this facilitated allogeneic stem cell transplantation. Our experience suggests further formal prospective evaluation of this agent is warranted.
No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Wednesday 2 November **0830-1000**
HSANZ Free Communications 9: Acute Leukaemia ***Auditorium B***

O136 0945

Acquired von Willebrand Disease (vWD), Cyclical Thrombocytosis and Hydroxyurea (HU)-Related Complications in Patients With Ph-Negative Myeloproliferative Neoplasms (MPNs)

Emma Verner [1], Cecily Forsyth [2], Andrew Grigg [3]
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2: Wyong Hospital, Kanwal, NSW, Australia
3: Austin Health, Melbourne, Victoria, Australia

Aim
To determine the incidence of less commonly reported disease and HU-related complications in an Australian cohort of patients with Ph-negative MPN.
Method
Retrospective audit of patients in a metropolitan tertiary referral centre and a regional community based practice.
Results
188 patients (M/F: 115/73) were included in the audit: 104 with essential Thrombocythaemia (ET), 62 with polycythaemia vera (PV), 18 with primary myelofibrosis (PMF), and 4 with MPN-unclassifiable (MPN-U). The median age at diagnosis was 66 (18-93, apart from 1 patient diagnosed in infancy) years with a mean length of follow-up of 7.6 (0.5-44) years. 139 (74%) patients were JAK2+ and 79% of patients had received HU, with a mean daily dose of 700mg and an average length of exposure of 4.2 years. 54 (29%) patients had been treated with non-HU cytoreduction including interferon, anagrelide, busulphan and P32.
Cyclical thrombocytosis (CT), defined as platelet counts that varied by over 100 x 10^9/L during a 6 week period while on a stable dose of cytoreductive therapy, was documented in 29 (15%) patients, the majority (86%) of whom were taking HU (with a mean dose of 920mg/day), and occurred in all MPN subgroups. The mean maximum difference between the lowest and highest platelet count during the 6 weeks was 350 x10^9/L (100-1346 x10^9/L). Acquired vWD, most frequently screened for in a subset of patients with platelets >1000 x10^9/L, was identified in 17 patients (20% of those screened) and was not associated with haemorrhagic complications in the absence of haemostatic challenge. Reversible HU-related toxicities identified included drug-fever (n=9), hepatitis (n=6, 4 of whom also experienced HU-induced fever), leg ulcers (n=8), mouth ulcers (n=6), digital gangrene (n=1). Non-melanoma skin cancers were common, occurring in 54 patients, and occurred multiply in 29. Thrombotic events occurred in 72 patients (93 thrombotic events in total) with the majority of these (66%) occurring prior to or at diagnosis.
Conclusion
Therapy of Ph-negative MPNs was associated with a number of clinically significant, albeit relatively infrequent, reversible complications related to HU. Cyclical thrombocytosis and acquired vWD were not uncommon, the recognition of which in individual patients is important for management.

No conflict of interest to disclose

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 10: ALL & Myeloma ***Bayside 103***

O137 0830

Low-Dose Lenalidomide and Dexamethasone (RD) Reduces Toxicity Without Compromising Efficacy Compared to Standard Dose Lenalidomide-Dexamethasone (RD) in Elderly Patients With Relapsed/Refractory Multiple Myeloma (RRMM): A comparison of RevLite to MM009/010

Hang Quach[1], Liam Fernyhough[2], Ross Henderson[3], Gillian Corbett[4], Dirk Honemann[5], Stefan Peinert[6], Bart Baker[7], Hiliary Blacklock[8], Peter Browett[9] Emma Link[10], Linda Cowan[10], Jacki Li[5], Arlene Swern[5], Mohamad Hussein[5], Kevin Lynch[5], Meletios Dimopolous[11], Miles Prince[1,12]
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Aim
The safety and efficacy of lower dose lenalidomide-dexamethasone (rd) was evaluated in a phase II RevLite study in RRMM patients at high risk for myelosupression including elderly patients. Results were compared to a similar cohort of patients receiving standard dose lenalidomide-dexamethasone (RD) from the two phase III MM009 and MM010 studies.
Methods
150 pts received 28-day cycles (C) of rd: 15mg lenalidomide, D1-21 (C1-4; C5+ if ≥stable disease [SD]) and 20mg dexamethasone (D1-4, 9-12, 17-20 [C1-4] and D1-4 [C5+] if ≥ SD) until progression. MM009/010 treatment has been described. 255 MM009/010 RD pts with ≥1 of these RevLite eligibility criteria: age ≥60, creatinine clearance (CrCL) ≤60 mL/min, platelets ≤75×10^9/L) were included in the analysis. PFS, TTP and OS were assessed by the Kaplan-Meier method and comparisons were made using the logrank test.
Results
Baseline characteristics were similar except for prior thalidomide (rd 65 vs RD 36%). With a median follow-up of 9.2 (rd) and 14.8 (RD) months, similar ORR (rd 67% vs RD 60%) was seen. PFS (rd 10 vs RD 11 mos; *P*=0.64) and time to progression (TTP; rd 12.5 vs RD 13 mos, *P*=0.36) were similar. OS was not significantly different for rd vs RD (25 mos vs not reached, *P*=.08). In pts with CrCL ≤60 mL/min, PFS (8.5 vs. 7.4 mos), TTP (10.3 vs 9.5 mos) and OS were similar (15.8 vs not reached mos) for rd vs RD. Pts receiving rd had lower incidence of Gr 3/4 AE vs RD, including neutropenia (19 vs 38%), thrombocytopenia (4.7 vs 13.7%) and thrombotic events (2 vs 13%).
Conclusion
These data suggests low-dose rd regimen is an option in elderly pts with RRMM or pts at high risk of myelosuppression, achieving similar efficacy with less toxicity vs RD.
This research was supported by Celgene Corporation. The company facilitated access to the raw data of from the MM009 and MM010 studies for the above comparison.

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 10: ALL & Myeloma ***Bayside 103***

O138 0845

Fat1 Cadherin as a Novel Minimal Residual Disease Marker in Acute Lymphoblastic Leukemia

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Aim
Despite improvements in chemotherapeutic regimes, a considerable number of patients with Acute Lymphoblastic Leukaemia (ALL) eventually suffer relapse due to minimal residual disease (MRD). Current MRD markers can be laborious and expensive to implement since these are either leukaemia specific (ALL with known chromosomal translocations) or patient specific (normal karyotype ALL). Here, we propose Fat1, a cadherin superfamily member whose altered expression has previously been implicated in a number of solid tumours, as a novel tumour marker in ALL.

Methods
In this report, we measured the expression of the Fat1 protocadherin in a range of leukemia cell lines and normal peripheral blood (PB), bone marrow (BM) and haematopoietic stem cells (HSC) both at protein and mRNA level. Extending this work to clinical leukaemias, we also carried out the *in silico* analysis of micro array data in both clinical correlates as well as normal haematopoiesis. Further to demonstrate proof of Fat1 as MRD marker, artificially spiked preB-ALL cells into normal peripheral blood was screened by qPCR.

Results
We demonstrate Fat1 expression in subsets of T-ALL, B-ALL and AML cell lines but not in normal PB, BM or HSC cells. *In silico* analysis revealed the presence of Fat1 mRNA transcript in 11% of AML, 29% of B-ALL and 63% of T-ALL. In similar microarray evidences of normal haematopoiesis, we also found Fat1 with little or no transcription level in all haematopoietic lineages. Artificially spiking samples showed experimentally robust detection of the Fat1 transcript at 1:100,000 cells using qPCR.

Conclusion
Since Fat1 is expressed by ALL cells but has low to absent expression on normal haemic cells, it may serve as a candidate generic marker of Minimal Residual Disease (MRD). Additionally, since Fat1 is a cell surface molecule, the development of an anti-Fat1 antibody may also be warranted and this finding could offer significant advantages in ALL therapy.
No conflict of interest to disclose

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 10: ALL & Myeloma ***Bayside 103***

O139 0900

Combination Mapatumumab and Low Dose Velcade Treatment Induce Myeloma Immunogenic Cell Death

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Aim
Immunogenic cell death (ICD) following novel therapy in multiple myeloma has the potential to incite a disease-controlling immune response. Velcade is a novel therapy with significant impact on refractory multiple myeloma; however, velcade doses used in clinical trials to date have a deleterious effect on the immune system. To address this problem, we evaluated whether combination therapy with an agonistic antibody to TRAIL-R1, Mapatumumab (Mapa) and low dose Velcade (ldV) would facilitate ICD by inducing myeloma apoptosis and preserve immune function.
Method
Human myeloma cell lines (n=6) were examined for sensitivity to mono- or combination therapy with Mapa and/or Velcade. The effect of Mapa-ldV was assessed on human monocyte-derived DC (MoDC) function including maturation, phagocytosis, antigen presentation and the generation of MM-specific cytotoxic lymyphocytes.
Result
Velcade induced apoptosis in all myeloma cell lines from 5nM dose upwards; this titration curve established each myeloma cell line's sub-optimal sensitivity to Velcade for subsequent studies. Mapa-induced apoptosis in 4/6 myeloma cell lines (RPMI8226, U266, OPM-2, LP-1) as a monotherapy, sensitivity to Mapa correlated with surface expression of TRAIL-R1. Subsequent combination therapy with ldV plus Mapa-induced apoptosis in 4/6 myeloma cell lines (RPMI8226, U266, LP-1 and OPM-2), in 3/6 (RPMI8226, U266 and OPM-2) ldV Mapa-induced apoptosis was significantly greater than that observed with Mapa alone. MoDC viability and function were unaffected by ldV-Mapa combination therapy. In the presence ldV-Mapa, immature MoDCs (iDCs) readily phagocytosed myeloma apoptotic bodies, responded to lipopolysaccharide by upregulating CD80, CD86, CD83, MHC class II and secrete IL-12p70. Combination Mapa-ldV induced enhanced cytotoxic lymphocyte response to autologous MoDCs primed with the myeloma cell line U266, this occurred specifically when the ldV-Mapa was present during MoDC endocytosis of apoptotic U266.
Conclusion
ldV-Mapa combination treatment shows promise as an anti-myeloma therapy. ldV-Mapa preserved DC function and induced endogenous anti-myeloma immune responses.

The authors have no conflict of interest to disclose.
This research was supported by Human Genome Sciences Inc. The company had no role in analysing the data or preparing the abstract.

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 10: ALL & Myeloma ***Bayside 103***

O140 0915

Minimal Residual Disease Testing by Flow Cytometry for Childhood Precursor B Cell Acute Lymphoblastic Leukaemia

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Aim
The presence of Minimal Residual Disease (MRD) at day 29 of treatment for precursor B cell Acute Lymphocytic Leukaemia (Pre B ALL) is the single most important prognostic factor in children treated on the Children's Oncology Group (COG) protocols used by the Royal Children's Hospital (RCH) [1]. MRD detection by flow cytometry for children enrolled on the COG study is performed by a reference laboratory in the USA. The MRD result is used to stratify patients for further treatment according to perceived risk of relapse. MRD assessment is now considered standard of care in the treatment of children with ALL so the RCH is in the process of validating the COG MRD detection by flow cytometry assay into routine clinical use.

Method
The assay examines differences from normal expression of CD9, CD10, CD13/33, CD19, CD20, CD34, CD38, CD45 and CD58. Clusters of events that fall outside the areas where normal cells occupy are considered as abnormal. The definition of MRD requires a sensitivity of at least 0.01%. 1 x 10^6 cells are labelled in order to collect enough events to achieve sensitivity in this range.

Results
Good correlations have been achieved with the COG results and molecular methods for MRD. A sensitivity of at least 0.01% has been achieved for all positive patients. All patients tested so far have had informative leukaemia aberrant markers enabling MRD detection by flow cytometry.

Conclusion
MRD detection by flow cytometry has the advantages of being applicable to almost all Pre B ALL patients, is a rapid test and is relatively more cost effective than other methods of MRD detection. It will become the standard of care for the treatment of all our paediatric patients with Pre B ALL.

No conflict of interest to disclose.

1. Borowitz et al. Blood 2008 111(12):5477

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 10: ALL & Myeloma ***Bayside 103***

O141 0930

Inhibition of Class I Deactylases Is Critical for Myeloma Cell Death

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Introduction
Deactylation of proteins by histone deactylases (HDACs) orchestrates gene expression, cell differentiation and survival. HDACs consist of 18 genes grouped into Class I (HDAC1-3 and 8), Class II (HDAC4, 5, 6, 7, 9 and 10), Class III (Sirtuins1-7) and Class IV (HDAC11). Altered HDAC expression is significantly associated with poor prognosis in malignancies; expression levels and relevance to patient outcome in multiple myeloma (MM) is unknown. Deactylase inhibitors (DACi) exhibit a diverse range of anti-MM effects, however it is not clear which HDAC requires inhibition for maximal MM cell death.

Aims
(i) Compare *HDAC* expression in purified MM cells to normal plasma cells and correlate to overall survival (OS – diagnosis to death) and progression-free survival (PFS – diagnosis to first relapse/progression) in MM patients,
(ii) Ascertain the most suited DACi for MM therapy.

Methods
Quantitative RT-PCR was performed for *HDAC1-11* in normal (n=6) and MM plasma cells (n=41) purified from patient bone marrow aspirates obtained following consent. The OS and PFS in MM samples with high gene expression levels ($\geq 75^{th}$ centile) compared to low levels ($\leq 75^{th}$ centile) were derived utilising Kaplan-Meier survival plots. Pan-inhibitors (LBH589 and SAHA), Class I inhibitor (FK228) and HDAC6 inhibitor (Tubacin) were evaluated in bone marrow mononuclear cells from MM patients (n=6). Proportion of MM cell death was determined by flow cytometric analysis for APO2.7 at 24 and 48 hours.

