

**Sunday 28 October**  
**HSANZ/Nurses Combined Symposium 1: Myeloma**

**0900-1030**  
**Plenary 2 (Auditorium)**

## **Induction Therapy in Newly Diagnosed MM**

Philip McCarthy  
*Roswell Park Cancer Institute, Buffalo, NY, USA*

### **Aim**

Understanding indications for treatment, treatment options and long term strategies for multiple myeloma (MM) patients

### **Results**

MM is a plasma cell cancer of the bone marrow. MM can manifest as a low grade condition (smoldering MM) with low tumor burden that may not require treatment. CRAB criteria are standard indications for therapy. These include hyperCalcemia, Renal failure, Anemia and Bone disease. Appropriate staging includes blood and urine testing for monoclonal immunoglobulin proteins, bone marrow (BM) testing for detecting the presence of malignant plasma cells and radiographic imaging by skeletal survey +/- PET and MRI. BM cytogenetic analyses by metaphase karyotyping and fluorescence *in situ* hybridization is an important component of risk stratification. Molecular tests such as gene expression profiling will help with further risk stratification. The clinical team determines treatment based on clinical factors such as age, performance status, organ involvement, disease risk and consideration for intensive therapy such as autologous hematopoietic stem cell transplant (HSCT). In the USA, MM induction regimens include older agents with known anti-MM activity including melphalan (M) and glucocorticoids (prednisone (P) or dexamethasone (D)) and cyclophosphamide (Cy). Incorporation of novel agents into induction regimens has improved response rates and duration of response. These novel agents include the proteasome inhibitor (PI) bortezomib (B, V or P) and the immunomodulatory drugs (IMiDs) thalidomide (T) and lenalidomide (R) have led to better responses for transplant-eligible patients before HSCT or before maintenance therapy. Older doublet therapies such as MP or TD may be replaced by newer doublets such as VD or Rd (low dose D). Triplet regimens such as RVD, PAD, CyBorD and VTD are appropriate regimens for HSCT-eligible patients. For the non-HSCT MM patients, MPT, MPR, Rd and VD are appropriate treatments.

### **Conclusion**

There are several approaches for induction therapy for MM patients. Inducing a complete or near complete response remains a primary goal of induction with the long term goal of maintaining response.

**Keywords**     Combination therapy for multiple myeloma

**Conflict of interest**     Advisory board for Celgene, Speaker Janssen Pharm.  
 Abstract not available at time of publication

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**0900-1030**  
**Plenary 2 (Auditorium)**

## **The Why, How and When of ASCT in MM**

H Miles Prince

*Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia*

Approximately 1000 new cases of myeloma are diagnosed in Australia each year and approximately 550 autologous stem cell transplants (ASCT) are performed in Australia and New Zealand annually. Although MM remains an incurable disease, survival outcomes have improved significantly owing to the introduction of first, high dose therapy (HDT) and ASCT in the late 1990s, then novel therapeutic agents including thalidomide, its immunomodulator derivative lenalidomide, and the first-in-class proteasome inhibitor, bortezomib.

Treatment options for MM are now diverse. Apart from basic treatment principles, there is no strict consensus for standard of care for both upfront or relapsed/refractory disease. Local treatment guidelines vary, based on both the local availability of novel therapeutic agents, and familiarity of the treating physician to these agents. This statement holds true for stem cell transplantation in MM.

Recently consensus guidelines were established by the Australian Medical Scientific Advisory Group (MSAG) to the Myeloma Foundation Australia (MFA), which consists of a panel of haematologists across Australia.

This presentation will review the guidelines for the management of MM in Australia with a focus on the place of ASCT.

**Keywords**     Myeloma, ASCT

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## **Supportive Care in Myeloma – A Challenge of Modern Myeloma Management**

Tracy King

*Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia & Sydney Nursing School, Cancer Nursing Research Unit, Sydney, NSW, Australia*

The management of multiple myeloma (MM) has improved substantially as a result of advances in our understanding of the disease biology as well as improvements in treatment and supportive care strategies (Ludwig et al 2012). Such advances have resulted in improved patient outcomes including overall survival (OS). Despite these significant therapeutic advances, patients often have pronounced symptoms and substantially reduced health related quality of life (HRQOL) with reduced functioning, fatigue and pain as major problems (Gulbrandsen et al 2004). Living longer in a relapsing, remitting state, patients can experience an increased symptom burden due not only to the disease itself but to being exposed to the cumulative effects of increasing lines of therapy (Snowden et al 2010). To achieve substantive improvements for MM patients, the prolonged survival associated with MM must be matched with attention to evidenced based effective supportive care measures (Snowden et al 2010). Central to the supportive care measures for those with MM is the appropriate management of the disease related morbidities of bone pain, bone fractures, recurrent bacterial infections, impaired renal function and anaemia. More recently there has been a focus on managing the common and compounding toxicities of therapy including peripheral neuropathy, myelosuppression, gastric effects, steroid effects and thrombotic events. This session will explore the dimensions of health related HRQOL in MM and present the evidence for best practice in the provision of supportive care measures.

### **References**

- Gulbrandsen, N, Hjermstad, M, Wisloff, F (2004). Interpretation of quality of life scores in multiple myeloma by comparison with a reference population and assessment of the clinical importance of score differences. *European Journal of Haematology*. 72 :172-80.
- Lugwig, H, Avet-Loiseau, H, Blade, J et al (2012). European perspective on multiple myeloma treatment strategies: Update following recent meeting. *The Oncologist*. 17 :592-606.
- Snowden, J, Ahmedzai, S, Ashcroft, J et al (2010). Guidelines for Supportive Care in Myeloma. British Committee for Standards in Haematology in conjunction with the UK Myeloma Forum (UKMF).

**Keywords** Myeloma, Supportive Care, Quality of Life **Conflict of interest** No

**Sunday 28 October**  
**ANZSBT Symposium 1: Transfusion Trials for Clinical Practice**

**0900-1030**  
**Rooms 219/220**

## **Clinical Trials in Transfusion Medicine**

Larry J Dumont

*The Geisel School of Medicine at Dartmouth, Lebanon, NH USA*

### **Aim**

We will review key questions on the safety and efficacy of transfusion practice, highlight recent and ongoing clinical trials, and discuss barriers to the implementation of new methods and practices.

### **Results**

Contemporary dogma is that transfusion practice should be informed and guided by the best available evidence. Although blood products have been used for decades, there remain areas where evidence is weak or lacking to support current practice. Progress has been made in understanding the role of white cells in alloimmunisation and transfusion reactions, platelet dose effectiveness in hypoproliferative thrombocytopenia, antibody mediated TRALI, plasma and platelet use in trauma, methods to reduce the risk of transfusion acquired sepsis, effective test strategies for infectious disease screening, RBC transfusion triggers in adult critical care and others. Questions still remain regarding transfusion triggers, dose, prevention of adverse events, and prevention of transfusion transmitted infectious diseases. As we scan the patient population served by transfusion medicine (such as, neonatology, trauma, neurology, pediatric intensive care, cardiac surgery, and hematologic oncology), it is apparent that one size does not fit all. Evidence across all patient groups is needed. Where solutions seem to be within our grasp, such as pathogen reduction, implementations are hampered because of uncertainties related to adverse events. Clinical trials have been opened world-wide to address some of these points.

### **Conclusion**

Important evidence has been provided in recent clinical trials, and important trials have been launched. There remain open questions. Collaborative effort is necessary to identify and overcome barriers to gaining important evidence and timely implementation of solutions.

**Keywords** clinical trial, evidence based medicine

**Conflict of interest** No

**Sunday 28 October**  
**ANZSBT Symposium 1: Transfusion Trials for Clinical Practice**

**0900-1030**  
**Rooms 219/220**

## **Patient Outcomes and Red Blood Cell Storage: the Clinical Studies**

Christopher P Stowell  
*Massachusetts General Hospital & Harvard Medical School, USA*

### **Aim**

The aim of this lecture is to provide a broad overview of the clinical studies which have been carried out to determine the effects of RBC storage on patient outcomes. In addition, the design of several ongoing, randomized, controlled clinical trials addressing this issue in critical care patients and cardiac surgery patients will be reviewed.

### **Results**

One of the most controversial topics in our field in recent years has been the question of whether or not the transfusion of stored RBC has adverse effects on patients. It has been proposed that the numerous changes which are known to occur to RBC during storage impair their function when transfused and, furthermore, be actively deleterious. A large number of retrospective clinical studies, and a few prospective studies, have been carried out to address this issue, but the results have not been conclusive. Within the past few years, several randomized clinical trials addressing the effect of RBC storage on patient outcomes have been initiated. In this session, the speaker will summarize our current understanding of this issue based on the results of published clinical studies, and describe several ongoing randomized, controlled clinical trials which have been designed to provide an answer to this question.

### **Conclusion**

Although it is firmly established that the RBC changes during storage, we remain in a state of clinical equipoise on the question of whether or not these changes affect patients in a clinically significant way.

**Keywords** – RBC storage, RCT, equipoise

**Conflict of Interest** – none

Sunday 28 October

Platelet Workshop 5: Animal Model for the Assessment of Platelet Number and Function

0900-1030

Room 210

## **Assessing the Antithrombotic Activity of Weak Agents Using in vivo Models**

Peter L Gross, Ran Ni

*Thrombosis and Atherosclerosis Research Institute, McMaster University, Canada*

### **Aim**

Most heart attacks and strokes are caused by platelet-rich thrombi superimposed on disrupted atherosclerotic plaques. Because of the central role of platelets in these disorders, platelet inhibitors, such as acetyl salicylic acid (ASA), are a mainstay of their prevention and treatment. Despite the importance of platelets, our knowledge about the dynamics of platelet activation comes mainly from in vitro studies of platelet aggregation in response to single agonists or from in vivo monitoring of platelet accumulation after vessel wall injury. Our objective was to monitor the spatial and temporal aspects of platelet activation that occur after laser injury to cremaster arterioles in mice using high-speed two-color confocal microscopy.

### **Results**

Platelets were labelled with an antibody against GPIIb/IIIa and platelet activation was monitored using fluorescently-labelled antibodies against platelet integrin CD41 that increases in number upon platelet activation, and P-selectin, a marker of alpha granule release, and DiI, a marker of phosphatidylserine (PS) exposure on the platelet surface. The ratio of fluorescence of the activation label to the platelet label at each pixel space within the thrombus was calculated. CD41 up-regulation occurs more rapidly than alpha-granule release. PS exposure is the slowest event. These activation events occur in distinct locations within the thrombus: whereas CD41 up-regulation occurs diffusely, P-selectin and PS exposure are localized to the vessel wall at the site of injury. ASA, low doses of DiI, and atorvastatin individually had no effect on platelet accumulation or P-selectin expression, but delayed CD41 up-regulation.

### **Conclusion**

This system appears capable of monitoring platelet activation events in vivo and is more sensitive to the effects of weak inhibitors than current methods.

**Keywords** intravital microscopy, asa, phosphatidylserine

**Conflict of interest** No

**Sunday 28 October**

**Platelet Workshop 5: Animal Model for the Assessment of Platelet Number and Function**

**0900-1030**

**Room 210**

## **Novel Regulators of Platelet Production and Function**

Benjamin T Kile

*The Walter & Eliza Hall Institute of Medical Research, Melbourne, Vic, Australia*

We have conducted a genetic screen for novel molecular regulators of hematopoiesis, with a focus on mutations affecting the myeloid lineage. Random ENU mutagenesis in mice was followed by automated hematological analysis and subsequent exome sequencing of pedigrees segregating heritable phenotypes. Mutations in a number of genes with largely uncharacterised roles in platelet production and function were identified. The proteins encoded by these genes include apoptotic regulators, ion channels, cytoskeletal modulators and components of the mRNA splicing and export machinery.

**Keywords:** Megakaryocyte, platelet, hematopoiesis

**Conflict of interest:** No

Sunday 28 October

0900-1030

Platelet Workshop 5: Animal Model for the Assessment of Platelet Number and Function

Room 210

## Transgenic, Inducible RNAi in Megakaryocytes and Platelets in Mice

Megumi Takiguchi<sup>1</sup>, Chloé James<sup>1</sup>, Emma C Josefsson<sup>1</sup>, Catherine L Carmichael<sup>1</sup>, Prem K Premrurut<sup>2</sup>, Scott W Lowe<sup>2</sup>, Justin R Hamilton<sup>3</sup>, David CS Huang<sup>1</sup>, Benjamin T Kile<sup>1</sup>, Ross A Dickins<sup>1</sup>

<sup>1</sup>Walter and Eliza Hall Institute of Medical Research, Parkville, Vic, Australia

<sup>2</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA

<sup>3</sup>Australian Centre for Blood Diseases, Monash University, Melbourne, Vic, Australia

### Aim

RNA interference (RNAi) is a powerful tool for suppressing gene function. The tetracycline (tet)-regulated expression system has recently been adapted to allow inducible RNAi in mice, however its efficiency in particular cell types *in vivo* depends on transgenic tet transactivator expression pattern and is often highly variable. We aimed to establish a transgenic strategy that allows efficient tet-regulated gene knockdown in particular haematopoietic lineages in mice.

### Results

Using a tet-regulated reporter gene strategy, we find that transgenic mice expressing the rtTA (tet-on) transactivator under control of the CMV promoter (CMV-rtTA) display inducible reporter gene expression with unusual and near-complete efficiency in megakaryocytes and platelets. To test whether the CMV-rtTA transgene can drive inducible and efficient gene knockdown within this lineage, we generated a novel mouse strain harbouring a tet-regulated short hairpin RNA (shRNA) targeting Bcl-x<sub>L</sub>, a pro-survival Bcl-2 family member known to be essential for maintaining platelet survival. Doxycycline treatment of adult mice carrying both transgenes induces shRNA expression, depletes Bcl-x<sub>L</sub> in megakaryocytes, and triggers severe thrombocytopenia, whereas doxycycline withdrawal shuts off shRNA expression, normalizes Bcl-x<sub>L</sub> levels, and restores platelet numbers. These effects are akin to those observed with drugs that target Bcl-x<sub>L</sub>, demonstrating that this transgenic system allows efficient and reversible inhibition of genes in megakaryocytes and platelets.

### Conclusion

We have established a novel transgenic strategy for inducible gene knockdown in megakaryocytes and platelets that will be useful for characterising genes involved in platelet production and function in adult mice.

**Keywords** RNAi, platelets, mouse

**Conflict of interest** No



Sunday 28 October

**Platelet Workshop 5: Animal Model for the Assessment of Platelet Number and Function**  
**Free Communication**

0900-1030

Room 210

## **Phosphoinositide 3--C2 $\alpha$ -Deficient Mice Have Impaired Platelet Function**

Jessica Mountford, Justin Hamilton, Shaun Jackson  
*Australian Centre for Blood Diseases, Melbourne, Vic, Australia*

### **Aim**

Platelets are critical for the development of thrombosis, and understanding signalling events involved in platelet activation may lead to novel anti-thrombotic approaches. Phosphoinositide 3-kinases (PI3Ks) are important signalling enzymes; yet the role of Class II PI3Ks in platelets remains entirely unknown. Therefore we used a mouse genetic approach to examine the function of Class II PI3Ks in platelets.

### **Method and Results**

The expression of two Class II PI3Ks, PI3KC2 $\alpha$  and PI3KC2 $\beta$ <sup>+/-</sup> was detected in human and mice platelets via Western blot. Platelets from PI3KC2 $\beta$ <sup>+/-</sup> mice had normal platelet function in all assays examined and PI3KC2 $\alpha$ <sup>+/-</sup> mice died *in utero* prior to haematopoiesis. However, PI3KC2 $\alpha$ <sup>+/-</sup> mice exhibited a platelet-dependent haemostatic defect *in vivo* and dysregulated thrombosis *ex vivo*. Specifically, anticoagulated PI3KC2 $\alpha$ <sup>+/-</sup> mice had a 3-fold increase in tail bleeding time vs anticoagulated wild-type littermates. An *ex vivo* whole blood thrombosis assay revealed that thrombi of PI3KC2 $\alpha$ <sup>+/-</sup> mice were highly unstable, with 75% of thrombi formed in blood from PI3KC2 $\alpha$ <sup>+/-</sup> mice embolising vs 0% in littermate controls. Finally, ~ 15% of platelets in PI3KC2 $\alpha$ <sup>+/-</sup> mice were dysmorphic, suggestive of a defect in platelet membrane/cytoskeleton structure and/or function. Strikingly, the *ex vivo* phenotypes observed in platelets taken from PI3KC2 $\alpha$ <sup>+/-</sup> mice were reproduced in wild-type platelets via pharmacological increases in membrane fluidity (DMSO, 0.5-1%).

### **Conclusion**

Our studies define a novel role for the Class II PI3K isoform, PI3KC2 $\alpha$ , in the haemostatic and thrombotic function of mouse platelets. We hypothesise that PI3KC2 $\alpha$  performs this by playing important roles in the maintenance of platelet membrane/cytoskeleton structure and/or function.

**Keywords** Platelets, thrombosis, PI3K, mouse.

**Conflict of interest** No conflict of interest to disclose.

**Sunday 28 October**  
**APSTH Education 1: vWF and the Microangiopathies**

**0900-1030**  
**Room 211**

## **Thrombotic Microangiopathies- A Diagnostic Approach**

Amanda Davis  
*The Alfred, Melbourne, Victoria, Australia*

Thrombotic microangiopathy (TMA) is characterized by fragmentation haemolysis with associated thrombocytopenia. More widespread disease may result in tissue ischaemia damaging organs such as the brain, kidneys and pancreas.

There are a variety of molecular mechanisms which can result in the TMA phenotype, but often there are distinct clinical and/or laboratory features which can help to establish the specific cause. Identifying the underlying aetiology is crucial to providing appropriate therapy. For example, malignant hypertension causing TMA will respond well to blood pressure control. In contrast, ADAMTS13 deficiency due to genetic mutation in congenital thrombotic thrombocytopenic purpura (TTP), or inhibitory antibodies as a result of acquired TTP requires plasma therapy. In the case of congenital TTP, replacement of ADAMTS13 enzyme with FFP is sufficient whereas, in acquired TTP the antibody must be reduced and the enzyme replaced. More recently, the role of complement regulatory proteins has been elucidated in the development of TMA due to atypical haemolytic uraemic syndrome. This discovery has helped identify a potential role for the anti-C5 monoclonal antibody, eculizumab.

This session will focus on the clinical and laboratory features which can help to identify the cause of TMA and as a result help the clinician to deliver the most appropriate therapy.

**Keywords** thrombotic microangiopathy, diagnosis, ADAMTS13

**Conflict of interest** No

**Sunday 28 October**  
**APSTH Education 1: vWF and the Microangiopathies**

**0900-1030**  
**Room 211**

## **TTP and Redox Regulation of VWF Cleavage by ADAMTS-13**

Jing-fei Dong

*Puget Sound Blood Center and Division of Hematology, Department of Medicine,  
 University of Washington, School of Medicine, Seattle, WA, USA*

Von Willebrand factor (VWF) is a multimeric glycoprotein that mediates platelet tethering to the subendothelium at sites of vessel injury. Activated endothelial cells release ultra-large (UL) VWF multimers that are prothrombotic. In contrast, plasma VWF (pVWF) multimers requires activation by shear or modulators to bind platelets. This difference suggests that ULVWF multimers have an active conformation, whereas pVWF multimers have an inactive conformation. It is unclear how shear activates pVWF multimers. We hypothesize that 1) ULVWF differs from pVWF in their thiol-disulfide states; 2) shear stress activates pVWF multimers by inducing a thiol-disulfide exchange (TDE) to form laterally associated fibrils; and 3) ADAMTS-13 acts as a reductase to prevent VWF activation mediated by TDE. We show that pVWF multimers contain surface-exposed thiols that form disulfide bonds upon exposure to high shear. TDE involves cysteine residues in the D3 and C domains and enhances pVWF binding to platelets. This thiol-mediated interaction also occurs between pVWF and ULVWF multimers as perfusing pVWF elongates ULVWF strings anchored to the activated endothelium. This pattern of propagating ULVWF strings is reduced by blocking thiols on pVWF. Together, these results suggest that thiol-disulfide state is a critical structural determinant for VWF activity and can be modified by shear. TDE also allows thrombogenic ULVWF strings to be formed covalently on the endothelium to capture platelets and to form thrombus. These data suggest that the activity of pVWF multimers could be enhanced by MPO-mediated VWF oxidation and reduced by the glutathione precursor N-acetyl-cysteine.

ADAMTS-13 is found to have a disulfide-bond-reducing activity that regulates the shear-induced TDE of pVWF. This reducing activity primarily targets disulfide bonds formed under high shear, with a limited impact on intra-multimer disulfide bonds. Cysteine thiols targeted by this reducing activity are located in VWF C-domains that are involved in shear-induced TDE. Consistent with this reductase-like activity, ADAMTS-13 contains active thiols that remain exposed under high hydrodynamic forces. Blocking these thiols abolishes ADAMTS-13 reductase-like activity, but not its VWF-cleaving activity. Mass spectrometry identified thiols in multiple domains of ADAMTS-13, but those in the C-terminal region are essential for this disulphide bond-reducing activity.

In summary, these data demonstrate a novel mechanism of regulating VWF adhesive activity and cleavage by ADAMTS-13 by redox. This mechanism may be active in triggering TTP episodes upon oxidative stress and impaired in acquired deficiency of VWF proteolysis under the condition of severe inflammation.