Results
Quantitative gene expression analysis revealed that *HDAC2*, *3*, *5* and *7* were elevated ($p \leq 0.03$), while *HDAC4* was repressed (p=0.03) in MM plasma cells compared to normal. Patients with elevated *HDAC7* demonstrated decreased PFS (median survival: 751 vs 1224 days; p=0.03) compared to patients with lower expression levels. Deactylase inhibition in primary MM cells demonstrated marked inter-patient variation in cell death at 24 hours, while maximal killing occurred with Class I inhibition and not HDAC6 inhibition alone at 48 hours.

Conclusion
This study is the first comprehensive confirmation that HDAC are deregulated in MM. Inhibiting Class I HDAC appears to be critical in inducing maximal cell death in primary MM samples.

No conflict of interest to declare

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 10: ALL & Myeloma ***Bayside 103***

O142 0945

Regulation of the Bone Marrow Microenvironment by G-CSF: Effects on Malignant Lymphopoiesis

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G-CSF is commonly used to treat chemotherapy-induced neutropenia and for the mobilization of hematopoietic stem cells for transplantation in patients with leukemia. Administration of G-CSF has profound effects on the bone marrow microenvironment including the cleavage of essential molecules required for the maintenance of hematopoietic cells.
G-CSF had no effect on the proliferation of patient derived ALL cell lines *in vitro*, regardless of the presence of supportive stroma or expression of the G-CSF receptor. Using a NOD/SCID xenograft model, G-CSF was found to increase disease progression in two of six xenografts investigated (1345 and 1999), while the remaining xenografts demonstrated a reduction in leukemia. Microarray analysis of the bone marrow from xenograft 2032, which decreased in progression, revealed that the expression of the genes encoding cathepsin G and neutrophil elastase were increased while expression of *CXCR4* decreased, consistent with our current understanding of G-CSF mobilisation. Surprisingly, a similar analysis of marrows from mice bearing xenograft 1999 showed an increase in the expression *CXCR4* and a decrease in cathepsin G and neutrophil elastase mRNA levels in response to G-CSF. The reason for such disparate results are not clear but may result from specific cytogenetic differences, however a simple correlation between genetic changes and responses were not apparent. Regardless it appears that some patient leukemias can profoundly alter the response of the bone marrow microenvironment to G-CSF.
In summary, G-CSF inhibited leukemia progression in the majority of patient xenografts, however, in a subset of samples G-CSF accelerated disease progression. Clinically, G-CSF administration to ALL patients has not been associated with any adverse outcomes. However our data suggest that a small subset of patients may experience accelerated disease. This suggests that the response of leukemias to G-CSF in vivo needs to be further characterised to develop optimal treatment protocols for all patients.

No conflict of interest to disclose

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 11: CML & Myeloproliferative ***Bayside 104***

O143 0830

Development of a Predictive Classifier for Poor Risk Chronic-Phase CML Patients at Diagnosis Using Immunophenotyping

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Background
Although the majority of chronic myeloid leukaemia (CML) patients are diagnosed in chronic-phase (CP), up to 35% of CP patients treated upfront with imatinib will have a suboptimal response, indicating heterogeneity within this patient group. Patient OCT-1 activity (OA; measurement of the functional activity of the organic cation transporter-1 (OCT-1) protein) currently provides the best prognostic indicator of imatinib response in CP-CML, but requires C^{14}-imatinb and specialised equipment. We have previously shown that very low OA (bottom 25%; poor-risk) is associated with poor molecular response and leukaemic transformation.
Aim
To determine the variation in immunophenotype of CP-CML patients at diagnosis and develop a classifier that predicts for poor-risk patients using flow cytometric immunophenotyping.
Method
Immunophenotyping of PB-MNCs from 34 newly diagnosed CP-CML patients was undertaken using a 39-marker antibody panel. Flow analysis was performed using the Beckman Coulter FC500 analyser. Statistical analysis was performed using SigmaPlot v12.0, with *R* v2.13.0 used for classification algorithms.
Results
A distinctive 6-marker classifier for patient OA prediction (poor- vs. standard-risk) based on immunophenotype was identified using a supervised, probabilistic method called Bayesian Model Averaging (BMA). This classifier had an overall accuracy of 88% (sensitivity: 77%, specificity: 95%). The populations identified by the classifier were $CD45^+$ (pan-leukocyte), $CD34^+HLA\text{-}DR^{neg}$ (haematopoietic progenitor), $CD3^+CD25^+$ (activated T-cell) and $CD45^{neg}GlyA^+$ (erythroid). Upon further investigation, the $CD45^+$ and $CD34^+HLA\text{-}DR^{neg}$ populations displayed significant increases in the standard-risk patients ($p=0.031$ and $p=0.02$, respectively), whilst $CD45^{neg}GlyA^+$ was significantly increased in the poor-risk patients ($p=0.002$). This suggests poor-risk disease is characterised by a more immature and erythroid cellular phenotype.
Conclusion
We have developed a classifier which differentiates the poor- and standard-risk OA groups of CP-CML patients with 88% accuracy using immunophenotyping. This classifier identified differential lineage involvement which may provide a valuable key towards dissecting the underlying disease biology and response heterogeneity observed in imatinib treated CP-CML patients.

No conflict of interest to disclose.

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 11: CML & Myeloproliferative ***Bayside 104***

O144 0845

Male Gender Bias in *FIP1L1-PDGFRA* Positive Chronic Eosinophilic Leukaemia and Demographics of Idiopathic Hypereosinophilc Syndrome

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Aim
Since 2004, our laboratory has been testing for the presence of the *FIP1L1-PDGFRA* gene rearrangement in referred samples from patients being investigated for chronic eosinophilic leukaemia (CEL). *FIP1L1-PDGFRA* positive CEL occurs almost exclusively in males with only few reported female cases. We sought to determine whether this male bias also exists in Australia and to analyse age and sex demographics in referred patients as a surrogate cohort of hypereosinophilic syndrome (HES) in the Australian population.
Method
FIP1L1-PDGFRA fusion transcripts were detected by reverse transcription and polymerase chain reaction. De-identified data including *FIP1L1-PDGFRA* positivity, gender, and age at first referral were collected from all patient samples referred to our laboratory from 2004 until the end of June 2010 from throughout Australia. The patient age distributions were compared to the general population using the chi-squared test. Incidence of *FIP1L1-PDGFRA* by gender was compared using Fisher's exact test.
Results
The patient cohort consisted of 596 patients referred from throughout Australia. There were 335 males (56%) and 261 females (43%) with respective median ages of 60 (range 0-91) and 53 (range 6-90). All fifteen *FIP1L1-PDGFRA* positive patients were male with a median age of 53 (range 24-70). The incidence of *FIP1L1-PDGFRA* positivity was 2.5% of all patients tested and 4.5% of all males tested, and the male gender bias was significant ($p=0.0002$). The age distributions of the referred male and female cohorts were significantly different to each other ($p<0.0001$) and to the respective Australian male ($p<0.0001$) and female ($p<0.0001$) populations.
Conclusion
When investigating female patients for CEL, causes other than the presence of *FIP1L1-PDGFRA* are likely to be significant. Differences in the age distributions of the male and female patient cohort suggest that hormonal factors may be involved in the aetiology of HES.
No conflict of interest to disclose.

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 11: CML & Myeloproliferative ***Bayside 104***

O145 0900

The TIDEL II Strategy of Imatinib Dose Intensification and Nilotinib Switch May Not Overcome the Negative Impact of a Low OCT-1 Activity in De-Novo CP-CML Patients

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Aim
Low OCT-1 activity (OA) is a poor prognostic indicator for CP-CML patients treated with imatinib (IM). The TIDEL II strategy of early dose escalation and/or switch to nilotinib (NIL) was designed to reduce the incidence of poor response/ failure in CP-CML patients, particularly those with low OA.
Method
Patients in Cohort I (n=105) were switched to NIL for IM intolerance, or early sub-optimal response (with no benefit of dose intensification). In cohort II (n=105) patients with early suboptimal response were switched immediately to NIL. OA was measured at diagnosis and a minimum of 6m NIL exposure was required for inclusion in this analysis.
Results
Cohort I: The overall rate of MMR at 12m was 64%. There was a significant difference in the rate of MMR between patients with low OA (n=47) and high OA (n=55)(51% vs 73% *p=0.023*). The overall rate of MMR at 24m was 83% (n=92).Patients with low OA (n=39) achieved MMR at a significantly lower rate (72%) compared with those with high OA (91% n=53 *p= 0.034*). To date 31 patients have switched to NIL 12/31 because of early sub-optimal response (med 15m). Only 1/12 has achieved MMR, and only 1/4 patients not in CCyR at the time of switch have achieved CCyR. Cohort II: 33/105 patients have switched to NIL, 17/33 for suboptimal response. While the follow up is short (med 9m) only 1 patient has achieved MMR following NIL switch and CCyR has been achieved in 2/8 patients not previously in CCyR. Importantly, the OA of suboptimal patients who switched to NIL was significantly lower than that of those who switched for intolerance(*p=0.002*), and of those patients remaining on IM(*p=0.004*).
Conclusions
While switching to NIL significantly improves response in IM intolerant patients, the response on NIL remains sub-optimal in most patients (90%) switching because of suboptimal IM response. This suggests that OA may delineate a group of CP-CML patients intrinsically insensitive to all TKIs.

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Wednesday 2 November: **0830-1000**
HSANZ Free Communications 11: CML & Myeloproliferative ***Bayside 104***

O146 0915

PPARγ Ligands Modulate OCT-1 Activity in BCR-ABL+ Cell Lines and Primary CML Cells

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Background
The organic cation transporter 1 (OCT-1) is the major active influx pump for imatinib (IM) in CML cells and the functional activity of OCT-1 (OA) is a key determinant of IM response in CP-CML patients. The peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear hormone receptor implicated in murine OCT-1 control. In this study we aim to identify the effect of PPARγ ligands in regulating OA in BCR-ABL positive and negative cells.

Methods
KU812 and HL60 cells were firstly incubated with PPARγ antagonist (diclofenac, GW9662) or agonists (GW1929, troglitazone, rosiglitazone) respectively for 1 hour. OA was then determined by intracellular uptake and retention assay (IUR) in the presence and absence of the OCT-1 inhibitor. The effect of diclofenac on OA was also assessed in the mononuclear cells (MNC) of CP-CML patients.

Results
A significant increase in OA was observed both cell lines when treated with PPARγ antagonists. Diclofenac also induced a significant increase in OA in the MNC of CP-CML patients. In contrast, PPARγ agonists significantly decreased the OA in both cell lines (Table 1).

Table 1. The effect of PPARγ ligands on OCT-1 activity (percentage of vehicle control)

	PPARγ antagonist		PPARγ agonists		
	diclofenac	GW9662	GW1929	troglitazone	rosiglitazone
KU812 (n=6)	122%*	126%*	68%*	49%**	57%**
HL60 (n=3)	129%**	123%*	72%*	80%*	77%*
CP-CML MNC (n=57)	169%**	-	-	-	-

*p<0.05, **p<0.001

Conclusion
These findings demonstrate the potential link between OA and PPARγ in BCR-ABL positive and negative cells. Ligand-activation or inhibition of PPARγ could therefore act as a regulator of OA. More importantly, clinically relevant drugs targeting PPARγ (diclofenac and thiazolidinediones) are demonstrated to mediate an effect on OA and hence may impact IM efficacy. Ongoing studies related to the expression levels and functional activity of PPARγ aim to reveal the exact mechanism of OA modulation by PPARγ.

No conflict of interest to disclose

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 11: CML & Myeloproliferative ***Bayside 104***

O147 0930

Constant Exposure to Low Dose Dasatinib Is Sufficient for Induction of Apoptosis in CML Cells

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Aim
Imatinib therapy founded the paradigm that continuous high dose (1uM imatinib) is required for optimal patient response. Although dasatinib has a short half-life of only 3-5hours, patients on the recently approved standard dose of once daily dasatinib (100mg) achieved similar cytogenetic and molecular responses as patients on twice a day dasatinib (50mg). To determine the underlying mechanism, we investigate the effect of transient and low level dasatinib exposure on inhibition of Bcr-Abl, and its downstream signalling and survival.
Methods
BCR-ABL+ KU812 cells were treated with 1nM, 10nM or 100nM of dasatinib either transiently for 30min (ie followed by washout), or continuously for up to 72hr. Protein was detected by western blot at multiple timepoints up to 48hr. Cell viability was assessed at 72hr by Trypan Blue staining and flow cytometry using Annexin V/7AAD staining.
Results
In vitro culture for 72hr with dasatinib resulted in decreased cell viability when cells were treated with either 1nM continuously (6-13% live cells), or 100nM transiently (8-17% live cells). Comparison of Bcr-Abl downstream targets has established p-STAT5, Bcl-xL and Mcl-1 as key determinants of the fate of cells treated with dasatinib under various conditions. Treatment with 10nM and 1nM continuous dasatinib resulted in the inactivation of p-STAT5 within 30min and 2hr respectively, and it did not reactivate throughout the 48hr culture period. 100nM transient exposure of dasatinib resulted in near complete p-STAT5 inhibition during the 30min of treatment and for 2hr following washout before gradual reactivation between 4-8hr post washout. For both treatments the expression of the anti-apoptotic proteins Bcl-xL and Mcl-1 decreased after 12-48hr.
Conclusion
Here we demonstrate that a low level of 1nM dasatinib is sufficient to result in cell kill at a level 1000x lower than for equivalent effects mediated by imatinib. Thus, we propose that it is the inactivation of pSTAT5 and its downstream pro-survival partners Mcl-1 and Bcl-xL which instigate the effect of dasatinib. This study adds support for the clinical efficacy of a once daily dosing whether dose is maintained, or drops to a low level.
No conflict of interest to disclose

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 11: CML & Myeloproliferative ***Bayside 104***

O148 0945

Bcr-Abl Dependent and Independent Mechanisms of Resistance to Nilotinib Are Observed in CML Cell Lines

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Background and Aims
Nilotinib (NIL) results in superior responses compared with imatinib in CP-CML, however, some patients develop resistance via over-expression of Bcr-Abl, ABCB1 and Lyn. This study aims to model NIL resistance *in vitro* to recapitulate possible mechanisms of *in vivo* resistance.
Methods
BCR-ABL+ K562 and K562-Dox (ABCB1+) cells were cultured long term in increasing concentrations of NIL. IC50's were based on p-Crkl protein expression. ABCB1 mRNA and protein levels were determined by RT-PCR and flow cytometry. Cell death was calculated by AnnexinV/7AAD and confirmed via trypan blue exclusion. Bcr-Abl quantitation, kinase domain mutation sequencing and ABCB1 substrate efflux (rhodamine) studies were also performed.
Results
Two resistant K562-Dox cell lines were generated, and both initially increased ABCB1 mRNA and protein expression. Expression peaked in cells cultured in 125nM NIL with IC50 and 3-day cell death assays confirming resistance. However, continued exposure to increasing concentrations of NIL (to 2μM) resulted in a significant decrease in ABCB1 expression, accompanied by a loss of ability to effectively efflux rhodamine. Resistance to imatinib (IM) and dasatinib (DAS) was also apparent in 3-day cell death assays, despite the IC50 values for all three TKIs being significantly decreased compared with control (Table1). Importantly, there was no overexpression of Lyn or BCR-ABL mRNA.
Similarly, K562 cells showed initial NIL resistance due to ABCB1 overexpression with a subsequent decrease in mRNA and protein levels. However, here the IC50 for all three TKIs remained high in cells cultured in 2μM NIL (Table1). There was also a significant increase in Lyn mRNA expression.

	K562-Dox				K562		
	Naive	$NIL^{2\mu M}$ #1	$NIL^{2\mu M}$ #2		Naïve	$NIL^{2\mu M}$	
$IC50^{NI}$ (nM)	424	127	91	*P*<0.001	212	1626	*P*<0.001
$IC50^{IM}$ (μM)	12	0.3	1.6	*P*<0.023	3.8	21	*P*=0.003
$IC50^{DAS}$ (nM)	97	2.4	4.3	*P*=0.012	5.9	7.5	*P*=0.081

Summary and Conclusions
Combined, these indications suggest ABCB1 overexpression acts as a precursor for both Bcr-Abl dependent and independent mechanisms of NIL resistance. To our knowledge this is the first report of a Bcr-Abl independent resistance mechanism not mediated by Lyn. With NIL now becoming a front-line treatment option in CML, better understanding of Bcr-Abl independent mechanisms of NIL resistance are needed.
This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract

Wednesday 2 November: **0830-1000**

HSANZ Free Communications 12: Acute Leukaemia & Myelodysplastic Syndromes

Bayside 105

O149 0830

Transposon Driven Leukaemogenesis Follows an Accelerated Darwinian-Like Evolution in a Mouse Model of Npm1c+ Acute Myeloid Leukaemia

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Heterozygous somatic mutations in the nucleophosmin gene *NPM1* are common in acute myeloid leukaemia (AML). We recently described a mouse model of these mutations in which one third of mice with conditional activation of a humanised *Npm1c*^{A} knock-in allele developed late-onset AML[1]. This model was subjected to insertional mutagenesis with *Sleeping Beauty (SB)*, through the mobilisation of 80 copies of the *GrOnc* transposon from a resident locus on chromosome 19. Rapid onset AML developed in 80% of mice in association with recurrent transposon insertions in known and novel leukaemia genes including Csf2, Flt3 and Nup98.

Aim

To understand the molecular basis of leukaemogenesis in a similar but novel model, by studying the serial acquisition of transposon insertions.

Methods

In mice carrying the humanised *Npm1c*^{A} knock-in, 15 copies of the *GrOnc* transposon were mobilised from chromosome 16. Fortnightly blood samples were collected from the time of *SB* activation to the onset of frank leukaemia. Transposon insertions from the serial samples and the final tumour were compared.

Results

Leukaemia onset was sudden and occurred without antecedent abnormalities in FBC parameters. Over fifty transposon integrations were identified within each tumour, however only a minority occurred early and persisted for several months during leukaemia development. Similarly only a few integrations persisted in tumours of transplant recipient mice. Common integration sites (CIS) showed striking overlap with those identified in the published study.

Conclusions

Transposon mobilisation continues throughout leukaemia evolution. Our data suggest only a subset of integrations behave as "driver" mutations. Continued mobilisation of transposons from passenger sites occurs without loss of proliferative potential. By contrast, mobilisation of driver insertions is immediately selected against. Our findings give important insights into how *SB* operates and validate many CIS identified in the published study, confirming the strong cooperativity of such insertions with *Npm1c* in leukaemogenesis. Future applications include the study of therapeutic resistance mechanisms.

No conflict of interest to disclose

1. Vassiliou, G.S., et al., *Mutant nucleophosmin and cooperating pathways drive leukemia initiation and progression in mice.* Nat Genet, 2011. 43(5): p. 470-5.

Wednesday 2 November: **0830-1000**
HSANZ Free Communications 12: Acute Leukaemia & Myelodysplastic Syndromes
Bayside 105

O150 0845

HIDAC-3 Chemotherapy Is Clinically Efficacious With Less Gastrointestinal Toxicity Than ICE Induction in Adult Acute Myeloid Leukaemia

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Background and Aim
Although ICE induction for adult Acute Myeloid Leukaemia (AML) produces a CR rate of 80%, gastrointestinal (GI) toxicity is significant, with 32% grade 3/4 nausea and vomiting (Bradstock *et al*, Blood 2005). Omission of Etoposide in HIDAC-3 induction may produce similar clinical outcomes with potentially less GI toxicity.
Methods
47 consecutive patients aged 15-60 with newly diagnosed AML receiving high dose Cytarabine induction between 2007 and 2011 at the Alfred Hospital, Melbourne, were retrospectively analysed. Regimens included ICE (Idarubicin 9mg/m^2 d1-3, Cytarabine 3g/m^2 bd d1,3,5,7, Etoposide 75mg/m^2 d1-7) or HIDAC-3 (Idarubicin 12mg/m^2 d1-3, Cytarabine 3gm/m^2 bd d1,3,5,7) Toxicity was assessed using Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.
Results
22 patients received ICE and 27 patients HIDAC-3 induction. Groups receiving ICE or HIDAC-3 were comparable in terms of baseline mean age (46 vs 41 years; p=0.16), frequency of poor risk karyotype/secondary AML (23% vs 32%; p=0.55) or FLT3 mutation (36% vs 14%; p=0.16). Patients receiving ICE had a longer median follow up than those treated with HIDAC-3 (30 vs 15 months). CR, DFS and OS were comparable between the groups. Time to neutrophil and platelet recovery was similar. Grade 3 or 4 non-haematologic toxicity was comparable, except for greater grade 3/4 nausea (41% vs 0%; p<0.001) and TPN use (7.3 vs 1.5 days; p=0.005) in those receiving ICE. There was a trend to increased enterocolitis in the ICE group (32% vs 8%; p=0.068). There were no induction deaths in those receiving HIDAC-3.
Conclusions
HIDAC-3 is a highly effective induction regimen for adult AML with less treatment related GI toxicity than ICE.

No conflict of interest to disclose

Wednesday 2 November: **0830-1000**

HSANZ Free Communications 12: Acute Leukaemia & Myelodysplastic Syndromes

Bayside 105

O151 0900

A Phase Ib Study Combining the mTOR Inhibitor Everolimus (RAD001) with Low-Dose Cytarabine in Untreated Elderly AML

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Background

Pre-clinical studies suggest that mammalian target of rapamycin (mTOR) is important for leukemic stem cell proliferation and self-renewal. Everolimus (RAD001) is an orally available ester derivative of rapamycin with modest clinical activity in myelodysplastic syndrome.

Aim

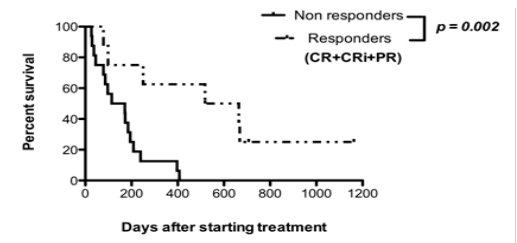
To determine the safety and preliminary of RAD001 in combination with low dose ara-C (LDAC) in untreated, elderly AML patients unfit for intensive chemotherapy.

Methods

Phase Ib open label dose escalation study. Patients were treated with LDAC 20 mg s.c. twice daily on days 1-10 of 28-day cycle for a maximum of 4 cycles and either 2.5, 5 or 10 mg everolimus per orally daily (8 patients treated at each level) for a maximum of 12 cycles.

Results

24 patients (M 14, F 10), median age 74 years, were included in the study. 71% had at least one poor risk factor defined by age >75 (33.3%), ECOG>2 (12.5%), secondary AML (38%) or adverse risk cytogenetics (25%). During cycle 1, grade 4 thrombocytopenia and neutropenia occurred in 58.3% and 66.6% respectively. Grade 3/4 non-haematologic toxicity occurred in 6 (25%) patients; 2.5 mg RAD001 (n=1; flare leucocytosis), 5 mg RAD001 (n=1; peripheral oedema) and 10 mg RAD001 (n=4; cough, mouth ulcers, septicaemia in 2). The maximum tolerated dose of RAD001 in combination with LDAC was 5 mg. After 2 cycles, the overall response rate (ORR) was 34% (CR 13%, CRi 4% and PR 17%). Stable disease was observed in 50%, while 4% had progressive disease. 30 and 60-day mortality was 8 and 25%, respectively. One and two-year survival was 29% and 18%, respectively. Median overall survival for the entire cohort was 179 days and was superior in responders compared to non-responders (591 vs 142.5 days; p=0.002: figure). There was no significant difference in overall survival between the 3 everolimus dosing cohorts (p=0.7). The median progression free survival was 76 days (range 13-475).



Conclusion

In an unfit elderly AML population, everolimus in combination with LDAC was tolerable and active with extended survival observed in those achieving hematologic response.

This study was supported by Novartis. The company had no role in analysing the data or preparing the abstract.

Wednesday 2 November: **0830-1000**

HSANZ Free Communications 12: Acute Leukaemia & Myelodysplastic Syndromes

Bayside 105

O152 **0915**

Results of a Phase-II Study of Thalidomide (Thal) and Azacitidine (Aza) in Patients With Clinically Advanced Myelodysplastic Syndromes (MDS)

Melita Kenealy[1,2], Pratyush Giri[3], Robin Filshie[4], Shir-Jing Ho[5], Andrew Nicol[6], Linda Cowan[2], Emma Link[2] and John Seymour[2], on behalf Australasian Leukaemia and Lymphoma Group

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Aim

Thal and Aza each have single-agent activity in MDS, but there is limited experience with the combination. Combining Thal/Aza may improve efficacy but side effects could limit tolerability. We report the safety analysis of pts on the Ph II single-arm ALLG MDS3 study of Thal/Aza.

Methods

All FAB MDS categories were eligible; RA or RARS required at least one clinically significant cytopenia. Patients with prior Thal/derivatives or demethylating agent were excluded. Patients received titrated Thal 50-100mg/d maximum 12 months and Aza 75mg/m^2/d x7d every 28d until progressive disease/unacceptable toxicity. All analyses are intention-to-treat. Primary end point was rate Gr3+ haematologic and non-haematologic toxicity.

Result

80 patients registered between 7/08 – 7/09 from 15 sites. Median age 68y (42-82), 67% male. FAB category: RA 13%, RARS 9%, RAEB 48%, RAEB-t 10%, CMML 19%. IPSS at entry: low 9%, Int-1 40%, Int-2 36%, high 15%. ECOG 0 42%, 1 45%, 2 13%. Median time from diagnosis was 13.4mths (0.7 -142.5). 78 patients evaluable for toxicity, 68 for response. Median follow up 22.5mths (1.2-32.1), median number of cycles was 8 (1-24+). 95% patients experienced at least one Gr3+ haematologic adverse event (AE) per NCI CTC (46% anaemia, 11% neutropenia, 19% thrombocytopenia.) 74% patients experienced Gr3+ non-haematologic AE; most frequently infection-related (32%), gastrointestinal-related (20%) and fatigue 10%. Most AEs occurred in the early cycles.

Best response by IWG2006 criteria: overall response rate 66%; 27% CR, 4% PR, 16% mCR, 19% HI (2HI-E, 10HI-N, 2HI-P); 18% stable disease, 10% progressive disease and 7% non-evaluable.