**Keywords:** VWF, ADAMTS-13, Redox regulation

**Conflict of interest:** The author claims no relevant conflict of interest.

Sunday 28 October  
 APSTH Education 1: vWF and the Microangiopathies

0900-1030  
 Room 211

## Management of Thrombotic Microangiopathy

Doyeun Oh

*Department of Internal Medicine, School of Medicine, CHA University, Korea*

Thrombotic microangiopathy (TMA) is a pathologic term characterized by intravascular aggregation of platelets resulted in thrombocytopenia and mechanical injury to erythrocytes. There are two major types in TMA; thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) which have different pathophysiology, prognoses and treatment strategies. TTP has traditionally been described as a pentad of thrombocytopenia, microangiopathic hemolytic anemia (MAHA), neurologic abnormalities, renal failure and fever. But currently, MAHA and thrombocytopenia in the absence of any other identifiable cause are sufficient criteria to establish a clinical diagnosis of TTP. Coagulation test are usually normal and ADAMTS13 activity is absent or severely decreased in plasma. PE is the essential treatment for TTP and more than 90 percent of patients can be successfully treated. Treatment with PE should be continued until full correction of laboratory abnormalities. Serologic tests for HIV, hepatitis, autoantibody screen and pregnancy test with ADAMTS 13 activity should be performed at presentation to differentiate the etiology. Corticosteroid is helpful to decrease the production of ADAMTS13 inhibitor or autoantibodies in TTP without evidence of infection. Rituximab or splenectomy is considered to treat refractory or relapsed TTP. Evidence for harm from platelet transfusion is uncertain in TTP.

The clinical utility of ADAMTS13 activity in TTP has been extensively studied: response rate, remission rate, and TTP-associated mortality appear better for patients with severe ADAMTS13 deficiency and the presence of severe ADAMTS13 deficiency or inhibitor after remission may predict relapse. Preclinical or clinical studies with recombinant ADAMTS13 or novel agents that target the A1 domain of von Willebrand factor (VWF) in TTP are underway.

HUS is characterized by TMA with acute renal failure primarily to describe the condition in children. There are two forms of HUS: diarrhea-associated HUS usually proceeded by bacterial hemorrhagic enterocolitis and atypical HUS which is not associated with a prodrome of diarrhea. In HUS, ADAMTS13 level is not severely deficient. Organ damage is limited to the kidneys. Therefore in HUS, supportive care with or without hemodialysis is the mainstay of treatment. Based on its pathogenesis in which overactivation of complement system due to deficiency of control protein, factor H, eculizumab (monoclonal antibody to C5) has been recently used in the management of atypical HUS.

**Key words:** Thrombotic microangiopathy (TMA), Thrombotic thrombocytopenic purpura (TTP), Hemolytic Uremic syndrome (HUS), Treatment

**Conflict of interest** No

**Sunday 28 October**  
**BMTSAA Symposium 1**

**0830-1000**  
**Room 213**

## **What is the Best Manipulation of the Graft for Haploidentical Stem Cell Transplant: Feasibility of CD3/CD19 Depletion**

Francoise Mechinaud

*Haemopoietic Stem Cell Transplant Programme, Children's Cancer Centre, The Royal Children's Hospital, Melbourne, Australia*

### **Aim**

The availability of a suitable HLA-matched donor is one of the major limitations to the widespread application of allogeneic hematopoietic stem cell transplantation (HSCT). The successful strategy of haploidentical allogeneic hematopoietic stem cell transplantation (haplo-HSCT) would eliminate the "lack of donor" problem. Therefore attempts to overcome the HLA-barrier are focused on strategies for effective graft; in-vitro, and host; in-vivo T-cell depletion. Graft T-cell depletion compromises the donor effect and is associated with significant increase in failure to engraft, infection and relapse. With these caveats in mind, our Programme provides haplo-HSCT utilizing donor CD3/CD19 depleted grafts, which not only contains CD34 positive stem cells, but also CD34 negative progenitors, natural killer (NK), dendritic and other graft-facilitating cells.

### **Results**

To date, our Programme has treated two patients who lacked HLA -matched donors. They were transplanted with haploidentical related donor products following CD3/CD19 depletion with anti-CD3 and anti-CD19 coated microbeads on a CliniMACS device. Both patients achieved engraftment at day+10 and day+12 respectively, without clinical symptoms of acute graft versus host disease. The final product characterisation, and early transplant outcome will be discussed.

### **Conclusion**

The benefits and limitations of this T-cell depletion system will be discussed in the context of other available options: CD34 positive selection and unmanipulated haploidentical graft with host in-vivo T-cell depletion.

**Keywords**     Haploidentical Transplant T-cell Depletion

**Conflict of interest**     NO

Sunday 28 October  
 BMTSAA Symposium 1

0830-1000  
 Room 213

## Strategies Towards Improving Cord Blood Transplant Outcomes

Ngairé Elwood<sup>1,2</sup>, Pei Tian<sup>2</sup>, Karin Tiedemann<sup>1</sup>

<sup>1</sup>BMDI Cord Blood Bank, The Royal Children's Hospital; <sup>2</sup>Cord Blood Stem Cell Research Group, Murdoch Children's Research Institute, Parkville, Victoria

### Aim

More than 25,000 unrelated donor cord blood (CB) transplants have now been performed; however, many patients who undergo a CB transplant experience a delay in haematological recovery. To date, the most important factor in predicting a positive outcome for a CB transplant is that the total nucleated cell (TNC) dose, or CD34<sup>+</sup> cell dose, in relation to the recipient's weight be  $> 3 \times 10^7$  / kg or  $> 2.0 \times 10^5$  / kg, respectively. However, even with ample numbers of cells transfused, many patients die following CB transplantation, usually of late opportunistic infection related to delayed immune recovery, or relapse of the underlying disease. There are now >500,000 CB units (CBU) stored in >130 public CB banks for unrelated use. This expanding pool of available CBU provides an increasing donor choice for many patients. Following selection of a CBU based on, firstly HLA-antigen match, and then secondly, on the available TNC number, there are now often several suitable CBU available for a particular patient. To date, no other parameter or characteristic of the CBU is available that will reliably predict which CBU will engraft quickly and provide rapid recovery. The aim of our study was to determine if telomere length of cord blood stem cells is a useful indicator of engraftment potential post-transplant.

### Results

A quantitative real-time PCR based method was used to measure telomere length in CBU released for transplant. Outcome data was available for 89 CBU used for single CB transplants for the treatment of malignant and non-malignant disorders. Patients receiving CB with short telomeres had significantly delayed engraftment compared to those receiving CB with long telomeres; median time to neutrophil recovery of 27d vs 21d ( $p<0.01$ ) and platelet recovery of 67d vs 43d ( $p<0.05$ ). Survival in those receiving CB with short telomeres was significantly reduced compared to those with long telomeres (54% vs 91% at 12 months) ( $p<0.05$ ).

### Conclusion

Screening and selection of CB units based on TL, in addition to those parameters already utilized, may significantly improve the likelihood of successful CB transplant outcome.

**Keywords** cord blood, transplant, telomeres

**Conflict of interest** No

**Sunday 28 October**  
**BMTSAA Symposium 1**

**0830-1000**  
**Room 213**

## **Introducing Automation to Live Cellular Processes**

Ian Fitzpatrick  
*Invetech Melbourne Australia*

### **Aim**

The rationale and practical methods for translating live cell processes from laboratory operations to robust systems will be described.

### **Results**

The benefits of process formalisation range from quality, labour demand through to cost. Benefits determined from example processes will be presented.

### **Conclusion**

Complex laboratory procedures can be translated into robust processes reducing dependence on skilled labour and cost.

**Sunday 28 October**  
**HSANZ Symposium 2: Focus on Allografting in Leukaemia**

**1100-1200**  
**Plenary 2 (Auditorium)**

## **Indications, Selection and Timing of Transplantation in ALL**

Nicola Goekbuget  
*Goethe University Hospital, Frankfurt, Germany*

Abstract not available at time of printing



**Sunday 28 October**  
**HSANZ Symposium 2: Focus on Allografting in Leukaemia**

**1100-1200**  
**Plenary 2 (Auditorium)**

## **Indications, Selection and Timing of Transplantation in AML**

Charles Craddock

*Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, UK*

Despite advances in chemotherapy and supportive care the majority of adults with AML remain destined to die of resistant or relapsed disease. Whilst allogeneic stem cell transplantation has the capacity to deliver maximal anti-leukaemic activity its curative potential has been restricted, until recently, to a limited population of younger patients in whom a suitable donor can be identified. In the last decade the advent of reduced intensity conditioning regimens and the increased availability of suitably matched unrelated or cord blood donors has resulted in allogeneic stem cell transplantation becoming an important consideration in the majority of adults with AML. As a consequence a rigorous assessment of relapse risk if patients are treated with chemotherapy alone is now mandatory in all newly diagnosed patients with AML so that candidates for allogeneic transplantation can be identified as quickly as possible. At present molecular and cytogenetic stratification coupled with morphologic and immunophenotypic assessment of response to induction chemotherapy coupled and patient age prove effective tools in risk stratification. In the future quantitation of leukaemic stem/progenitor cells, assessment of the depth of molecular response to chemotherapy and genome sequencing strategies are likely to improve the identification of patients whose relapse risk with standard chemotherapy is high enough to justify allogeneic transplantation. Importantly between 15 and 40% of patients fail to achieve a CR and there is increasing evidence that allogeneic transplantation, using either a myeloablative or reduced intensity conditioning regimen, has curative potential in primary refractory AML emphasising the importance of early detection of transplant candidates so that donor identification can be expedited.

The other major factors determining the optimal deployment of allogeneic transplantation in AML relate to factors determining transplant outcome in particular donor selection and conditioning regimen. The increased availability of adult unrelated donors coupled with the growth of high quality cord blood banks has substantially increased the availability of stem cell donors for patients who do not have an available matched sibling. The choice of both adult unrelated and cord blood donors is now informed by the results of retrospective analyses of the impact of stem cell source, allelic disparity and donor factors such as sex, age and parity on outcome. Furthermore there is now compelling evidence that post-transplant immunosuppression is an important determinant of relapse risk and graft-versus-host disease and this coupled with choice of conditioning regimen and stem cell source are critical determinants of long term survival. The benefits of implementing such an integrated patient specific approach to allogeneic transplantation in optimising outcome in patients with AML will be discussed.

**Keywords** Risk stratification; donor selection; alternative donors; conditioning regimen

**Conflict of interest** No

**Sunday 28 October**  
**ANZSBT Symposium 2: Transfusion Safety Today and the Future**

**1100-1130**  
**Rooms 219/220**

## **How Safe is Safe Enough?**

Cees van der Poel  
*The Netherlands*

Abstract not available at time of going to print

Sunday 28 October  
 ANZSBT Symposium 2: Transfusion Safety Today and the Future

1130-1200  
 Rooms 219/220

## What Have We Learned From Haemovigilance?

*TRIP Dutch National Hemovigilance and Biovigilance Office, The Netherlands*

### Aim

- To review results from implementing a national haemovigilance reporting system, based on the Dutch experience
- To note lessons concerning the most frequently reported transfusion hazards
- To recognise strengths and limitations of national haemovigilance data

### Introduction

The national hemovigilance office in The Netherlands commenced data collection in 2003.

### Methods

The hemovigilance office is run by a foundation governed by representatives of professional societies. It captures reports all types of transfusion reaction, both serious and non-serious, and incidents in the transfusion chain. Expert review is in place for all serious reports. For serious transfusion reactions and errors reporting to the healthcare inspectorate is mandatory under EU legislation; this can be effected using the TRIP a digital reporting system.