Conclusion

The combination Thal/Aza in clinically advanced MDS is tolerable, with gastrointestinal and fatigue the most common non-haematologic and non-infection AEs reported. The rate of AEs is not in excess of that expected from the underlying disease or treatment with Aza alone. There was no unexpected toxicity of the combination. ORR was 65%, which is comparable to ORR of single-agent Aza reported in MDS studies CALGB-9221 & AZA-001. The high rate of CR is promising and warrants further investigation of combination IMiD+Aza therapy.

This research was supported by Celgene. The company had no role in analysing the data or preparing the abstract.

Wednesday 2 November: **0830-1000**

HSANZ Free Communications 12: Acute Leukaemia & Myelodysplastic Syndromes

Bayside 105

O153

0930

Sphingosine Kinase Inhibition Represents a Promising Novel Strategy for the Treatment of Acute Lymphoblastic Leukaemia

Craig Wallington-Beddoe[1], David Ho[1], Julia Dobbins[2], Stuart Pitson[2], Kenneth Bradstock[1], Linda Bendall[1]

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Introduction

Acute lymphoblastic leukaemia (ALL) is the most common form of childhood cancer, which usually responds to chemotherapy. Long-term survival in adults is poor with most developing disease relapse, whilst Ph^+ ALL has a particularly poor prognosis. Sphingosine 1-phosphate (S1P) is a lipid mediator of diverse cellular functions, most notably of lymphocyte trafficking, angiogenesis, cell proliferation and survival. S1P is produced intracellularly by the sphingosine kinases (SK) of which there are two isoforms, SK1 and SK2. Inhibition of SKs may have potent anti-leukaemic effects.

Methods and Results

Application of the combined SK1/SK2 inhibitor SKI II and the selective SK2 inhibitor ABC294640 to ALL cells produced a reduction in both proliferation as assessed by ^{3}H-thymidine incorporation and viability, determined by annexin V/PI staining, of all cell lines except the Ph^+ ALL1 and HARM cells treated with SKI II. IC_{50} values for loss of cell viability ranged from 2μM to >10μM for SKI II and 50-60μM for ABC294640, whilst those for inhibition of proliferation were 1μM - 7μM for SKI II and <40μM for ABC294640. SKI II resulted in caspase-dependent cell death, as determined by flow cytometric assessment of caspase-3 cleavage and light microscopy. However, ABC294640 induced caspase-3 cleavage at lower than expected levels and cell death was not prevented by the caspase inhibitor Z-VAD-FMK. A ^{32}P-S1P labelling assay demonstrated that SK1 or SK2 enzyme activity was reduced with stable SK1 protein levels and both agents resulted in a reduction in intracellular S1P as determined by ELISA. Synergistic cell death was observed when Ph^+ ALL cells were treated with the combination of imatinib and either ABC294640 or SKI II thereby overcoming resistance seen with SKI II alone. Preliminary results of ABC294640 administered to NOD/SCID IL2$\gamma_c^{-/-}$ mice engrafted with human ALL are promising with experiments ongoing.

Conclusion

SK1 and SK2 inhibition, through a reduction in intracellular S1P, is an exciting novel anti-leukaemic strategy potentially adding to the repertoire of non-chemotherapeutic agents for the treatment of ALL.

No conflict of interest to disclose

Wednesday 2 November: **0830-1000**

HSANZ Free Communications 12: Acute Leukaemia & Myelodysplastic Syndromes

Bayside 105

O154

miR-10a is a Pro-Survival Factor in Acute Myeloid Leukaemia Bearing the *Nucleophosmin1* Mutation

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[1]Blood, Stem Cells and Cancer Research, St Vincent's Hospital, Sydney, Australia; [2]School of Mathematics and Statistics, Sydney Bioinformatics Centre for Mathematical Biology, University of Sydney, Sydney, NSW, Australia

Aim

To investigate the role of microRNA dysregulation in the initiation and development of acute myeloid leukaemia (AML) with *Nucleophosmin1* (*NPM1*) mutation, which is a common AML type with an unknown pathogenesis.

Method

microRNA microarray of bone marrow samples from 28 CN- AML patients and confirmation by qRT-PCR demonstrated a unique microRNA signature in mutated *NPM1* AML samples dominated by miR-10a over-expression. Functional assessments were performed in the human OCI-AML3 cell line which harbours the *NPM1* mutation. miR-10a repression was induced by transfection with miRCURY LNA microRNA knockdown probes (Exiqon). Functional effects were assessed by AnnexinV/PI and Caspase 3 assays (apoptosis), BrdU uptake (proliferation), MTS (cell growth) and colony forming assays. Potential mechanisms were elucidated by determining miR-10a target genes by bioinformatics and confirmed by luciferase reporter assays.

Result

We demonstrate that a signature composed of five microRNAs can accurately delineate AML samples based on their *NPM1* mutational status. Of these, miR-10a was highly differentially expressed between wild type *NPM1* and mutated *NPM1*AML patients. Knockdown of miR-10a in OCI-AML3 cells resulted in increased cell death as detected by AnnexinV binding but not Caspase3 activation, and reduced clonogenic capacity. These effects are thought to occur through miR-10a mediated modulation of *ARNT, GTFH1, ID4, KLF4, MAPRE1, NR4A3, RB1CC1* and *TFAP2C*, all of which are associated with neoplastic transformation, were confirmed as miR-10a targets.

Conclusion

Taken together, we report for the first time that aberrant miR-10a expression in AML with mutated *NPM1* patients promotes cell survival, positioning miR-10a as a potential drug target.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
ANZSBT Symposium Symposium 9: Haemovigilance in the Asian-Pacific Region
Auditorium A

Haemovigilance in Japan

Hitoshi Okazaki
Japanese Red Cross, Tokyo, Japan

The Japanese haemovigilance system was implemented by the Japanese Red Cross (JRC), the sole manufacturer of labile blood products in Japan. More than seventeen years have passed since we launched the infection and adverse reaction reporting system in 1993. The haemovigilance system was set up from its inception as a voluntary reporting system for adverse reactions and infections following transfusion. Adverse events following transfusion are reported by hospitals through over 150 medical representatives (MRs) nationwide assigned in JRC blood centers. The adverse event notification and reporting forms are submitted by hospital doctors. MRs collect these forms from hospitals, and then send them to the headquarters. These forms are used for transfusion-transmitted infections and other adverse reactions/events. Because the blood products in Japan are regulated by the Pharmaceutical Affairs Law, the JRC blood service headquarters (BSHQ) is also classified as a pharmaceutical company and has a responsibility to act in accordance with the law. In this sense, hemovigilance is part of pharmacovigilance. We do not actually define the types of adverse event, because some newly recognized adverse events may occur anytime. Because we must keep up to date with advances in the field of transfusion medicine, adverse reactions are classified by JRC specialists including medical doctors. The basic concept of our haemovigilance system is evidence-based hemovigilance, which can be achieved not only by the collection of reports on adverse reactions, but also by the rigorous analysis of objective data obtained by laboratory tests of patient and donor blood samples.
Donor vigilance has also been systematically implemented in Japan. Errors and near misses are not currently managed by our haemovigilance system and these issues will also be discussed.

Wednesday 2 November **0830-1000**
ANZSBT Symposium Symposium 9: Haemovigilance in the Asian-Pacific Region
Auditorium A

The New Zealand Haemovigilance System

Dorothy Dinesh
New Zealand Blood Service, Wellington, New Zealand

Blood transfusion is an important component of modern day medicine. As doctors our first consideration must always be the interests and safety of patients. Haemovigilance programmes collect and analyse data on untoward events associated with transfusion. The information collated must be shared with health professionals who prescribe and administer blood products so that they can continue to deliver the good without unintended negative consequences.
In New Zealand the National Haemovigilance Programme was established in 2005. Reporting is voluntary and usually mediated via the hospital blood banks. New Zealand is a country member of the Internal Haemovigilance Network (IHN) and utilises the definitions of reporting categories agreed upon by the IHN. The majority of reports involve reactions that are mild. The overall rate of an adverse event is approximately 1 in 300 units transfused. Platelet concentrates are more frequently associated with reactions compared with other blood components and reactions are predominantly of the allergic type.
The Haemovigilance Programme has demonstrated a reduction in the reported rate of transfusion related acute lung injury (TRALI) since the introduction of male donor fresh frozen plasma (FFP) 3 years ago. The programme has also identified that bacterial contamination of blood components, incorrect blood component transfused (IBCT), wrong blood in tube (WBIT) and acute haemolytic transfusion reactions due to transfusion of passive haemolysin, are risks that require further attention.
Complications associated with blood donation are also included in haemovigilance. Vasovagal reactions are the most frequently occurring donation associated event and are higher in donors under the age of 20 years.
Ongoing surveillance and review of untoward events associated with transfusion is vital so that we can continue to minimize risks related to blood products.

No conflict of interest to disclose.

Wednesday 2 November **0830-1000**
ANZSBT Symposium Symposium 9: Haemovigilance in the Asian-Pacific Region
Auditorium A

Haemovigilance in Hong Kong

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Hong Kong Red Cross Blood Transfusion Service, Hong Kong SAR

Background
In 2001, we implemented the haemovigilance system for reporting of transfusion incident and adverse transfusion reaction in Hong Kong. It is mandatory for all public hospitals to report incidents whereas reporting of adverse reaction is voluntary.
Findings
From 2002 to June 2010, the number of transfusion incidents per 6 month was initially more than 600 but gradually dropped to 273 in 2010. 90.7% of the incidents were related to errors in blood request or sampling. The notable data were: 43 cases of blood sample taken from the wrong patient, 34 were near-miss but 8 reached severity index (SI) 1 and one 4; 5 cases of wrong patient label on blood units, two of which were of SI 1 and 2 respectively; 14 cases of wrong blood products issued or taken, three of which were of SI 1; 2 cases of transfusion given to wrong recipients, though they did not result in serious reaction in the recipients. There was a serious event of severity index 4 reported in 2004. Root cause analysis revealed that the resident performing the type and screen misidentified the patient.
For adverse transfusion reaction reporting, the number per 6 month was initially around 300 but gradually dropped to around 100 in 2010. Majority reported were minor allergic reaction (43.8%) and febrile non-haemolytic transfusion reaction (43.7%). In addition to one case of ABO mismatch, there were two more cases of acute haemolysis due to other causes, 3 confirmed and two suspected TRALI. In 2008, a patient was given a bacterial contaminated red cells resulting in fatality.
Conclusion
With ten years of experience, reporting transfusion incident and adverse transfusion reaction has become a way of life among the front line clinical staff. However, the quality of the reporting needs to be improved. To achieve that, we need to revamp our system by adopting the latest international classification and to train our staff with the emphasis on the investigation to find out the cause clearly.

HAA 2011 HSANZ ANZSBT ASTH

Wednesday 2 November **0830-1000**
ASTH Free Communications 3: Thrombosis ***Bayside 204***

O155 0830

A Two-Centre (AUS-NZ) Retrospective Audit of Pregnancy Related Venous Thromboembolism (VTE): Failure of Current Guideline to Identify At Risk Patients in the Postpartum Period

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Aim
To explore the epidemiology, VTE risk profile and management of pregnancy related VTE in 2 major obstetric centres in Australia and New Zealand.
Method
Retrospective chart review of 60 patients with objectively diagnosed pregnancy related VTE as identified by ICD-10 codes at Monash Medical Centre and from a pre-existing database at North Shore hospital (NZ) in the period of Jan 2007 to March 2011. Data was extracted and collected in a standardised case report form
Result
The median age and weight were 31 years (18-43 years), and 80 kg (50-120 kg) respectively. Antenatal and postpartum VTE was observed in 31 and 29 women respectively, giving an incidence of 9 per 10,000 deliveries/year. Pulmonary embolism (PE) occurred more frequently in postpartum than antenatal period (40% vs 20% respectively, p=0.10; n.s). Antenatal risk factors included twelve (20%) women with a thrombophilia and 3 with a prior VTE history. Most antenatal women (92%) received therapeutic doses of enoxaparin based on their weight at diagnosis. Subsequently, wide variability in maintenance dosing was observed including 8 women who had a 25% dose reduction. There were no antepartum recurrences but one major bleed (3.3%) was observed. In the 29 women with confirmed postpartum VTE none were receiving postpartum prophylaxis and more concerning was that less than a third would have been considered for prophylaxis based on their risk factors (MMC thromboprophylaxis guideline requires presence of ≥ 3 risk factors). One woman suffered a major bleed and 2 recurrences occurred, both in women with distal DVT treated with inadequate anticoagulant therapy.
Conclusion
The epidemiology and antepartum outcomes for VTE in this cohort are similar to the reported literature. Of most concern is that over 2/3 of postpartum women with VTE were not identified by current thromboprophylaxis guidelines.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
ASTH Free Communications 3: Thrombosis ***Bayside 204***

O156 0845

Apixaban versus Acetylsalicylic Acid in Stroke Prevention: The AVERROES Study

John Amerena[1], Stuart Connolly[2], John Eikelboom[2], Greg Flaker[3], Scott Kaatz[4], Alvara Avezum[5], Leopoldo Piegas[5], Antonio Gonzalez-Hermosillo[6], Stefan Hohnloser[7], Sergey Golitsyn[8], Salim Yusuf[2]

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Aim
The purpose of AVERROES was to evaluate apixaban for the prevention of stroke or systemic embolism in patients with atrial fibrillation at risk of stroke who were unsuitable for therapy with a vitamin K antagonist (VKA), in comparison to acetylsalicylic acid (aspirin), which is the standard therapy for these patients. Comparison with VKA use is being investigated in a separate study (ARISTOTLE) due to report in the near future.