### Results

Participation by hospitals has run at over 95% since 2006. The annual number of reports has shown a slight continued rise since then to 2601 in 2011, for an overall rate of 3.9/1000 blood components distributed. The largest categories are those of new allo-antibody formation, febrile non-hemolytic transfusion reactions and minor allergic reactions. The annual rate of serious reactions is approximately 0.15 per 1000 components distributed. A drop in TRALI reports was observed following implementation of male-only plasma. The most frequent serious reactions in 2011 were other reaction (reactions not meeting criteria for recognised reaction categories), anaphylactic reaction and transfusion-associated circulatory overload. The overall rate of reported incorrect blood component transfused was 0.07/1000 components. This has dropped from 0.09/1000 in 2009.

### Discussion and conclusion

Collecting information about transfusion reactions and errors in the transfusion chain has highlighted the risks associated with blood transfusion. Hemovigilance data capture is subject to variable and under-reporting. Only some adverse reactions can be prevented.

**Keywords** reporting system, transfusion error, adverse reaction

**Conflict of interest** None

**Sunday 28 October**  
**Platelet Workshop 6: Rheology and Platelet Activation**

**1130-1200**  
**Room 210**

**Australian Haemodynamics During Thrombosis – Impact on Platelet Reactivity and Thrombus Growth**

Warwick Nesbitt

Abstract not available at time of going to print

**Sunday 28 October**  
**Platelet Workshop 6: Rheology and Platelet Activation**

**1130-1200**  
**Room 210**

**Platelet Activation During Coronary Passage**

Len Kritharides

Abstract not available at time of going to print

**Sunday 28 October**  
**Platelet Workshop 6: Rheology and Platelet Activation**

**1130-1200**  
**Room 210**

**cAMP Exerts Its Inhibitory Effects of GPIIb/IX/V by Disengaging the Complex from Lipid Rafts**

Adam Munday

Abstract not available at time of going to print

Sunday 28 October  
APSTH Education 2: Microparticles and Their Messages

1100-1200  
Room 211

## **Microparticles in Thrombosis and Inflammation: Pathogenic? Diagnostic? Therapeutic**

Peter Gross

*Thrombosis & Atherosclerosis Research Institute, Hamilton, Canada*

### **Aim**

The roles of microparticles derived from platelets and leukocytes will be reviewed.

Microparticles circulate in the blood of healthy people and levels and can be altered in diseases. Some microparticles, but not all, are enriched in exposed phosphatidylserine, and some circulating microparticles contain tissue factor. Most circulating microparticles are of platelet origin. Although microparticles are formed when platelets are activated there is no evidence that the levels of circulating platelets are altered in thrombosis, rather the evidence suggests that inflammatory states increase the levels of megakaryocyte microparticles.

Microparticles formed from neutrophils and monocytes also circulate. They can be increased in disease states and have been demonstrated to promote a variety of pathological processes including thrombosis.

Microparticles derived from cancer cells also circulate and in a limited number of cancers have been associated with progression and with venous thrombotic disease.

**Keywords** microparticles, platelets, cancer

**Conflict of interest** No

Sunday 28 October  
 APSTH Education 2: Microparticles and Their Messages

1100-1200  
 Room 211

## **The Nature and Origin of Circulating CD36 in Type 2 Diabetes: A Role for Microparticles**

Lisa Lincz

*Hunter Haematology Research Group, Calvary Mater Newcastle, Waratah, NSW Australia*

CD36 is an integral membrane glycoprotein expressed on the surface of a variety of cells; including platelets, monocytes, red blood cells and endothelial cells. It is involved in the uptake of long chain fatty acids and oxidized lipoproteins and its altered expression has been associated with atherosclerosis, platelet aggregation, and insulin resistance. A circulating form of CD36 in human plasma was recently shown to constitute a novel biomarker for Type 2 Diabetes Mellitus (T2DM). Originally thought to be a cleaved cell free variant of the receptor, we have shown, based on our prior studies of CD36 structure, that CD36 in plasma is entirely associated with circulating microparticles (MPs). These small membrane-bound vesicles are shed from activated or damaged cells and have the capacity to transfer bioactive proteins and nucleic acids, including microRNA, to distant tissues within the body. Thus rather than being a simple biomarker, CD36 may in fact be the *mediator* of transcellular exchange responsible for many of the complications of diabetes. To further explore this concept, we have used flow cytometry to determine the levels and cellular sources of these CD36+MPs in plasma samples from 33 obese diabetics (BMI=39.9±6.4, age 57±9 years, 18M:15F) and age and gender matched lean and obese healthy controls (BMI=23.6±1.8 and 33.5±5.9, respectively). Our results confirmed that CD36 bearing MPs are significantly elevated in T2DM, and are positively correlated with levels of plasma CD36 protein concentration measured by Enzyme Linked Immunosorbent Assay. Overall levels of circulating CD36+MPs were relatively high, corresponding to approximately 50% that of platelet derived MPs. Surprisingly, the primary source of CD36+MPs in obese diabetics was from erythrocytes (35.8 ± 14.6%), as compared to endothelial cells in non-diabetics (40.9 ± 8.3% and 33.9 ± 8.3% for lean and obese controls, respectively). Thus our results have shown that people with T2DM have altered levels and a unique cellular profile of CD36+MPs, suggesting that these specific vesicles may provide a novel axis contributing to the pathophysiology of T2DM and its complications.

**Keywords** CD36, Type 2 Diabetes Mellitus, microparticles

**Conflict of interest** No conflict of interest to disclose



**Sunday 28 October**  
**Nurses Symposium 1: Focus on Allografting in Paediatrics**

**1100-1200**  
**Room 212**

## **Current Trends in Paediatric Transplants**

Peter J Shaw

*BMT Service, The Children's Hospital at Westmead, Sydney, NSW, Australia*

Although done in smaller numbers, trends in paediatric BMT often precede the same trend emerging in adult BMT. Topical issues include:

Two thirds of transplants use alternate donors. For many years, paediatric indications for BMT are absolute; if no matched sibling is available, an unrelated or mismatch related donor is used, hence the ratio of alternate donors. This trend has significant impact on resources and is now being seen in more adult centres.

GvHD is bad for you. Part of the drive behind adoption of PBSC was the perception that cGvHD and GvT or GvL would improve outcome. This trend is now reversing and bone marrow harvesting, which was always maintained in paediatric practice, is coming back. Because of the large numbers of BMTs done for non-malignant disease in paediatrics, cGvHD has always been seen as more foe than friend, and so strategies to reduce GvHD, such as T cell depletion, remain more prevalent in our practice.

Patients come to BMT very immunosuppressed. The intensity of pre-BMT therapy continues to increase for certain diagnoses, and this can be associated with a broad range of opportunistic infections, such as EBV-PTLD and Adenovirus infection. As adult therapies also escalate, these infections are being seen in both the transplant and non-transplant populations.

BMT is a Platform for T Cell Therapy. Because of the prevalence of opportunistic infections, particularly viral infections, in our patients, they have been early candidates for adoptive immunotherapy, such as CTLs against CMV, EBV and ADV.

BMT is a Platform for Gene Therapy. It is no coincidence that some of the first diseases treated by allogeneic BMT are also diseases where gene therapy is being tried. Such patients often require conditioning so safe conditioning for an allograft will be safe conditioning for a gene modified "autograft".

Follow up is for life. The concept of 2 year, even 5y survival is outmoded. As our patients survive longer, survivorship issues of quality of survival and long term consequences spill over into adult practice as we transition our survivors and they join the increasing number of adults who have survived BMT and whose long term health is our final goal.

**Keywords** BMT, allogeneic transplant, GvHD

**Conflict of interest** No

Sunday 28 October

Nurses Symposium 1: Focus on Allografting in Paediatrics

**Long Term Follow Up in Paediatric BMT**

1100-1200

Room 212

Ida O'Brien

*BMT Service, The Children's Hospital at Westmead, Sydney.*

Given the early and high rate of success of paediatric BMT, it is inevitable that long-term follow up (LTFU) is well advanced in paediatric practise.

Severe aplastic anaemia is the leading non-malignant indication for BMT, and has been treated successfully for decades. There are minimal consequences of therapy when just CPM is used, problems are more a result of chronic GvHD. Issues remain of psychological adjustment and insurability. In contrast, the leading diagnosis for allogeneic BMT in children is ALL. These patients are intensively treated; almost all receive either cranial and/or total body irradiation (TBI). TBI has major long terms consequences, on growth, hypothyroidism, cataracts, and thyroid cancer. More recent studies have highlighted metabolic syndrome, diabetes mellitus, dyslipidaemia, second malignant neoplasms including breast cancer, hypertension and cardiovascular disease as long term effects. The toxicity profile is much less in patients who have not had TBI and, although the consequences of cGvHD and its treatment are the same in children, its lower incidence means it is less of a problem. Our service has evolved to encompass the following elements:

- Initial engagement at 6 months post-BMT, to align with adult practise, supervise early and successful **re-immunisation** and, importantly in paediatrics, advocate for successful (re) **integration into the school** environment.
- Comprehensive BMT-directed follow up starting at 2 years is directed initially towards **growth** issues and to monitor progress through **puberty**.
- Maintaining contact with families to ensure they contact us for assistance with **school integration, learning issues, psychological and social support** in the early years post BMT leads naturally into such support for the adolescent years, HSC support and then **vocational guidance**.
- From late adolescence, we play a vital role in transferring the responsibility taken by the parent to ensure the now young adult is aware of their past diagnosis, the treatment and its consequences.
- Knowledge of those consequences enables to educate the young adult on **healthy life-style**, recreational activity (smoking, alcohol and drugs) **fertility options** and the importance of long term health monitoring, now being **Life long**, not just Long term.
- **Transition** of the patient into the adult health care system.

**Keywords** BMT, Late Effects, Long term follow up

**Conflict of interest** No

Sunday 28 October  
 ASTH: Barry Firkin Oration

1200-1300  
 Plenary 2 (Auditorium)

## Novel Platelet Functions Beyond Hemostasis

Yukio Ozaki

*Department of Laboratory Medicine, Faculty of Medicine, University of Yamanashi, Chuo, Yamanashi, Japan*

Platelets play a number of important roles in thrombosis and hemostasis, and its functions have long been considered to be related to these realms. However, recent findings suggest novel functions of platelets beyond the conventional concept of this tiny cell: platelets are required for liver regeneration. Platelets are important in defence against various pathogens. Platelets are related to tumor growth and metastasis. We recently identified a platelet-specific protein, CLEC-2 which plays a critical role in vein-lymphatic vessel separation.

Rhodocytin, purified from snake toxins of *Calloselasma rhodostoma* was found to activate platelets with a pattern similar to that of collagen. Through a series of studies, we found that rhodocytin binds an as-yet unidentified protein leading to platelet activation. We used rhodocytin affinity chromatography and TOF-MASS spectrometry and identified a novel class of platelet activation receptor, c-type lectin-like receptor 2 (CLEC-2), which belongs to c-type lectin superfamily. Although its physiological ligand had not been identified, CLEC-2 attracted attention of researchers as a novel target of anti-platelet drugs because of its ability to stimulate powerful platelet aggregation and its specific expression in platelets and megakaryocytes. We subsequently revealed that the physiological ligand of CLEC-2 is podoplanin, which is a sialoglycoprotein present in renal podocytes and lymphatic endothelial cells. It is also present on the surface of certain tumor cells and is involved in tumor cell-induced platelet aggregation and tumor metastasis. Using antibodies against podoplanin or CLEC-2, we demonstrated that platelets serve to facilitate tumor metastasis by way of CLEC-2 and podoplanin interactions.

In order to better understand the role of CLEC-2, we produced CLEC-2 knockout mice, which were lethal at the fetal stage. It had edema, lymphatic vessel dilatation, and the presence of blood cells in lymphatic vessels. Thus, CLEC-2 knockout mice have the phenotype of blood vessel-lymphatic vessel mal-separation. We found that growth, migration, and tube formation of lymphatic endothelial cells is inhibited by releasates from platelets activated by the interaction between CLEC-2 on the platelet membrane and podoplanin on lymphatic endothelial cells interactions, and recently found that BMP-9 which belongs to the family of TGF-, is the major factor in platelet releasates which is responsible for blood vessel-lymphatic vessel separation.

**Keywords** platelets, metastasis, lymphatics

**Conflict of interest** No

**Sunday 28 October****1400-1530****HSANZ/ANZSBT/APSTH Workshop 7: Goldilocks and the 3 Platelets Plenary 2 (Auditorium)**

## **Platelets in MPD Clinical Syndromes and Management**

Claire Harrison

*Guy's and St Thomas' Hospitals, London, UK*

A raised platelet count, bleeding or thrombosis are often the first manifestation of a myeloproliferative disorder (MPD) triggering an investigation and indeed all of these issues may also guide management decisions for these patients. A raised platelet count may also be a manifestation of other clinical states such as myelodysplasia or iron deficiency. How can we best reach a more certain diagnosis of MPD? In this session we will also discuss the role of platelets in the pathogenesis of clinical complications of the MPDs – innocent bystander or smoking gun ? In particular for essential thrombocythaemia our management has been driven by the height of the platelet count.

In collaboration with colleagues in Australia, New Zealand, Ireland and France since 1997 we have been accruing patients into the Primary thrombocythaemia -1 trial. With well over 1000 patients entered this trial is now yielding important insights into this disease from pathogenesis, diagnostics, impacts of newer molecular markers, prognostic factors and treatment decisions. I will share the latest information from this trial and data from other authors in this session.

**Keywords** Platelet, MPD, Thrombosis

**Conflict of interest** Received speaker fees from Novartis, Shire, Cellgene, Sanofi Avensis; consultancy work for YM Bioscience, S\*Bio, Sanofi Avensis and research funding from Shire and Novartis.

Sunday 28 October

1400-1530

HSANZ/ANZSBT/APSTH Workshop 7: Goldilocks and the 3 Platelets Plenary 2 (Auditorium)

## Platelet Function Defects

Paul Harrison

*Oxford Haemophilia & Thrombosis Centre, Churchill Hospital, Oxford University Hospitals NHS Trust, Oxford, UK*

Inherited platelet defects are a heterogeneous group of rare bleeding disorders presenting with a classical mucocutaneous bleeding pattern. They may be difficult to diagnose and manage (and are likely to be under-diagnosed). They are generally characterised by a range of molecular defects affecting either platelet function and/or number. Most defects result in deficiencies and/or dysfunction within platelet receptors (e.g. GPIb-IX-V, Bernard-Soulier syndrome and integrin  $\alpha\text{IIb}\beta_3$ , Glanzmann thrombasthenia), defects in receptors for agonists (e.g. P2Y<sub>12</sub>, collagen and thromboxane receptors), various signalling pathways, cytoskeletal proteins, alpha and dense granule contents and procoagulant activity. With Chediak-Higashi, Hermansky-Pudlak, Wiskott-Aldrich and Scott syndromes the molecular defect also affects other cells. There is a failure of platelet production in Familial thrombocytopenia that sometimes results in macrothrombocytopenia (e.g. MYH9-related diseases). Diseases of platelet production can also interfere with the development and function of major organs. Acquired platelet defects are most commonly caused by the administration of various anti-platelet drugs e.g. aspirin/NSAID's. Numerical and/or functional platelet disorders are also prevalent amongst patients with abnormal bleeding and may be clinically indistinguishable from other haemostatic disorders, particularly von Willebrand disease (VWD). An evaluation of patients with abnormal bleeding symptoms requires objective clinical assessment of bleeding history, a physical examination and if appropriate a suitable panel of platelet function investigations. Laboratory testing can identify a low platelet count, abnormal platelet size and morphology, the loss or abnormal functioning of receptors, downstream signalling pathways, storage organelles, or enzymatic activities essential for platelet adhesion, activation, aggregation and procoagulant activity. A full diagnosis is only complete when the genetic mutation(s) have been defined for each patient although the molecular basis of some defects remains to be fully characterised. Platelet disorders can also sometimes co-exist with other coagulation factor defects or VWD. Laboratory investigations of platelet number and function are therefore recommended in any patient where bleeding symptoms are not fully explained by standard clinical laboratory investigations.

**Keywords** Blood Platelet Disorders/diagnosis, Platelet Function

**Conflict of interest** Consultant for Sysmex UK, Research Grants from Eli Lilly and Siemens Diagnostics. Wife is an employee of IL-UK.

**Sunday 28 October****1400-1530****HSANZ/ANZSBT/APSTH Workshop 7: Goldilocks and the 3 Platelets Plenary 2 (Auditorium)****Platelet Support: Best Practice in Malignant Haematology**

Terry B Gernsheimer

*Puget Sound Blood Center, University of Washington School of Medicine, Seattle, Washington, USA*

Platelets, along with reactive vasoconstriction, are the first response to bleeding and the need to maintain haemostasis. Without adequate numbers of platelets to preserve vascular endothelial integrity spontaneous bleeding occurs, and when tissue is damaged, bleeding occurs more readily and may be uncontrollable. Since storage of platelets for transfusion became possible in the 1960's investigators have sought the optimum strategy to prevent bleeding in thrombocytopenic patients with haematologic malignancy.

The two main variables in the approach to platelet transfusion have been the "transfusion trigger" or "threshold" at which platelets should be administered, and the appropriate dose of platelets necessary to maintain vascular integrity while minimizing exposure. More recently, whether platelet transfusions could be postponed or avoided until signs of bleeding occur has been explored as a potential management option.

This session will review the most recent data on the transfusion management of the patient with haematologic malignancy and thrombocytopenia. Alternative strategies, including the use of antifibrinolytic agents and thrombopoietic receptor agonists, will be discussed.

**Keywords** Platelet transfusion, thrombocytopenia, platelet alloimmunization**Conflict of interest** No

Sunday 28 October  
 Nurses Symposium 2: Focus on Adolescents and Young Adults (AYAs)

1400-1530  
 Room 212

## **Developmentally Appropriate Psychosocial Screening for Adolescent and Young Adult Patients**

K Thompson

*ONTrac at Peter MacCallum Cancer Centre, Victorian Adolescent & Young Adult Cancer Service, Melbourne, Vic, Australia*

There are approximately 280 new diagnoses of cancer in adolescent and young adult (AYA) patients aged 15-25 each year in Victoria. A cancer diagnosis and its treatment have significant physical and psychosocial impacts in young people which are unique from those observed in children and older adults. This is related specifically to the unique development that young people face during these years.

For this reason, age-based psychosocial screening and assessment is crucial to best practice psychosocial care for young people. However, to date, a paucity of appropriate tools has resulted in the perception that paediatric or adult measures are good enough. This is in direct contrast to the recognised principles of best practice adolescent medicine and care.

In 2011 a psychosocial screening tool was developed through Australian collaboration based on four essential elements of AYA care including developmentally appropriate communication, confidentiality, engagement and a family-systems approach. Several currently available measures were used as a foundation for the development of this tool including the HEADSS assessment [1] and the distress thermometer, with specific revisions made for relevance to AYA oncology. This tool was piloted by ONTrac at Peter Mac Victorian Adolescent and Young Adult Cancer Service in 2011 as the first step in implementing a standard screen for young people with cancer in Victoria.

This paper will discuss the rationale behind developmentally appropriate psychosocial screening including the foundations of adolescent development and the impact of development on adjustment to a cancer diagnosis. Principles and benefits of screening young people in a developmentally appropriate manner will also be discussed in relation to the pilot undertaken by ONTrac at Peter Mac.

### **References**

- [1] Goldenring, J.M and Cohen, E. (1988). Getting into adolescents' heads. *Contemporary Pediatrics*; July: 75-90.

**Keywords** Cancer, young people, psychosocial screening

**Conflict of interest** No

**Sunday 28 October****Nurses Symposium 2: Focus on Adolescents and Young Adults (AYAs)****1400-1530****Room 212**

## **The Rural and Remote AYA Experience**

Allan Hayward

*Youth Cancer Service SA/NT*

Adolescence and young adulthood are certainly challenging times for us all as we negotiate the necessary developmental tasks of this age, such as our increasing independence and autonomy, completing school, starting work, going to university and so on. Add to this the challenge of cancer and on top of that, the need to have treatment that takes you far from home.

The Youth Cancer Service SA/NT provides a state and territory wide service for young people 15-25yo with cancer. Providing services over such vast geography certainly has its challenges and requires good communication between health care providers and patients alike.

Individual case studies will be used to illustrate some of the challenges faced by rural and remote AYA's with cancer.

**Keywords**    AYA, rural, remote**Conflict of interest**    No



**Sunday 28 October****Nurses Symposium 2: Focus on Adolescents and Young Adults (AYAs)****1500-1530****Room 212****AYA Lived Experience of Cancer Diagnosis and Treatment****Kylie Lewis***Cancer Survivor, Co-Chair of the Victorian and Tasmanian Youth Cancer Advisory Board, Melbourne, Vic, Australia*

At age 19, I was diagnosed with a Ewings Sarcoma. Not knowing whether the cancer had spread, I underwent 10 months of chemotherapy in an adult ward as well as having prior and post surgery. My chemotherapy treatment meant that I was no longer able to attend my university degree, my friends were no longer able to relate to my new situation, my parents hovered over me like buzzing helicopters and then, of course, my hair fell out. Things then changed again when a year later my brother was diagnosed.

There are numerous issues that face adolescent and young adults when they are diagnosed with cancer. Some issues are particular to an individual while many similarities occur. Through discussing my own experiences as a patient, bereaved sibling, carer and my experiences on the board, I hope to make evident some of the common issues facing adolescent and young adults living with cancer from their perspective.

**Sunday 28 October**  
**BMTSAA Symposium 2**

**1400-1530**  
**Room 213**

## **The Facts about FACT**

Nancy Messino

*Haemopoietic Stem Cell Transplant Programme, Children's Cancer Centre, The Royal Children's Hospital, Melbourne, Vic, Australia*

### **Aim**

In accordance with best practice and international standards, the Haemopoietic Stem Cell Transplant (HSCT) Programme of the Children's Cancer Centre (CCC) at the Royal Children's Hospital (RCH), Melbourne, Australia, decided to obtain FACT Accreditation. Aims of accreditation include;

- A Quality Management Plan with functional robust quality systems providing a framework for clinical, collection and laboratory services provided by the HSCT Programme.
- Compliance with both international and legislated national benchmarks.