Method
AVERROES was a double-blind, randomized, active controlled trial of apixaban compared to aspirin. Patients with documented atrial fibrillation and at least one risk factor for stroke who were also unsuitable for therapy with a VKA were randomized 1:1 to receive apixaban 5 mg twice daily (2.5 mg twice daily in selected patients) or aspirin (81-324 mg per day). The study was performed in 522 sites worldwide (14 in Australia) with 5,599 patients enrolled. Following the first Data Monitoring Committee formal interim analysis, showing overwhelming evidence of efficacy against stroke or systemic embolism, together with an excellent safety profile, the study was terminated early and all patients were offered open label apixaban.

Result
The annual rate of stroke or systemic embolism was 3.7% per year on aspirin and 1.6% per year on apixaban (Hazard Ratio 0.45, 95% CI, 0.32-0.62, $p<0.001$). The rate of major haemorrhage was 1.2% per year on aspirin and 1.4% per year on apixaban (Hazard Ratio 1.13, 95% CI, 0.74-1.75, $p=0.57$). There were 11 cases of intracranial bleeding with apixaban and 13 with aspirin

Conclusion
In patients with atrial fibrillation at risk of stroke and unsuitable for therapy with a VKA, apixaban reduces the risk of stroke or systemic embolism with no significant increased risk in major haemorrhage. Apixaban offers an advantage over aspirin for prevention of stroke in these patients.

This research was supported by Bristol-Myers Squibb and Pfizer. The study was designed by the steering committee members, together with the sponsors, Bristol-Myers Squibb and Pfizer. The data were collected, validated and analysed at the Population Health Research Institute at Hamilton Health Sciences and McMaster University, Hamilton, Canada, with on-site monitoring by the sponsors.

Wednesday 2 November **0830-1000**
ASTH Free Communications 3: Thrombosis ***Bayside 204***

O157 0900

Apixaban Versus Enoxaparin for Thromboprophylaxis After Joint Replacement Therapy: Pooled Analysis of Major Venous Thrombembolism and Bleeding in 8,564 Patients From the ADVANCE-2 and -3 Trials

Alexander Gallus[1], Gary Raskob[2], Graham Pineo[3], Dalei Chen[4], Luz Margarita-Ramirez[4], Michael Rud Lassen[5]
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4 Research and Development, Bristol Myers Squibb, Princeton, NJ, USA. 5 Orthopedics, Spine Clinic, Clinical Trial Unit, Horsholm Hospital, University of Copenhagen, Glostrup, Denmark

Aim
Apixaban, a novel orally administered factor Xa inhibitor, has been evaluated in three phase 3 randomized, double-blind, double-dummy clinical trials (the ADVANCE studies) for the prevention of venous thromboembolism (VTE) after hip or knee replacement. The aim of this analysis was to combine data from the two trials comparing apixaban 2.5 mg twice daily with the same enoxaparin regimen of 40 mg once daily (ADVANCE-2 and -3).
Method
Study medications were continued for 10 to 14 days after knee arthroplasty in ADVANCE- 2, and for 32 to 38 days after hip replacement in ADVANCE-3. In both studies, mandatory bilateral venography was done at the end of the intended treatment period to assess the presence or absence of asymptomatic deep-vein thrombosis, and clinically suspected VTE was confirmed or excluded by objective testing. Patients were followed-up 30±5 and 60±5 days after the last dose of study medication. A total of 8,564 patients were randomized in the ADVANCE-2 and 3 trials.
Result
Major VTE occurred in 23 of 3,394 evaluable patients (0.68%) in the apixaban group and in 51 of 3,394 (1.50%) evaluable patients in the enoxaparin group (absolute risk difference, -0.76%, 95% CI, -1.23% to -0.30%). Major bleeding occurred in 31 of 4,174 patients (0.74%) who received apixaban (18 occurred before the first dose) and in 32 of 4,167 patients (0.77%) given enoxaparin (absolute risk difference -0.02%, 95% CI, -0.40% to 0.35%).
Conclusion
The apixaban regimen was more effective than enoxaparin 40 mg once daily for preventing major VTE, without increased bleeding, and has the clinical advantages of oral administration and later initiation 12 to 24 hours post-operatively.

This research was supported by Bristol-Myers Squibb and Pfizer. The study was designed and supervised by the steering committee members. Data were collected and analysed by the study sponsors, Bristol-Myers Squibb and Pfizer. The statistical analysis plan was approved by the steering committee before the database was locked and unblinded.

Wednesday 2 November **0830-1000**
ASTH Free Communications 3: Thrombosis ***Bayside 204***

O158 0915

Investigation of Differences Between Warfarin and Dabigatran in Atrial Fibrillation – a RE-LY Trial Sub-study

Brian Dale[1], Jack Hirsh[2], John Eikleboom[2]
1 School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia. 2 McMaster University, Hamilton, Ontario, Canada.

Aim
The RE-LY study was a clinical trial comparing dabigatran with VKA in patients with atrial fibrillation (AF). Sub-analysis revealed that there was a marked decrease in intracranial haemorrhage (ICH) in patients assigned dabigatran. We hypothesized that the inhibitory effects of dabigatran on thrombin-mediated coagulation are overcome by high concentrations of tissue factor, which occurs at sites of vascular disruption.
Method
Pooled warfarinized plasma (PP-INR 2.6) was obtained as well as pooled normal plasma (PNP) and PNP with dabigatran added at 250 ng/ml (PNP-D250). A fluorogenic thrombin generation assay was used to evaluate the haemostatic effect of the anticoagulants. Assays were performed in quadruplicate without tissue factor or with dilutions of recombinant thromboplastin (1/10, 1/20, 1/100, 1/500, 1/2000). Student's t test was used to determine statistical significance.
Result
Inhibition of peak thrombin production was significantly higher in PP-INR 2.6 compared to PNP-D250 at all tissue factor concentrations. In the absence of tissue factor PP-INR 2.6 inhibited peak thrombin by a mean of 78.8% versus 25.8% for PNP-D250 ($p=0.003$). As tissue factor concentration increased, levels of inhibition decreased. At a 1/20 dilution of thromboplastin PNP-D250 had minimal inhibitory effect upon peak thrombin (mean 3.3%) whereas PP-INR2.6 demonstrated a mean inhibition of 47.5% ($p<0.001$).
Conclusion
Our results are consistent with the hypothesis that the difference in the rate of ICH in the RE-LY trial is caused by reduced capacity of dabigatran to counter thrombin generated at sites of vascular damage compared to warfarin. Our findings do not explain why dabigatran and warfarin demonstrated similar benefits in preventing stroke in AF patients. One possibility is that there is a ceiling to the beneficial effects of any anticoagulant and this may have been achieved at the higher dose of dabigatran in the RE-LY trial.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Wednesday 2 November **0830-1000**
ASTH Free Communications 3: Thrombosis ***Bayside 204***

O159 0930

Thromboprophylaxis in Hospitalised Patients with Haematological Malignancies – A Single Centre Audit

RE de Wet, HC Lai, IM Irving, E Morris
The Townsville Hospital/Queensland Health, Townsville, QLD, Australia

Aim
Hospitalised patients with haematological malignancies have an increased incidence of thrombosis[1,2]. We sought to determine how many haematology inpatients received thromboprophylaxis (medical or mechanical) during their hospital stay.
Methods
Chart review of patients admitted under haematology in the months of October to December 2010. Data collected included patient age, ECOG status, diagnosis, reason for admission, length of admission, treatment received during hospitalisation including chemotherapy, antibiotic and thromboprophylaxis, platelet count at admission, nadir and days below $50x10^9$/l and thrombotic events.
Results
Sixty patients were admitted from October to December 2010. The average age of patients was 60.6 years and the baseline ECOG status was one. The average length of hospital admission was 17 days. 83% of patients admitted had a haematological malignancy. The main reasons for admission were for administration of chemotherapy (25%), management of febrile neutropenia (17%), stem cell transplant (8%), thrombosis (6%) or acute renal failure (6%). Only 18% of patients received thromboprophylaxis. During the course of admission, either at presentation or during the hospital stay, 9% of patients developed a thrombosis. The mean admission platelet count was 157 $x10^9$/l. The platelet count was below 50 $x10^9$/l for an average of 11.7 days per patient admission.
Conclusion
The results of this audit confirm a significant incidence of thrombosis in hospitalised patients with haematological malignancies. Although thromboprophylaxis is complicated by thrombocytopenia, low molecular weight heparin prophylaxis is considered safe when platelets are >50 $x10^9$/L and mechanical prophylaxis is almost always acceptable. We have initiated a protocol for all patients to have their thrombotic risk assessed on admission and appropriate thromboprophylaxis initiated.
References
1. *Venous thromboembolism in the haematologic malignancies, Flanaga and Marchetti, JCO, 27;29, 10 October 2009*
2. *Thomboembolism in hospitalised neutropenic cancer patients,* Khorana et al, JCO 24:3, 20 January 2006

There was no conflict of interest

Wednesday 2 November **0830-1000**
ASTH Free Communications 3: Thrombosis ***Bayside 204***

O160 0945

Monitoring Hemostasis With Global Assays During Lenalidomide and Dexamethasone (LEN/dex) Therapy for Relapsed/Refractory Multiple Myeloma (R-MM)

Caroline Reddel[1], Kate Burbury[2], Peter Gambell[2], Amanda Choo[2], Judith Trotman[1], Kevin Lynch[4], Hang Quach[2], H Miles Prince[2,3], David Westerman[2,3], Jennifer Curnow[1]
[1] Concord Hospital, Concord, New South Wales, Australia. [2] Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia. [3] University of Melbourne, Parkville, Victoria, Australia. [4] Celgene Pty Ltd, Melbourne, Australia

Aim
Myeloma is associated with a hypercoagulable state (HCS) and risk of thromboembolism (TE) which therapy, such as LEN/dex, may exaggerate. Utilising global assays to integrate haemostatic effects in individuals may enable targeted thromboprophylaxis, adjusted to variable TE risk. The overall haemostatic potential (OHP) evaluates fibrin generation (FG) and fibrinolysis (FL) in plasma, identifying HCS.
Method
A subgroup of patients, in a phase II clinical trial of LEN/dex in R-MM, had prospective sequential assays of OHP, thrombin generation, PAI-1 and TAFI, prior to cycles 1, 2 and 4. Results for the first 44 patients are reported here.
Result
Results are presented as mean±SD compared with a group of healthy control subjects. Before commencing LEN/dex (cycle 1), thrombin generation (TG) was elevated (endogenous thrombin potential, ETP, 1523.3±424, control 1330.5±190, $p<0.05$), FL reduced (overall fibrinolytic potential, OFP, 69.35±14.2%, control 77.27±8.5%, $p<0.05$) and PAI-1 elevated (30.70±12.0ng/mL, control 16.52±4.5ng/mL, $p<0.001$), indicating a HCS in R-MM. After starting LEN/dex (cycle 2), FL was further reduced (61.52±19.9, lower than control, $p<0.01$ and cycle 1, $p<0.05$) and FG increased (43.05±14.1 control 36.08±5.2) . The increase in FG persisted at cycle 4 but FL trended towards baseline (65.15±16.7, no significant difference from cycle 1) as PAI-1 (24.74±19.6ng/mL) and TAFI (7.11±2.0ug/mL vs cycle 1 8.23±1.9ug/mL, $p<0.05$) fell. TG remained elevated (ETP 1582.0±484). Analysis of results according to either prophylaxis received (LMWH or aspirin) or evidence of disease response, showed no significant differences.
Conclusion
Thrombin generation and the OHP provide insights into mechanisms underlying the HCS in R-MM. Global assays may provide a simple, convenient method of monitoring haemostatic changes during R-MM therapy. These initial results may indicate an increase in HCS during early treatment cycles of lenalidomide when TE risk is highest.
This research was supported by Celgene. The company had no role in analysing the data or preparing the abstract.

HAA 2011 HSANZ ANZSBT ASTH

Wednesday 2 November **0830-1000**
ISCTA Free Communications 2: Cell Delivery ***Bayside Gallery B***

O161 0830

Allogeneic HPC Collection With Neupogen Induced Mobilization: Revisit Cost, Time and Complications

Abdus Salam[1], K Plewacki[1], S Fritzel[1], J Miller[2], J Ng[3]
[1]Apheresis Associate of Northern Virginia, Annandale, Virginia, USA, [2]NMDP, Minneapolis, Minnesota, USA. [3]DOD Marrow Donor Program, Rockville, Maryland, USA

Background and Aim
Hematopoietic progenitor cell (HPC) transplantation is routinely used for the treatment of hematologic malignancies. HPC mobilization with Neupogen (filgrastim) for 5 days is the preferred method of stem cell harvesting. However, neupogen induced mobilization is not free of risk. Common side effects include bone and muscle pain, nausea, vomiting, etc. Rare but serious effects include allergic reactions, splenic rupture. These side effects are dose dependent. Cost of neupogen is high. The aim of the present study is to evaluate neupogen related CD34 mobilization and dose collected and to look for opportunities to modify the collection profile to potentially reduce side effects and costs.
Method
Of 500 charts, 50 HPC donor charts are randomly selected and reviewed. Minimum criteria of 50 CD34+ cells/µl in the peripheral blood were used as the initiating point for the collection. HPC mobilization in all donors was performed using a standard 5-day neupogen injection (10µg/kg). On average, 12-18L of total blood volume was processed using the Cobe Spectra. Pre-collection and mid-collection peripheral CD34+ cells were used to predict the final HPC product outcome.
Result
In all 50 cases reviewed, the final CD34+ cells/kg in the HPC product met the target of $5x10^6$/kg (100%) with >90% viability. The range of CD34+ cells varied between 5.7 to $25.6x10^6$/kg (data not shown). In 85% of donor, $>9x10^6$/kg of CD34+ cells were achieved (data not shown). The engraftment data were not reviewed.
Conclusion
Enumeration of pre-collection CD34 and number of total blood volumes processed are two valuable criteria that correlate well with final CD34 in HPC products. Large numbers of CD34+ cells may be collected after 5 days of neupogen: a prospective validation study is underway using 4-day neupogen protocol to determine if sufficient cells may be collected with reduced cost, time and complications.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
ISCTA Free Communications 2: Cell Delivery ***Bayside Gallery B***

O162 **0845**

Trial of a Cell Therapy for Recessive Dystrophic Epidermolysis Bullosa (Fragile Skin)

Supriya Venugopal[1], Hellen Yan[1], Anna Liza Chan Agero[2], Lesley Rhodes[1], Kim Tran[1,5], Marian Sturm[4], Janice Fogarty[4], Janis Nelson[3], Akemi Ishida-Yamamoto[5], Peter Marinkovich[6], Dedee Murrell[1]
1 Department of Dermatology, 2 Department of Anatomical Pathology, 3 Pharmacy Department, St George Hospital, Sydney, 4 Cell and Tissue Therapies WA, Royal Perth Hospital, Perth, Australia, 5 Dermatology, Asahikawa Medical University, Japan, 6 Department of Dermatology, Stanford University, USA

Recessive dystrophic epidermolysis bullosa (RDEB) is a serious, rare condition of extremely fragile skin due to the absence of a structural protein, collagen VII. Individuals present with widespread trauma-induced blisters and painful erosions that are slow-healing. Life expectancy is shortened due to an increased (50-fold) risk of aggressive squamous cell carcinoma of the skin. No definitive treatment is currently available. This is the first study to assess wound healing in RDEB using a cell therapy administered intradermally.

Aims

This study is a double blinded, intra-patient, placebo controlled randomised controlled trial to determine the clinical response of intradermal allogeneic culture expanded fibroblast injections in chronic erosions of patients with RDEB.

Methods

Allogeneic skin fibroblasts were culture expanded from skin biopsies from a healthy unrelated male donor in a GMP accredited manufacturing facility. Cells were supplied in a solution of Plasma-Lyte 148 with 2% Albumex.

Five adult patients, each with up to 6 pairs of symmetrical wounds were recruited and biopsied at baseline. Paired symmetrical wounds were then randomised for injection of the cell therapy or placebo solution. Ulcer size was assessed using a Visitrak device and skin biopsied to determine collagen VII protein and mRNA expression, anchoring fibril numbers and morphology, and inflammatory markers at 2 weeks, 3, 6 and 12 months.

Results

All patients had rapid improvement in wound healing of both paired wounds treated with the cell therapy and the placebo solution which lasted up to 6 months. Healing was not observed in untreated wounds. Some of the wounds were 100% healed at 6 months. Collagen VII expression increased to a similar degree in both study arms for 3 patients but remained negative in 2 patients despite similar levels of wound healing.

Conclusions

RDEB patients can benefit from wound injection therapy. Injection of both allogeneic fibroblasts and suspension solution alone encouraged wound healing in chronic non-healing wounds. Additional studies to determine if administration of the cell therapy was responsible for placebo solution healing or if injection of placebo alone can result in wound healing are warranted.

No conflict of interest to disclose

HAA 2011 HSANZ ANZSBT ASTH

Wednesday 2 November **0830-1000**
ISCTA Free Communications 2: Cell Delivery ***Bayside Gallery B***

O163 0900

Manufacture of Cellular Therapies in a Regulated Environment – A Ten Year Report

Pamela Dyson[1] Judith Stevens[1] Malgorzata Badowicz[1] Michael Vo[1] Kerry Munro[1] Peter Harrison[1] Ian Lewis[1,2]
[1]SA Pathology & [2]Bone Marrow Transplant Unit, Royal Adelaide Hospital, Adelaide, South Australia

The Therapeutic Products Facility (TPF) was constructed to facilitate the processing of a range of human blood and tissue products in compliance with the Australian Code of Good Manufacturing Practice (GMP). A quality system, maintained by a quality manager, was developed incorporating the elements required by the TGA, NATA, and ISO 9002. TPF staff are trained in the principles and practice of GMP, clean room practice, and in the relevant technical protocols.
Planning for the facility commenced in 1997, building and fit out was completed in 1999 and the facility has been operational since early 2000. The first ten years of operation have seen changes to products and to manufacturing processes to meet regulatory requirements. Some of the greatest changes required have been in staff training and culture.
Products manufactured in the facility under TGA license include haemopoietic progenitor cells, autologous chondrocytes and autologous keratinocytes. Other products manufactured include mesenchymal stromal cells and autologous serum eye drops. In addition to tests required for product assessment post-transplant chimerism analysis and AML marker analysis are also performed.
After ten years of operation, a review was deemed necessary for effective future planning and to optimise allocation of resources. We assessed the effectiveness of the quality system, the strengths and weakness of the management processes, and their impact on staff, clients and contractors. We reviewed shifts in workload statistics, product demand and training requirements. We also examined the impact of licensed manufacturing processes on other manufacturing activities within the facility. Another key factor considered during this review was the expected effect of the newly implemented regulatory framework for biologicals.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
ISCTA Free Communications 2: Cell Delivery ***Bayside Gallery B***

O164 **0915**

Cell Therapy Medical Tourism: Time for Action

Kurt Gunter[1], Edwin Horwitz[2]
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[2]*Children's Hospital of Philadelphia, Philadelphia, PA, USA*
On behalf of the International Society for Cellular Therapy (ISCT)

Aim
Cell therapy medical tourism is defined as travel for purposes of acquiring cell therapy-related health care. Many countries offer cell therapies to international patients. Patients seeking cellular therapies need to understand the difference between 1) formal clinical trials and the innovative practice of medicine and 2) fraudulent cell therapy practice.

Method
The ISCT is working to mitigate and reduce patient risks by:
- Promoting scientific development of the field
- Enabling ethical and compassionate early access to promising cellular therapies
- Engaging in outreach to other industry and relevant scientific/professional organizations to leverage/share existing processes and resources with potential patients
- Providing tools to the consumer that can be used as guidance in evaluating a potential treatment
- Being available to the media to discuss claims of efficacy using cellular therapy

ISCT proposes a cell therapy guide, accessible on line. The guide could explain the hierarchy of evidence and data supporting cell therapies. In addition, the guide would clearly define and differentiate among:
- Approved/standard therapies (eg, hematopoietic stem cell transplant and other cellular therapies approved for marketing)
- Controlled clinical trials
- Valid compassionate use of unapproved therapies
- Treatments not subject to independent scientific and ethical review

ISCT should partner with and provide resources to patient advocacy groups to ensure a personalized and empathetic connection to patients searching for information on novel cell-based therapies.

Conclusion
Cellular therapy medical tourism is here to stay. ISCT members are uniquely positioned to use their scientific, translational, ethical and regulatory expertise to help patients and the field by ensuring the highest standards and ethical principles are employed. We have proposed several actionable steps to bring safe and effective cellular therapies to patients and to enable early access to promising experimental therapies and medical innovations.

No conflict of interest to disclose

Wednesday 2 November **0830-1000**
ISCTA Free Communications 2: Cell Delivery ***Bayside Gallery B***

O165 0930

Building a Good Manufacturing Practice Facility Within NSW Health

Janet L Macpherson[1], Angela Biggs[2],Karina Schwarz[1], Angel Jaramillo[1], Craig Wright[1], Ross Brown[1], Stephen Larsen[1], John EJ Rasko[1]
[1]*Cell & Molecular Therapies, Royal Prince Alfred Hospital, Camperdown NSW, Australia*
[2]*Australian Red Cross Blood Transfusion Service, Alexandria, NSW, Australia*

Aim
There is a shortage of Good Manufacturing Practice (GMP) laboratories in New South Wales for the manufacture of cell therapy and regenerative medicine products for clinical use. We have designed and built a state of the art facility at Royal Prince Alfred Hospital. A Validation Master Plan is required to track and document the commissioning process.
Method
The cGMP facility was designed to meet both ISO 14644 clean room standards, and the physical containment class 2 (PC2) requirements of the Office of the Gene Technology Regulator. In association with consultant building engineers, we developed a Project Users Requirements Specification (PURS) document that detailed our requirements. Integral to the design is the concept of maximum flexibility and real-time monitoring of environmental parameters. The PURS was expanded into tender specifications for electrical, mechanical and environmental monitoring services, and contractors engaged for construction. Hospital engineering services were responsible for co-ordinating construction, fit out and finish. Contract service providers were required to provide documentary evidence of conformance with the specifications and instructions for on-going maintenance of installed services.
Result
Contractors and staff engineers constructed and fitted out the space according to the tender documents and were ready to hand over the facility. Documentation to certify that the facility had been built to specification was requested, and the consultant engineers were engaged to facilitate the handover. It was evident that some aspects of the engineers' interpretation of the PURS were at variance with the intention of the designers at the time of writing, and gaps were identified. To rectify deficiencies, flooring, mechanical and electrical services all required remedial work. The different interpretations of the PURS caused substantial delays, and resulted in both direct and indirect cost implications. Drafting of the Validation Master Plan and Standard Operating Procedures is progressing. Both Quality Management and Operational procedures have been developed, building on a license to manufacture haematopoietic progenitor cells that has been successfully maintained since 2007.
Conclusion
In general, engineers and scientists speak different languages. The construction of a cGMP clean room facility is considerably more complex than the building of other areas within the Healthcare Service, requiring specialist knowledge and skills. Good communication is essential for execution of the plan.
No conflict of interest to disclose

Wednesday 2 November **1100-1200**
HSANZ Symposium 10: Viruses in Haematology ***Auditorium B***

The Importance of CMV in Haematological Diseases

Rajiv Khanna
Australian Centre for Vaccine Development, Queensland Institute of Medical Research, Herston, Qld, Australia

Allogeneic stem cell transplantation (SCT) remains the most effective curative therapy for the majority of haematopoietic malignancies. Unfortunately SCT is limited by its toxicity and infectious complications as a result of profound immunosuppression. Herpes virus infections are an extremely common and predictable problem in these patients. SCT recipients who are seropositive for cytomegalovirus (CMV) and suffering from acute graft-versus-host disease are at highest risk of developing CMV-associated complications. Antiviral drugs such as ganciclovir given either prophylactically or as early therapy for patients detected to be shedding CMV appear to be an effective strategy for reducing CMV infections. However, long-term treatment with these drugs is associated with significant toxicity, expense and the appearance of drug resistance virus isolates ultimately resulting in treatment failure. While the expansion and adoptive transfer of HCMV-specific T-cells from the healthy original donor can be an effective strategy to control viral replication, this is not possible when donors are seronegative or are subsequently inaccessible. Recent studies from our group have demonstrated for the first time, the successful expansion of CMV-specific T-cells from seropositive transplant recipients of a seronegative graft with active CMV disease and the long term reconstitution of protective anti-viral immunity following their adoptive transfer back into the patients. We have now developed a single platform technology to extend this immunotherapeutic strategy for multiple pathogens including CMV, Epstein-Barr virus, adenovirus and BK polyoma virus. T cells directed towards multiple pathogens can be rapidly expanded and can be used for adoptive immunotherapy. Finally, we are also testing a novel T cell-based immune monitoring technology (QuantiFERON-CMV) which will allow us to identify high risk SCT patients who may develop CMV-associated complications post-transplantation.

Wednesday 2 November **1100-1200**
HSANZ Symposium 10: Viruses in Haematology ***Auditorium B***

EBV Related Lymphomas

Maher Gandhi

Abstract not received at time of going to print

Wednesday 2 November **1100-1200**
HSANZ Symposium 10: Viruses in Haematology ***Auditorium B***

Evolving Adoptive Immunotherapy for Viral Disease after Allogeneic Stem Cell Transplant

Kenneth Micklethwaite
Westmead Hospital, Westmead, NSW, Australia

Haemopoietic stem cell transplantation is a potentially curative procedure for patients with otherwise untreatable haematological diseases. Post-transplant immunodeficiency related viral infections such as the latent herpes viruses, the adenovirus group, and a range of respiratory tract viruses continue to cause significant morbidity and mortality. Efficacy of current pharmacological agents is limited by their cost, side effects and resistant viral strains. Ultimately, pathogen-specific immune reconstitution is required for the long term prevention of infectious disease.

Adoptive immunotherapy with pathogen-specific T-cells can rapidly and safely reconstitute virus-specific immunity post-transplant; and can prevent or treat viral infections.

Experiments in the early 1990's targeted cytomegalovirus (CMV) using cytotoxic T-lymphocyte (CTL) clones. CTLs administered to transplant recipients expanded post-infusion and appeared to prevent CMV infection. However, the generation of these clones was time consuming, laborious and utilised live virus making its routine application problematic.

Subsequent clinical trials have confirmed the safety and efficacy of adoptive T-cell therapy in treating CMV and other important pathogens, especially Epstein Barr virus and Adenovirus. Concurrently, protocols used to generate these CTLs have been simplified, shortening the time to production and bringing them in line with current good manufacturing practice regulations. Broad spectrum antiviral cover for transplant recipients can now be provided through a single T-cell product targeting multiple viral pathogens. Efforts are being directed at making this technology broadly applicable through clinical grade isolation of pathogen reactive T-cells in less than 48 hours for immediate infusion in patients with viral infections resistant to standard therapy. Others have focussed on the generation of well characterised third party CTL banks for administration to closely matched recipients on demand.