### **Results**

While in general the RCH participates in the Australian Council of HealthCare Standards accreditation system to ensure quality of clinical care, the CCC made the strategic decision that a higher level of quality management was required to support the HSCT Programme. In particular, it was paramount that the HSCT Programme obtain FACT accreditation and international recognition in the field of cellular therapy, to ensure access to clinical trials, involving cellular therapies, supported by the Children's Oncology Group (COG). Compliance with FACT standards encompass close links with external facilities and the following transplantation services within RCH ;

- Clinical Programme [Inpatient and Outpatient Services]
- Collection Facilities [Day Medical Unit (DMU) for apheresis collections and Theatre for bone marrow harvesting]
- Processing Facility [Cell Therapy and Flow Cytometry (CT&F) Laboratory] and
- Multi-disciplinary team liaison.

### **Conclusion**

In March 2012, the HSCT Programme attained FACT Accreditation. The introduction and implementation of the HSCT Programme's quality system has provided a model and impetus to introduce a higher level of quality management to support all clinical services in the Children's Cancer Centre.

**Keywords**     Accreditation FACT Regulation

**Conflict of interest**     No

**Sunday 28 October**  
**BMTSAA Symposium 2**

**1400-1530**  
**Room 213**

## **Clinical Indications for Stem Cell Transplantation**

Trish Walker

*Malignant Haematology and Stem Cell Transplantation Service, The Alfred,  
Melbourne, Australia*

For many patients with blood and bone marrow disorders, a stem cell transplant will form an important part of the management plan.

Autologous stem cell transplantation relies on high dose therapy (for example chemotherapy and radiotherapy) to exert an antitumor effect with the aim of the stem cell infusion being to facilitate haemopoietic recovery. Allogeneic stem cell transplantation on the other hand often utilises a modified immune system for more long lasting immunological control of malignancy/disease processes. Important complications include graft failure, organ dysfunction, infections, graft versus host disease and relapse.

This session will discuss the current Australian and worldwide trends in autologous and allogeneic stem cell transplantation with a focus on clinical indications for transplantation. Important considerations include recipient disease (including response to prior treatment and risk stratification), age, co morbidity as well as donor availability and characteristics. Advances in medical management include reduced intensity conditioning (including very reduced intensity conditioning approaches and outpatient autologous and allogeneic stem cell transplants), monitoring of chimerism, and predictors of disease relapse (for example CD34 chimerism).

**Keywords** stem cell, transplantation, clinical indications

**Conflict of interest** No

Sunday 28 October

1600-1730

**HSANZ/Nurses Combined Symposium 4: The Challenges of Palliative Care in Haematology**  
**Plenary 2 (Auditorium)**

## **The Burden of Symptoms of Patients with Haematological Malignancy**

Jennifer Philip<sup>1</sup>, Vanessa Manitta<sup>1,2</sup>, Merrole Cole-Sinclair<sup>2</sup>, Harshal Nandurkar<sup>2</sup>

<sup>1</sup>*Palliative Care Service, St Vincent's Hospital, and Centre for Palliative Care, Collaborative Centre of University of Melbourne, Vic, Australia*

<sup>2</sup>*Haematology Service, St Vincent's Hospital, Melbourne, Vic, Australia*

Patients with haematological malignancy access palliative care less frequently (11% vs 89%), and for those who do, with a shorter interval between palliative care referral to death (0.6 vs 2.0 months) compared to those with solid tumours. There are likely to be multiple reasons for this, including the unpredictable nature of treatment responses, the lack of clear goals of care, and the potential for fatal treatment toxicity. In addition the burden of symptoms for patients with haematological cancer is not well understood, and may be a factor in low levels of engagement with palliative care.

### **Aim**

We sought to document the symptoms and levels of distress in consecutive patients with haematological malignancy using the Memorial Symptom Assessment Scale-Short Form (MSAS-SF).

### **Results**

180/190 (RR 95%) inpatients and outpatients completed the MSAS-SF including 37% with a diagnosis of lymphoma, 16% myeloma, 15% chronic and 11% acute leukaemia, 13% bone marrow failure and 9% with myeloproliferative disease. Most (72%) had performance status of ECOG 0 or 1, and 76% were deemed to have 'active' disease, with the remainder in remission. The most common symptoms, reported by 40-50% of patients, were lack of energy, feeling worried, difficulty sleeping, drowsiness, dry mouth and feeling sad. Psychological symptoms were present at least 'occasionally' or 'frequently' in more than 75% of patients.

### **Conclusion**

Patients have very significant symptom burden, particularly those with poorer performance status, with new diagnosis, and relapsed or refractory disease. However, even those with stable disease or disease assessed as in remission, reported more than 7 symptoms (mean 7.7). When comparing with populations with metastatic cancer referred to palliative care services, though the patterns of symptoms differ, both groups have substantial symptom levels.

**Keywords** palliative care, symptoms, distress

**Conflict of interest** No

Sunday 28 October

1600-1730

HSANZ/Nurses Combined Symposium 4: The Challenges of Palliative Care in Haematology  
Plenary 2 (Auditorium)

## **Improving Access to Palliative Care for Haematology Patients**

Michael Collins

*Department of Pain and Palliative Care (DPPC) at Peter MacCallum Cancer Centre,  
Melbourne, Victoria, Australia*

### **Introduction/ Background**

Patients with haematological malignancies are less likely to access specialist palliative care services than cancer patients from other clinical streams. In 2007 most patients with haematological malignancies were referred from the inpatient setting mostly late in the disease trajectory.

### **Method**

The DPPC at Peter MacCallum developed a Palliative Care Rapid Response Team (PCRRT) based in ambulatory care to meet increasing demand for services outside of designated palliative care clinics. The team consisted of a nurse practitioner and palliative care registrar who were able to access as needed support from a palliative care physician.

### **Aims:**

- Improve access for haematology patients to specialist palliative care.
- Improve integration of care.
- To provide timely access to palliative care within ambulatory care settings.
- To improve follow up for patients and families by facilitating coordination and maximizing continuity of care for patients with complex needs.

### **Results**

Following the establishment of the PCRRT there was a 200% increase in haematology patients referred to palliative care from ambulatory care areas which assisted in the development of an integrated approach to care. Haematology referrals to palliative care at Peter MacCallum represented 16.5% of patient referrals compared with an average referral rate of 5.3% (Palliative Care Outcomes Collaboration, 2011). Patients were referred earlier in the disease trajectory and provided patients with the opportunity to discuss advanced care planning issues.

### **Conclusion**

The development of the PCRRT improved access and integration of care for haematology patients. Further evaluation needs to be completed to establish overall impact on care.

**Key Words:** Haematological malignancies, Palliative Care, Referral and Consultation

**Conflict of interest:** No

**Sunday 28 October**

**1600-1730**

**HSANZ/Nurses Combined Symposium 4: The Challenges of Palliative Care in Haematology  
Plenary 2 (Auditorium)**

## **Impact on Carergivers**

Karen Syrjala

Abstract not available at time of going to print

**Sunday 28 October**  
**ANZSBT Symposium 3: Age of Red Cell - Blood Storage Lesion**

**1600-1730**  
**Rooms 219/220**

## **Stored Red Blood Cells: Old or Vintage?**

Christopher P Stowell  
*Massachusetts General Hospital & Harvard Medical School, USA*

### **Aim**

The aim of this lecture is to review the biochemical and structural changes that occur in RBC during storage under conventional blood bank conditions including some of the studies done in animal model systems to assess the functional impact of these storage-related changes.

### **Results**

RBC stored under conventional blood bank conditions undergo numerous biochemical change such as reduction in the levels of intra-erythrocytic 2,3-diphosphoglycerate, adenosine triphosphate and nitric oxide (and its adducts). These observed changes have generated physiologically plausible hypotheses about how they might affect the ability of the RBC to function once transfused. For example, a decrease in the 2,3-DPG level would decrease the  $P_{50}$ , and perhaps impair oxygen unloading. NO-depleted RBC might not be able to maintain the degree of vasodilatation required to permit adequate blood flow to the tissues. In addition, numerous changes in the structure and characteristics of the RBC plasma membrane have also been described, including the loss and oxidation of membrane lipids and proteins and the rearrangement of some membrane constituents. These changes are accompanied by the formation of microvesicles and a loss of membrane elasticity which could interfere with the movement of the RBC through the microcirculation. Evidence from animal model systems also suggests that stored RBC do not function as well as native RBC. These studies have pointed out the importance of blood viscosity and shear stress for maintaining microvascular blood flow, although differences in the biology of RBC from different species, notably in the pace of their “aging”, warrant some caution in extrapolating these data to the clinical setting.

### **Conclusion**

The biochemical and morphological changes which occur to RBC during storage have led to the formulation of several hypotheses about how they might affect their function, and some data from animal model systems support these hypotheses.

**Keywords** – RBC storage, biochemical changes, membrane changes

**Conflict of Interest** – none

**Sunday 28 October**  
**ANZSBT Symposium 3: Age of Red Cell - Blood Storage Lesion**

**1600-1730**  
**Rooms 219/220**

## **What Research is Telling Us About the Storage Lesion**

Rosemary Sparrow

*Research and Development, Australian Red Cross Blood Service, Melbourne, Victoria, Australia*

The current concerns about the 'age of blood' has encouraged renewed interest in laboratory-based research to better understand the biological effects of storage on red cell components and the potential implications of the red cell storage lesion on transfusion outcomes. Despite decades of research, it is clear that there is still much to learn about the basic biology of red cells together with the effects of manufacturing and storage. It is also clear that red cells are not just oxygen-delivery vehicles, but rather they contribute to blood dynamics through interaction and modulation of other blood elements.

This presentation will highlight some of the new approaches being used by researchers to better understand the red cell storage lesion and how these research findings may offer new insights into the potential in vivo responses of transfusion recipients.

**Keywords** red cells, storage lesion, transfusion

**Conflict of interest** No



Sunday 28 October  
 Platelet Workshop 8: Platelet Thrombosis and Inflammation

1600-1730  
 Room 210

## **Oxidative Stress and Endothelial Dysfunction in Cerebrovascular Disease**

Christopher G Sobey

*Department of Pharmacology, Monash University, Clayton, Victoria, Australia*

### **Aim**

Oxidative stress and vasomotor dysfunction in cerebral arteries may increase the risk of cognitive impairment or stroke. However, mechanisms underlying cerebrovascular abnormalities are poorly understood. We have tested whether augmented superoxide-dependent dysfunction occurs in the cerebral circulation in response to angiotensin II (Ang II), during hypercholesterolemia or following ischemia-reperfusion, and have evaluated the role of Nox2 oxidase.

### **Results**

Ang II: Ang II stimulated superoxide production and constriction in cerebral arteries of C57Bl6 (wild-type; WT) but not Nox2<sup>-/-</sup> mice. Nox2 immunofluorescence was found to be localised in adventitial and endothelial cells of cerebral arteries. The SOD mimetic, tempol, potentiated contractions to Ang II, whereas the SOD/catalase mimetic, EUK-134, virtually abolished contractions suggesting that Ang II-induced cerebral vasoconstriction is mediated by hydrogen peroxide generated from Nox2 oxidase-derived superoxide.