Future directions include further expansion of the number of pathogens targeted by single cultures and enhancing T-cell function through genetic modification, incorporating resistance to immunosuppressives, "safety switches" and anti-tumour activity.

Wednesday 2 November **1100-1200**
ANZSBT Symposium 10: Haemovigilance in Australia ***Auditorium A***

Serious Transfusion Incident Reporting (STIR) - Five Years On

Lisa Stevenson, Gerald Bates, Peter Beard, Philip Crispin, Merrole Cole-Sinclair, Slavica Curcic, Bridget Glazebrook, Richard King, Geoff Magrin, Ellen Maxwell, Jo Perillo, Richard Rogers, Carole Smith, Deane Wilks, Tina Noutsos and Erica Wood
STIR expert group and Blood Matters program, Department of Health, Victoria, and Australian Red Cross Blood Service, Australia

Background and Aim
STIR (Serious Transfusion Incident Reporting) is the voluntary haemovigilance reporting system developed by the Department of Health funded Blood Matters program based in Victoria, Australia and based on the Serious Hazards of Transfusion (SHOT) system in the UK. This presentation will review the first five-years experience of STIR.
Method and Results
Since 2006, STIR has received notification of 866 transfusion episodes resulting in 877 adverse events and incidents: with 48 institutions across Victoria, Tasmania, ACT and NT, reporting at least one event. The majority of cases (56%) were associated with red cell transfusion and acute reactions were the most frequently reported event type (52% of total events). Consequences ranged from no clinical impact to serious adverse reactions. Procedural errors accounted for 42% of all events, with “wrong blood in tube” the most common of these at 23 % and “incorrect blood component transfused” at 7.5% events, including 6 ABO incompatible transfusions. No deaths were reported in any of the events reported to STIR. Themes of failure to correctly identify the patient prior to sample collection and/or transfusion administration were prominent, in line with international experience. Additionally, problems relating to use of off-site blood storage refrigerators were common. During 2010-11 further steps were taken towards online reporting and automation of data management to simplify the process and improve the quality of data. Case review at an institutional level (e.g. by local transfusion committee) prior to reporting to STIR also contribute to data quality and completeness. All cases are reviewed by members of a multidisciplinary expert group, which also oversees the work of the system broadly. An annual report of general demographics and deidentified case studies, is utilised to disseminate information widely to health services and availably publicly. Its aim is to provide recommendations and safety tips, to assist health services to improve local transfusion practice.
Discussion
Acute transfusion reactions are common within the data set, and although many are not serious, serve as evidence of clinical vigilance and participation in the program. Awareness and feedback is improving the understanding and management of the different types of transfusion reactions. Although many events were unavoidable providing that the transfusion was appropriate, the number of “near misses’ and incorrect blood component transfused highlights the potential for serious consequences and need for ongoing practice improvement. The ongoing collection and collation of this data for Blood Matters will be a key driver for implementing improvement processes for procedural steps of transfusion and to assist in understanding the types of risks of transfusion that are less easy to quantify.
No conflict of interest to disclose

Wednesday 2 November **1100-1200**
ANZSBT Symposium 10: Haemovigilance in Australia ***Auditorium A***

Queensland Incidents in Transfusion (QiiT). A Haemovigilance system for Queensland

Simon Brown
Royal Children's Hospital, Brisbane, Qld, Australia

The introduction of a haemovigilance system in Queensland commenced with a pilot project in June 2007. The system was based on the concept of voluntary reporting of de-identified events related to the transfusion of fresh blood components from both the Public and Private Healthcare systems. The haemovigilance system, Queensland incidents in transfusion (QiiT), was approved for implementation across Queensland by Queensland Health in 2008. The uptake of QiiT across Public and Private Healthcare systems, including the main Pathology providers, has been excellent with over 100 hospitals across Queensland participating. To date over six hundred events have been reported to QiiT, and the contribution from stakeholders across the State has exceeded initial expectations. The implementation of QiiT has allowed the contribution of haemovigilance data to the National Haemovigilance system, which has been included in both the first and second national haemovigilance reports. An initial report for QiiT has been drafted and includes analysis of the first 229 events reported. It is hoped that on-going reports will provide the necessary feedback to all clinical staff across Queensland. Utilisation of the haemovigilance data has proven to be of utility in engaging clinical staff and initiating discussions of the link between appropriate utilisation of fresh blood components and patient safety. The data from QiiT has highlighted the variability in the management of transfusion reactions, an issue recognised by other haemovigilance systems. This presentation will outline the development of QiiT, findings from the initial analysis of events and the future challenges for haemovigilance in Queensland.

Wednesday 2 November **1100-1200**
ASTH Symposium 9: A Clot is Born ***Bayside 204***

New Concepts in Platelet Activation and Thrombosis

Xiaoping Du
Department of Pharmacology, University of Illinois at Chicago, Chicago, USA

At sites of vascular injury, platelets quickly adhere to injured endothelial cells and exposed subendothelial matrix, become activated and mediate formation of primary thrombi. Platelet activation is also important in facilitating the activation of the coagulation cascade. Platelet activation is induced by two major categories of agonists: the adhesive proteins and soluble agonists. The major adhesion receptors that transmit platelet activation signals include the glycoprotein Ib-IX-V complex that recognizes immobilized von Willebrand factor (VWF), glycoprotein VI that is activated by collagen, and integrins that interact with many matrix and cell surface adhesive proteins including fibrinogen, fibronectin, collagen, vitronectin, and laminin. While these receptors have their own unique signaling mechanisms, they also share similar signaling pathways, particularly the Src family kinases and immunoreceptor tyrosine-based activation motif (ITAM) signaling pathway. Soluble agonists such as ADP, thrombin, thromboxane A2, and serotonin bind to their respective G protein-coupled receptors and often serve to amplify initial platelet activation signals and to recruit circulating platelets into thrombi. Signaling induced by various platelet activation receptors eventually converge into common signaling events such as platelet shape change, granule secretion, and ultimately induce the “inside-out” signaling process leading to activation of the ligand binding function of integrin $\alpha_{IIb}\beta_3$. Platelet activation involves both the rapid positive feedback loops, which greatly amplify initial activation signals, and enable robust platelet recruitment and thrombus stabilization, and subtle negative feedback loops, which regulates the size of thrombus to prevent unwanted thrombosis during hemostasis. Recent studies have provided novel insight into the molecular mechanisms of these processes.

Wednesday 2 November **1100-1200**
ASTH Symposium 9: A Clot is Born ***Bayside 204***

Visualising the Thrombus: Out of the Test Tube and Into the Living Vessel

Vivien MY Chen[1,2], Reema Jasuja[1], Barbara Furie[1], Bruce Furie[1]
[1]*Beth Israel Deaconess Medical Center, Harvard Medical School, Boston USA*
[2]*Lowy Cancer Research Institute, University of New South Wales, Sydney, Australia*

Research into the coagulation system using *in vitro* assays has identified enzymes, cofactors, cell receptors and associated ligands important to the hemostatic process and its regulation. However, *in vitro* assays cannot simultaneously reproduce the interactions of all of the components of the hemostatic process that occur *in vivo* nor do they reflect the importance of hemodynamic factors resulting from blood flow. Visualisation of *in vivo* platelet and endothelial cell activation and fibrin generation has increased our understanding of hemostasis and thrombosis. Data from in vivo models has challenged long held items of dogma developed from models based on in vitro assays. For example, using cre-lox floxed tissue factor mice, we show that tissue factor is not confined to extravascular tissues in health, instead it is clear that intravascular tissue factor contributes to platelet accumulation and fibrin deposition in minimal injury models of thrombosis. We demonstrate the potential roles for the activated endothelium in initiation of platelet accumulation and as an important early surface for formation of the blood coagulation complexes that lead to thrombin generation. We demonstrated the importance of protein disulfide isomerase in thrombus formation and the role of tissue factor activation. We are able to visualize the heterogeneity of platelet activation within the platelet thrombus to show that not all platelets are activated equally during thrombosis. The continuing development and improvement of imaging techniques using widefield and confocal microscopy adapted to living animals provide powerful tools to study in depth physiologic processes associated with human cardiovascular diseases, providing new insights into the mechanisms of thrombus formation.

Wednesday 2 November **1100-1200**
ISCTA Workshop

Bayside Gallery B

Clinical Trials in a Changing Regulatory Environment

Chairs: Dominic Wall & Pam Dyson

Presenter: Tony Gill

Wednesday 2 November **1200-1300**
ISCTA Symposium 4 ***Bayside Gallery B***

Large Animal Models of Haematopoietic Stem Cell Gene Therapy

Brian Beard, Patrick Younan, Hans-Peter Kiem
Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Haematopoietic stem cell (HSC) gene therapy has remained an attractive treatment option for many genetic, infectious and malignant diseases. However, given the documented and potential adverse complications, careful evaluation of novel vectors of treatment strategies are important. Here we discuss how large animal models have contributed to the advancement of HSC gene therapy. We will discuss safety studies in large animal models, in particular long-term follow up with commonly used retroviral vectors and integration site patterns. There are several canine disease models like X-linked severe combined immunodeficiency, leukocyte adhesion deficiency, and pyruvate kinase deficiency that can be used to study both safety and efficacy of a particular treatment approach. We will discuss HSC gene therapy approaches for HIV/AIDS in the nonhuman primate model and how in vivo selection strategies can be used to improve therapeutic potential of HSC gene therapy. Finally, we will discuss how these large animal studies have led to the development of clinical trials.

Wednesday 2 November **1200-1300**
ISCTA Symposium 4 ***Bayside Gallery B***

MicroRNA Regulation for Gene and Stem Cell Therapy

Luigi Naldini

Abstract not received at time of going to print

Wednesday 2 November **1400-1530**
HSANZ Symposium 11: Molecular Mechanisms in Acute Leukaemias ***Auditorium B***

The Application of New Genomic Technology in Leukaemia

Charles Mullighan

Abstract not received at time of going to print

Wednesday 2 November **1400-1530**
HSANZ Symposium 11: Molecular Mechanisms in Acute Leukaemias ***Auditorium B***

Molecular Detection of MRD

David Grimwade
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Comprehensive molecular and cytogenetic analysis can distinguish biologically and prognostically distinct subsets of acute leukaemia that demand differing treatment approaches. Definition of these pretreatment characteristics coupled with morphological response to induction chemotherapy provides the framework for current risk-stratification schemes, aimed at identifying subgroups most (and least) likely to benefit from allogeneic transplant. While this has proved very useful, such parameters still serve to distinguish relatively large subgroups of patients with broadly differing risk of relapse, and hence there has been considerable interest in development of multiparameter flow cytometry, identifying leukaemia-associated aberrant phenotypes, and real-time quantitative polymerase chain reaction (RQ-PCR) detecting leukaemia-specific targets (eg, fusion gene transcripts, *NPM1* mutation, *IgH/TCR* gene rearrangements) or overexpressed genes (eg, *WT1* in AML), to provide a more precise measure of disease response. Minimal residual disease (MRD) assessment using such approaches can be used at early timepoints to assess kinetics of disease response, which has been shown to provide independent prognostic information, which is now being used to inform risk-stratified treatment approaches in ALL and AML. Although MRD assessment at early timepoints enhances prediction of disease outcome it lacks the capacity to precisely pinpoint which patients are destined to relapse following frontline therapy and those who will be cured. This however, is feasible in patients with a leukaemia-specific molecular marker (e.g. fusion gene, *NPM1* mutation). We have established proof of principle in acute promyelocytic leukaemia (APL), showing that serial monitoring of *PML-RARA* fusion transcripts to guide pre-emptive molecularly-targeted therapy with arsenic trioxide leads to a significant reduction in frank relapse rate, associated with improved survival. We are now investigating within the National Cancer Research Institute (NCRI) AML17 trial whether MRD monitoring is clinically useful in other subsets of AML, allowing more rational deployment of allogeneic transplant and lead to meaningful improvements in outcome.

Wednesday 2 November **1400-1530**
ANZSBT/ASTH Combined Symposium: ITP ***Auditorium A***

Primary Immune Thrombocytopenic Purpura (ITP): Diagnosis and Treatment, 2011

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Diagnosis of ITP is only achieved by excluding other causes of isolated thrombocytopenia. A common alternative cause is drug-induced immune thrombocytopenia (DITP). Many commonly used drugs, as well as some foods and quinine-containing beverages, can cause DITP. A careful history with explicit questions is required to identify potential drug etiologies. Identification of drug-dependent, platelet-reactive antibodies can confirm the diagnosis of DITP. Data on DITP from published case reports and detection of drug-dependent antibodies is available at www.ouhsc.edu/platelets. *Treatment* of ITP has changed with the availability of thrombopoietin (TPO) receptor agonists, romiplostim and eltrombopag. Previous treatments focused on preventing platelet destruction: immunosuppressive agents, intravenous immunoglobulin, and splenectomy. By increasing platelet production, TPO receptor agonists are effective in most patients, even after failure of multiple previous treatments. The current key issue is the appropriate place for TPO receptor agonists in the sequence of treatment. Corticosteroids remain the standard initial treatment for adults. For 50 years, splenectomy was the standard treatment for patients who did not achieve a sustained, safe platelet count with corticosteroids; splenectomy consistently achieves a durable complete remission in two-thirds of patients. Within the past 10 years, rituximab has become a popular alternative to splenectomy, but the frequency and durability of complete remissions is less than with splenectomy. The most important value of the TPO receptor agonists is for treatment of patients who fail to respond to splenectomy and rituximab; many of these patients will respond. Should TPO receptor agonists be used as an alternative to splenectomy or rituximab? Advocates of this strategy promote their simplicity and safety. However TPO receptor agonists only sustain platelet counts; they do not induce a remission; long-term treatment is required; safety and expense are important issues.