Hypercholesterolemia: Morphology of cerebral arteries was similar in WT and hypercholesterolemic ApoE<sup>-/-</sup> mice. In ApoE<sup>-/-</sup>, but not Nox2<sup>-/-</sup>/ApoE<sup>-/-</sup> mice, superoxide production by cerebral arteries was ~50% greater than in WT mice. Moreover, the magnitude of L-NAME-induced contractions of cerebral arteries from ApoE<sup>-/-</sup> mice was <50% of that in WT mice (P<0.05), whereas in Nox2<sup>-/-</sup>/ApoE<sup>-/-</sup> mice the contractile response was comparable to WT responses. However, in the presence of the superoxide scavenger, tempol (1 mM), L-NAME-induced contractions of MCA were similar between WT and ApoE<sup>-/-</sup> mice. Expression of p47phox was ~2-fold higher in ApoE<sup>-/-</sup> versus WT mice.

Ischemia-reperfusion: Analogous to findings in hypercholesterolemic mice. After ischemia and reperfusion, superoxide production was also markedly increased in the cerebral arteries of WT, but not Nox2<sup>-/-</sup> mice. In WT mice, L-NAME-induced constriction was reduced by ~50 % in ischemic arteries, whereas ischemia had no effect on responses to L-NAME in vessels from Nox2<sup>-/-</sup> mice.

### **Conclusion**

Thus, excess superoxide and impaired NO-mediated dilatation occurs in mouse cerebral arteries exposed to Ang II, hypercholesterolemia or ischemia-reperfusion, and appears to be exclusively due to increased activity of vascular Nox2 oxidase.

**Keywords** Superoxide, NADPH oxidase, stroke.

**Conflict of interest** No

**Sunday 28 October**  
**Platelet Workshop 8: Platelet Thrombosis and Inflammation**

**1600-1730**  
**Room 210**

## **Membrane “Strings” from Dying Platelets Promote Leukocyte Aggregation and Vascular Occlusion During Ischemia-reperfusion Injury**

Yuping Yuan, Zane Kaplan, Katrina Ashworth, Imala Alwis, Shaun Jackson  
*Australian Centre for Blood Diseases, Monash University, Alfred Medical and Research Education Precinct, Melbourne, Vic, Australia*

### **Aim**

Ischemia-reperfusion (I/R) injury to the intestines can result in remote organ injury to lung, leading to the acute respiratory distress syndrome (ARDS), a condition associated with considerable morbidity and mortality. Resident leukocytes in the pulmonary parenchyma are thought to play a major role in this process, although leukocytes in the mesenteric circulation may also be involved. In this study we have examined the possibility that the interaction between platelets and leukocytes in the ischemic mesenteric circulation may contribute to remote organ injury.

### **Results**

Utilising intravital microscopy we have identified a new leukocyte phenomenon, termed heterotypic leukocyte aggregation in mouse mesenteric circulations during I/R injury. Rolling leukocyte aggregates (up to 10 cells) develop in the venous circulation within the first 30-60 minutes of intestinal I/R injury. The extent of leukocyte aggregation in mesenteric vessels correlated with the degree of leukocyte aggregation in pulmonary vessels, resulting in vasculature obstruction in up to 20% of vessels. Confocal intravital microscopy revealed that aggregates contained both leukocytes and platelets with the majority of platelets expressing surface phosphatidylserine (PS+ve). Leukocyte aggregates were absent in platelet-depleted mice and in bone marrow transplanted chimeric mice deficient in platelet P-selectin, suggesting a critical role for platelets in this process. Analysis of platelet thrombi formed in vitro or in vivo, confirmed that PS+ve platelets were both necessary and sufficient for leukocyte aggregate formation. PS+ve platelets have unstable platelet membranes and under the influence of hemodynamic drag force of flowing blood extrude long filamentous membrane structures termed ‘strings’, that support leukocyte adhesion. These P-selectin positive strings, in combination with other platelet membrane fragments, bridge adjacent leukocytes and promote aggregation.

### **Conclusion**

These studies define a new mechanism of vascular occlusion that is triggered by PS+ve platelets and culminates in the formation of extensive heterotypic leukocyte aggregates. These aggregates have the potential to promote downstream vascular occlusion and remote organ injury following intestinal I/R injury.

**Keywords** Platelets, Leukocytes, ischemia-reperfusion

**Conflict of interest** No

**Sunday 28 October**  
**Platelet Workshop 8: Platelet Thrombosis and Inflammation**

**1600-1730**  
**Room 210**

## **Actions of Platelets in Promoting Inflammatory Leukocyte Trafficking in the Glomerulus**

Sapna Devi, Michael Kuligowski, Michael Hickey  
*Centre for Inflammatory Diseases, Monash University, Melbourne, Vic, Australia*

### **Aim**

The aim of this study was to examine the contribution of platelets to leukocyte recruitment and inflammation in the glomerulus in an animal model of glomerular inflammation.

### **Results**

We used intravital microscopy to examine the glomerular microcirculation in kidneys of mice undergoing glomerular inflammation, induced by an antibody against the glomerular basement membrane (anti-GBM Ab). Neutrophils recruited to the glomerulus were visualised by fluorescent dyes, whereas platelets were isolated from a donor mouse, labelled with CFSE, and transfused into mice undergoing intravital microscopy experiments. During the glomerular inflammatory response, neutrophil recruitment to the glomerulus was dependent on adhesion molecule P-selectin. Using a variety of approaches we demonstrated that glomerular P-selectin was derived from platelets recruited to the glomerulus rather than glomerular endothelial cells. We subsequently used a range of inhibitors and other approaches to identify the molecular basis of platelet recruitment to the glomerulus. Platelet recruitment was initiated within five minutes of administration of anti-GBM antibody. This was unaltered by inhibition of platelet GPIIb/IIIa, but was prevented by the absence of platelet GPVI. Fibrinogen was deposited in glomerular capillaries during the inflammatory response, and inhibition of  $\alpha$ IIb $\beta$ 3, fibrinogen and ICAM-1 inhibited platelet recruitment.

### **Conclusion**

Together these data indicate that platelet recruitment to the glomerulus in the anti-GBM antibody-induced model of acute glomerular inflammation is critical to retention of leukocytes in the glomerulus, and glomerular injury. Platelet recruitment in this model is dependent on the combined actions of GPVI and the  $\alpha$ IIb $\beta$ 3/ fibrinogen/ ICAM-1 pathway, and platelet P-selectin is crucial for subsequent leukocyte recruitment

**Keywords** platelets, leukocyte recruitment, inflammation

**Conflict of interest** No conflicts of interest

**Sunday 28 October**  
**Platelet Workshop 8: Platelet Thrombosis and Inflammation**  
**Free Communication**

**1600-1730**  
**Room 210**

### **NMDA Receptor: A Potential Novel Mediator of Glutamate in Platelets**

Maggie Kalev-Zylinska<sup>1,2</sup>, Marie-Christine Morel-Kopp<sup>3,4</sup>, Taryn Green<sup>1</sup>, Paul Sun<sup>1</sup>, Young-Eun Park<sup>1</sup>, Annette Lasham<sup>1</sup>, Matthew During<sup>1,5</sup>, Christopher Ward<sup>3,4</sup>

<sup>1</sup>*Department of Molecular Medicine and Pathology, The University of Auckland;*

<sup>2</sup>*LabPlus Haematology, Auckland City Hospital, Auckland, New Zealand;*

<sup>3</sup>*Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney;* <sup>4</sup>*Northern Blood Research Centre, Kolling Institute, University of Sydney, Australia;* and <sup>5</sup>*Departments of Molecular Virology, Immunology and Medical Genetics, Neuroscience and Neurological Surgery, Ohio State University, Columbus, Ohio, United States*

#### **Aim**

Glutamate is stored in platelet dense granules and released on platelet activation to high serum concentrations. However, targets and effects of glutamate in platelets are poorly understood. The *N*-methyl-D-aspartate receptor (NMDAR) represents one of the main glutamate receptors in other tissues but its relevance in platelets is unknown. The aim of this study was to determine if NMDARs contribute to glutamate effects in platelets.

#### **Methods**

NMDAR expression was examined in human platelets at the protein and mRNA levels. Potential contribution of NMDARs to platelet function was investigated in platelet aggregation and activation studies using chemical modulators of NMDARs.

#### **Results**

We found that platelets expressed three of the total repertoire of possible seven NMDAR subunits. Flow cytometry and electron microscopy studies revealed that in non-activated platelets, NMDAR proteins were predominantly located intracellularly. On the other hand, in activated platelets, NMDARs were found on platelet extensions and at points of contact between platelets. NMDAR antagonists inhibited platelet aggregation induced by ADP, collagen and epinephrine. Exposure of platelets to NMDAR inhibitors also reduced measures of  $\alpha$ IIb $\beta$ 3 integrin activation (PAC-1 binding) and  $\alpha$ -granule release (CD62P expression), as measured by flow cytometry.

#### **Conclusion**

NMDARs contribute to glutamate effects on platelets and may participate in contact-dependent platelet functions.

**Keywords** *N*-methyl-D-aspartate receptor, NMDAR, platelet

**Conflict of interest** No conflict of interest to disclose

Sunday 28 October  
 APSTH Education 3: Novel Anticoagulants

1600-1730  
 Room 211

## Novel Anticoagulants: The Evidence-Base

Alexander Gallus

SA Pathology at Flinders Medical Centre, Adelaide

New oral anticoagulants inhibit thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban).  $t_{1/2}$ 's are about 12 hours but renal clearance differs (80% for dabigatran,  $\approx$ 30% for rivaroxaban and apixaban). Drug-drug interactions are few and dosing is once or twice-daily without laboratory testing. Clinical trials include venous thromboembolism (VTE) prophylaxis, VTE therapy, preventing stroke and systemic embolism in non-valvular atrial fibrillation (AF), and acute coronary syndrome.

*Preventing VTE:* Dabigatran (220 or 150 mg od), rivaroxaban (10 mg od) and apixaban (2.5 mg bid) were 'non-inferior' to 40 mg od enoxaparin in patients having elective hip or knee replacement (rivaroxaban and apixaban were more effective). Overviews suggest a small excess of bleeding over enoxaparin with rivaroxaban not seen with apixaban. Starting rivaroxaban 6 – 8 hours after surgery (compared with 12 – 24 hours for apixaban) may have contributed.

*Initial Treatment after DVT or PE:* Dabigatran (150 mg bid) and rivaroxaban (20 od) were both 'non-inferior' to standard anticoagulation with a heparin followed by warfarin. Dabigatran was preceded by at least 5 days of low molecular weight heparin, in a double-blind trial. Rivaroxaban was given without initial heparin, starting with 3 weeks of 15 mg bid, in an open-label evaluation.

*Ongoing Treatment after Unprovoked VTE:* Starting after  $\approx$  6 – 12 months of initial therapy, dabigatran was 'non-inferior' to warfarin, while dabigatran and rivaroxaban were greatly superior to placebo (with more 'clinically relevant non-major' bleeding).