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Wednesday 2 November **1400-1530**
ANZSBT/ASTH Combined Symposium: ITP ***Auditorium A***

New Insights in the Pathogenesis of ITP

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Primary immune thrombocytopenia (ITP) has been traditionally thought as an antibody-mediated autoimmune disease involving platelet destruction by macrophages in the reticuloendothelia system. More recently it has become obvious that ITP is a more complex disorder in which T cell mediated immunity plays important roles in platelet destruction. Antiplatelet autoantibody production is under the control of platelet-specific helper T-cells, and loss of tolerance to self antigen by T cells is the critical step of the immune dysregulation in ITP. Our group has demonstrated that blocking the B7-CD28 interaction could induce platelet glycoprotein (GP)-specific T-cell tolerance. Defects of the apoptotic pathways in T cells could promote the survival of autoreactive T cells in ITP patients. T-cell subset polarization in ITP has been attributed to increased Th1, and Th17 cells or reduced number or function of $CD4^+CD25^+Foxp3^+$ T-regulatory cells (Tregs). In addition, a parallel body of aberrant cytokine patterns, such as the elevated ratio of interleukin (IL) -18/IL-18BP, the increased expression of B cell activating factor (BAFF), the decreased expression of IL-17 has been reported in active ITP patients. $CD8^+$ cytotoxic T lymphocytes (CTLs)-mediated platelet lysis or apoptosis are another mechanism of platelet destruction, especially in patients with negative GP-autoantibody. Besides the accelerated platelet clearance, the production of platelets has demonstrated to be impaired recently in ITP. Autoantibodies may impair megakaryocyte development, induce apoptosis, impede platelet release, or promote intramedullary phagocytosis. $CD8^+$ T cells in bone marrow of ITP have also been reported to impair platelet production via suppressing megakaryocyte apoptosis. In a word, ITP is a very heterogeneous autoimmune disorder, and further studies of disease pathogenesis will help to provide more rational and personalized therapeutics for ITP patients.

Wednesday 2 November **1400-1530**
ANZSBT/ASTH Combined Symposium: ITP ***Auditorium A***

TPO Receptor Agonists: the Australian Experience

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The use of TPO receptor agonists (TPORAs) to increase the patients' platelet counts is a new approach in ITP treatment. There are two TPORAs in clinical use: (1) romiplostim (a Fc-peptide fusion protein), given once a week by s/c injections and (2) elthrombopag (a small molecule), given orally once daily.

Haematologists in Australia have gained experience with the use of these drugs mainly through participation in clinical trials. The AMG 531 60131 study was a randomized controlled study which evaluated the efficacy and safety of romiplostim vs standard-of-care in ITP patients. The overall response rates in romiplostim-treated ITP patients were 88% in non-splenectomised and 79% in splenectomised patients, compared with 0% and 14% respectively in those receiving standard-of-care treatment. Response to treatment was associated with reduced bleeding, decreased use of concurrent ITP therapies and improved quality of life. AMG 531 Extention study showed that long-term therapy with romiplostim maintained the mean platelet counts above $50x10^9$/L for more than 204 weeks.

Clinical trials with elthrombopag showed similar results. The double-blind, randomized, placebo-controlled study (TRA100773 ITP) demonstrated a dose-dependent response rates of 28%, 70% and 81% with 30, 50 and 75 mg daily of elthrombopag respectively. In comparison, the response rate in placebo-treated patients was 11%. In the open-label repeat dosing study (TRA108057 ITP REPEAT), ITP patients continued to respond with each of the 3 repeated cycles of elthrombopag. In the TRA105325 ITP Extend study, ITP patients were treated for more 104 weeks with elthrombopag. 78% of patients achieved platelet counts of > $50x10^9$/l for at least 50% of the time. Response to treatment was also associated with reduction in bleeding, discontinuation of concomitant ITP medication and improved quality of life.

Both romiplostim and elthrombopag were well tolerated with only side-effects of mild and moderate severity, most were also seen in the placebo-treated patients. Thrombo-embolic events and increase in bone marrow reticulin were present in a small number of TPORA-treated patients but it is still unclear whether these were due to the underlined ITP or to the drugs. Further studies are needed to address these issues.

Wednesday 2 November **1400-1530**
ISCTA Symposium 6: The Tissue Interface: Bioreactors and Scaffolds
Bayside Gallery B

Articular Cartilage Cell Therapy

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Cartilage damage is one of the most common musculoskeletal injuries, affecting millions of people each year worldwide. As cartilage injury leads irreversibly to degeneration and osteoarthritis (OA) in later life, it is a significant socioeconomic burden in both developed and developing countries. An ideal solution to repair articular cartilage is the utilization of cell-based functional tissue engineering technology to generate biomechanically suitable hyaline or hyaline-like cartilage tissue *in situ*. Our laboratory, in collaboration with experts in the field and industry partners (Verigen AG, Germany and later Genzyme, USA, Orthocell Ltd Australia) has developed the cell-scaffold combined tissue engineered construct (the Matrix induced autologous chondrocyte implantation – MACI/ACI) using autologous chondrocytes seeded to a type I/III collagen scaffold. In 2000, MACI was the first combined cell-scaffold tissue engineered product approved for human use in Australia. To date it is estimated that over 25,000 patients in Australia, Asia and Europe have received MACI/ACI treatment in last 15 years. We hereby release the protocol of composition of ACI for research and clinical use. These steps include arthroscopic biopsy of cartilage, storage of cartilage tissue, liberation and cultivation of autologous chondrocytes, formulation of cell-collagen patch construct and acceptance criteria for the use of critical reagents. The current status on the study of clinical efficacy of MACI/ACI has also been reviewed.

Wednesday 2 November **1400-1530**
ISCTA Symposium 6: The Tissue Interface: Bioreactors and Scaffolds
Bayside Gallery B

Scaffolds Having Flow Channels, Oxygen Carriers and Oxygen Permeable Membranes

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Our main concern is how to organize cultured organ-derived cells such as liver cells in 3D manners in various scales and shapes for regenerative medicine from the standpoint of mass transfer such as oxygen supply. Artificial arrangement of functional vascular systems in vitro is still a very difficult issue and it thus becomes a serious problem to optimize simultaneously 3D high-cell-density organization while securing oxygen supply. When we intend to organize large tissue equivalents, the tissue should at least be arranged with a 3D branching/joining flow channel network as an in vivo vasculature and the channels should be perfused with suitable culture medium containing oxygen carriers. We proposed a design criteria based on oxygen diffusion-consumption around a flow channel in macroporous 3D scaffolds, fabricated them, and evaluated their efficacy and limitation in perfusion culture of liver cells. To meet the oxygen demand of large tissues, we examined the feasibility of existing nano-encapsulated hemoglobin. As a practical approach, sheet-like tissues without prevascularization are the best design when we consider that our body has a strong capability to induce vasculatures to implanted tissues. To grow such thick seudo-3D sheet-like tissues, we demonstrated the high efficacy of direct oxygenation through oxygen-permeable membranes in static culture. We noticed that meeting the cellular oxygen demand completely at appropriate physiological concentrations enables highly-efficient aerobic cellular respiration with less oxidative stresses, leading to spontaneous 3D cellular organization that has never been observed in vitro. As such, focusing on oxygen supply to cells should give a firm basis for the design of scaffolds and culture systems in engineering various tissues.

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Bayside Gallery B

Vascularization in Three-Dimensional Tissue Engineering

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Three-dimensional tissue engineering involves the incorporation of living cells into a matrix to create a functioning tissue or organ. Traditional models of tissue engineering use ex-vivo seeding of biological synthetic scaffolds which are then implanted into the body where the tissues incorporates and functions.

Tissues grown ex-vivo can be sustained by nutrient fluid in a bioreactor environment but to transfer this into a living animal, the tissue must rapidly connect to the microcirculation for survival. This is one of the major limiting steps in clinical application of tissue engineering.

One approach to overcome this problem is to grow new tissue in combination with its blood supply. When a hollow chamber containing a vascular pedicle is inserted into the body, new blood vessels spontaneously respond to ischaemia and biomechanical stimuli. If cells are also seeded into the chamber space they have an enhanced capacity to implant and become vascularised during this intense angiogenic period. Other vascularising techniques include co-culture with endothelial cells or vasculogenic stem cells, vessel imprinting and endothelial seeding ex-vivo of the vascular tree of decellularized tissue.

We have been able to grow fat, muscle, cardiac tissue, pancreatic islets and other tissues in animals using these techniques. Many issues remain with respect to translating this research into human application including scale, differentiation and cancer risk assessment.

Wednesday 2 November **1600-1700**
HSANZ Symposium 12: CLL ***Auditorium B***

Chronic Lymphocytic Leukaemia (CLL): The Elderly, Unfit and Refractory

Stephen P Mulligan
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The elderly, unfit and relapsed or refractory patients represent the major therapeutic challenges in CLL following the major advances in progression free and overall survival achieved with FCR based therapy for young and fit patients.

Most CLL patients are elderly with a median age over 70 years but their management and most appropriate therapy remains a controversial and debated topic. Chlorambucil remains commonly used in this group. The CLL5/OFOCIR Trial of the CLLARC and Australasian Leukaemia and Lymphoma Group (ALLG) is investigating the safety and tolerability of FCR-based therapy in patients ≥65 years with a Cumulative Illness Rating Scale (CIRS) ≤6. Interim analyses to date suggest this treatment is generally well tolerated and achieves high response rates. Elderly and other patients with comorbidities with a CIRS score ≥6 are being investigated by the German Cooperative CLL11 trial comparing chlorambucil alone or in combination with either rituximab or GA-101 (Obinutuzumab).

Relapsed and refractory CLL remains a major therapeutic challenge but is highly dependent on the prior treatment regimen. The REACH study showed a significant benefit adding rituximab (R) to fludarabine and cyclophosphamide (FC) with overall response of 69.9% for FCR compared to 58% with FC in patients who relapsed following one prior therapy, mainly alkylating agents. Novel CD20 antibodies, especially ofatumumab and GA-101 are under investigation. Alemtuzumab is clearly active in CLL but frequently used late in the disease and associated with toxicity, especially immunosuppression and infection, and hence its role, dose and optimal schedule remain unresolved. Other agents in clinical trials include pentostatin, bendamustine, lenalidomide, and flavopiridol (alvocidib). An historically high number of novel agents with activity in CLL are under investigation including inhibitors of bcl-2, Bruton's tyrosine kinase, Akt, PI3 kinase and HSP-90. P53 tumour suppressor gene deletion or mutation occur in many patients with refractory disease and is typically associated with a very poor outcome. Drugs bypassing the p53 pathway such as alemtuzumab and high-dose steroids are under investigation in these poor risk patients. Finally, stem cell transplantation offers potential benefits for patients with refractory disease who are eligible candidates.

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Wednesday 2 November **1600-1700**
HSANZ Symposium 12: CLL ***Auditorium B***

Management of the Young, Fit, Chemotherapy-tolerant Patient. Do We Change the Treatment Paradigm in the Post-FCR Era?

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The treatment of chronic lymphocytic leukaemia (CLL) has seen a massive paradigm shift from symptom palliation, to the pursuit of maximal disease eradication. Modern chemoimmunotherapy is capable of eradicating minimal residual disease (MRD) in a substantial proportion of patients, raising hopes that the cure of CLL may finally be within reach. However, subgroups of patients (eg. IgVH unmutated or 11q deletion) continue to relapse from complete remission, and others have detectable MRD at the end of induction therapy. These patients constitute high-risk patients who may benefit from targeted consolidation strategies, in order to eliminate MRD, prolong remission and maximise survival.

Wednesday 2 November **1600-1700**
ISCTA Symposium 6: Emerging Cell Therapies ***Bayside Gallery B***

Embryonic Stem Cells and Induced Pluripotency

Martin Pera

Abstract not received at time of going to print

Wednesday 2 November **1600-1700**
ISCTA Symposium 6: Emerging Cell Therapies ***Bayside Gallery B***

Use of ES and iPS in Clinical Therapies? Practical and Ethical Aspects

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If pluripotent cells can be differentiated ex vivo to recreate and repair mature human tissues and organs then regenerative medicine will become a reality. However embryonic stem cells have been mired in controversy and clinical development has been forestalled. In the past few years, relatively straightforward laboratory techniques have been developed to reprogram normal body cells to enter an embryonic stem cell–like state. Patient-specific cells for regenerative therapies will also be of use in studying diseases in vitro, testing new treatments for these diseases, and creating artificial gametes for use in reproductive medicine. Although cell reprogramming may transform regenerative and reproductive medicine there are still significant technical medical, moral, and political hurdles. Infact induced pluripotent stem cells are more ethically problematic than most people believe. Some key practical and ethical problems that this new field of research presents will be highlighted.

Power C and Rasko JEJ. Will Cell Reprogramming Resolve the Embryonic Stem Cell Controversy? *Annals of Internal Medicine*, 2011;155:114-121.

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