*Non-valvular atrial fibrillation (AF):* Dabigatran 150 mg bid was more effective than warfarin, with a similar rate of major bleeding, while effectiveness of 110mg bid was similar to warfarin with less bleeding. There was less intracranial bleeding with each dose than warfarin. Relative effectiveness seemed related to time in therapeutic range (TTR) for warfarin. Rivaroxaban 20 mg od was more effective than warfarin with less intracranial bleeding, as was apixaban 5 mg bid which also caused less major bleeding. Apixaban was greatly superior to aspirin  $<160$  mg/day in patients unwilling to take or considered unsuitable for warfarin, without more bleeding.

*Introduction to clinical practice:* will need caution and clear guidance on how to best manage overdose, major bleeding, or urgent invasive procedures.

**Keywords** new oral anticoagulants, VTE prevention, VE therapy, atrial fibrillation

**Conflict of interest** Steering Committees for trials of rivaroxaban, apixaban and edoxaban. Consultant to BMS, Boehringer-Ingelheim, CSL, Daiichi-Sankyo, Pfizer

**Sunday 28 October**  
**APSTH Education 3: Novel Anticoagulants**

**1600-1730**  
**Room 211**

## **Novel Oral Anticoagulants in Coronary Artery Disease**

Maria Teresa B Abola  
*The Philippine Heart Center, The Phillipines*

It has long been postulated that there is a link between blood coagulation and atherogenesis. Indeed, it has been shown that occlusive thrombosis in the setting of plaque rupture has caused acute complications of coronary atherothrombosis. The role of anticoagulants and antiplatelet drugs in preventing this process has been investigated early on in trials that showed that Vitamin K antagonists (VKAs) can reduce the risk of recurrent ischemic events, both as monotherapy and in combination with aspirin, but with the cost of an increased risk for bleeding. Despite proven efficacy, VKAs are not recommended for secondary prevention of cardiovascular events for patients with coronary artery disease because of the attendant bleeding risk and difficult monitoring. The novel oral anticoagulants are easier to manage than VKAs and therefore may have potential advantages. The advent of novel oral anticoagulants (OACs) is a welcome addition to the pharmacologic options for prevention of stroke and systemic thromboembolic events in patients with nonvalvular atrial fibrillation and in the prevention of venous thromboembolic events among those at risk for or have deep venous thrombosis or pulmonary embolism. They have been shown to have at least similar efficacy but with better safety profile when compared to VKAs. These drugs may therefore also play a role in the reduction of atherothrombotic events, specifically, acute coronary syndromes. This review will focus on the novel anticoagulants that have been investigated for use among patients with coronary artery disease.

**Sunday 28 October**  
**APSTH Education 3: Novel Anticoagulants**

**1600-1730**  
**Room 211**

## **Dabigatran – The New Zealand Experience**

Paul Harper

*Department of Clinical Haematology, Palmerston North Hospital, Palmerston North, New Zealand*

### **Aim**

To present an overview of the impact of the introduction of dabigatran in New Zealand with particular reference to the incidence of bleeding and change in warfarin use.

### **Method**

On 1st July 2011 dabigatran became available in New Zealand for the management of atrial fibrillation. It was fully funded through Pharmac and there were no restrictions on prescribing. Pharmac convened an expert panel to prepare guidelines to control bleeding and assist with the management around surgery. Concerns were raised by several haematologists that complications could arise and no mechanism was in place to monitor these. An *ad hoc* process was set up to report bleeding events.

An audit of 3,500 patients on warfarin has been performed to monitor the number of patients changing to dabigatran and to measure the number of bleeds associated with dabigatran and warfarin over 6 months.

### **Results**

During July and August 2011, 78 episodes of bleeding were identified with 12 major bleeds. Four major factors were identified that contributed to bleeding; prescriber error, impaired renal function, patient age and lack of a reversal agent. Bleeding from the gastrointestinal tract was the commonest site of bleeding. The management of bleeding episodes was reviewed.

Results of the audit showed that the number of patients taking warfarin fell by only 16%. The incidence of bleeding will be reviewed.

### **Conclusions**

As a result of the bleeding episodes changes have been made to the prescribing information to ensure safe patient selection. Additional educational material has also been circulated to General Practitioners. The guidelines to manage bleeding are undergoing review.

**Keywords** dabigatran, anticoagulants, warfarin

**Conflict of interest** Director of INR Online Ltd, a software company that produces a warfarin management tool.

**Notes:**



Monday 29 October

HSANZ Free Communications 1: AML - Clinical Interventions

O001

0830-1000

Plenary 2 (Auditorium)

0830-0845

## Intensive Treatment of AML in Elderly Patients

Kathryn Jackson<sup>1,2</sup>, Glen Kennedy<sup>1</sup>, Peter Mollee<sup>2</sup>, Paula Marilton<sup>2</sup>, Kirk Morris<sup>1</sup>

<sup>1</sup> Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.

<sup>2</sup> Princess Alexandra Hospital, Brisbane, Queensland, Australia

### Aim

AML incidence increases with age, yet treatment of elderly patients has reduced efficacy compared with younger patients and is often poorly tolerated. We aimed to determine the outcome of elderly patients with AML treated with intensive chemotherapy with or without allogeneic stem cell transplantation at our institutions.

### Methods

All patients with *de novo* AML (excluding APML) aged  $\geq 60$  years treated with induction chemotherapy at our institutions between February 1999 and July 2011 were retrospectively identified from institutional databases. Information on cytogenetic risk (using Intergroup criteria), chemotherapy protocols, response to therapy, disease-free survival (DFS), and overall survival (OS) were then determined retrospectively by review of individual medical records.

### Results

Induction chemotherapy was received by 128 patients (58% of elderly patients diagnosed with *de novo* AML), including 105 patients (82%) treated with standard-dose cytarabine (SDAC) and 14 patients (11%) treated with high-dose cytarabine (HiDAC). Median age was 67 years (range 60-83 years). Based on cytogenetic profile, 3% of patients had favourable, 55% had intermediate, and 27% had adverse-risk disease. Responses to 1-2 cycles of induction chemotherapy were complete remission (CR1) in 73% of patients, refractory disease in 15%, and induction death in 12%. 83% of patients who achieved CR1 received consolidation chemotherapy, incorporating SDAC in 76% and HiDAC in 22%. Thirteen patients proceeded to allogeneic transplantation in CR1; median age was 63 years (range 60-66 years). At a median follow-up of 22 months for survivors, intensive induction chemotherapy resulted in a median DFS of 11 months, and median OS of 13 months; 3 year OS for the entire cohort was 28%, with favourable, intermediate, and adverse risk groups having 50%, 32%, and 17% 3 year OS, respectively. Patients who received allogeneic transplantation in CR1 did not reach median DFS or OS; 11 patients (85%) remain alive and disease-free at a median follow-up of 27 months.

### Conclusions

Despite intensive chemotherapy, the majority of patients  $\geq 60$  years with AML have poor outcomes. However, a proportion of these patients experience long-term survival. In particular, patients selected for allogeneic transplantation in CR1 had high DFS and OS.

**Keywords** Acute myeloid leukaemia, Elderly. **Conflict of interest:** No

Monday 29 October

HSANZ Free Communications 1: AML - Clinical Interventions

O002

0830-1000

Plenary 2 (Auditorium)

0845-0900

**Progress Findings on a Novel Treatment Strategy Using Prolonged, Low-dose Cytarabine and Thioguanine in Combination with Peg-filgrastim for Acute Myeloid Leukaemia in Elderly Patients**

Charmaine Wong, Brandon Aubrey, Eunice Liu, William Stevenson, Gary Soo, Luke Coyle, Keith Fay, Matthew Greenwood, Naomi Mackinlay, Christopher Ward, Christopher Arthur  
*Royal North Shore Hospital, Sydney, Australia*

**Aim**

The incidence of AML increases with age, however elderly pts usually have a poor prognosis due to poor risk disease, medical comorbidities, and high treatment-related mortality. This is an updated report of our success with a novel outpatient based low-dose treatment strategy.

**Method**

A retrospective single-centre study of pts diagnosed with AML between April 2009-April 2012 who received the treatment protocol. Pts with refractory disease as well as *de novo* AML were included. Treatment consisted of daily sc cytarabine 20mg/m<sup>2</sup> and oral thioguanine 80mg for 14-21 days, monthly for 2 years with supportive Peg-filgrastim. Response was assessed by bone marrow aspiration.

**Results**

A total of 21 pts (13 *de novo* and 9 refractory/relapsed) received the low-dose protocol. The median pt age was 75 (52-89). The median follow-up time is 11.9 mths. A CR/CRi was achieved in 13 (62%) pts. 12 (57%) pts had secondary or MDS-related AML. 5 of 7 pts had poor risk cytogenetics and achieved CR/CRi, with 2 achieving cytogenetic remission. Of those that achieved any CR/CRi, 9 pts (43%) have remained in remission with a mean disease-free survival of 23.3 mths (14-32.5, 95%CI). For all pts, mean overall survival is 22.4 mths (14.3-30.5, 95%CI).

**Conclusion**

The results highlight the efficacy of this low-dose chemotherapy protocol and feasibility in the outpatient setting. Remission rates are comparable to those reported for intensive therapy with potential benefits of reduced toxicity, cost, hospital admissions and improved quality of life. This protocol may be an option for elderly patients unsuitable or ineligible for intensive chemotherapy or azacitidine. A prospective study is underway to further evaluate this protocol.

**Keywords** AML, elderly, chemotherapy **Conflict** No conflict of interest to disclose

Monday 29 October

HSANZ Free Communications 1: AML - Clinical Interventions

O003

0830-1000

Plenary 2 (Auditorium)

0900-0915

**Deferasirox (Exjade®) is Ineffective in Preventing Iatrogenic Iron Overload in Patients Undertaking Chemotherapy for Acute Myeloid Leukaemia (AML) and is Associated with Excess Gastro-intestinal and Infectious Toxicity: Results of a Prospective Randomized Study (HREC/10/QRBW/135)**

John Casey<sup>1</sup>, Manunath Narayana<sup>1</sup>, Elango Pillai<sup>1</sup>, Cameron Curley<sup>1</sup>, Jason Butler<sup>1</sup>, Kirk Morris<sup>1</sup>, Glen Kennedy<sup>1,2</sup>

<sup>1</sup>Department of Haematology, Royal Brisbane and Women's Hospital, Herston QLD, Australia. <sup>2</sup>University of Queensland, St Lucia QLD, Australia

**Background/Aims**

Iron overload, as measured by raised serum ferritin, is a significant risk factor for excess transplant related mortality (TRM) in allogeneic stem cell transplantation (SCT) for AML. We aimed to determine the efficacy of deferasirox (Exjade®) in preventing iatrogenic iron overload in patients receiving chemotherapy for AML.

**Methods**

Prospective randomised phase II study in AML patients planned to receive induction and/or consolidation chemotherapy. Iron studies and CRP were measured pre, mid and post each chemotherapy cycle. Patients were randomised to receive either therapy with deferasirox (starting at 5-10mg/kg/day PO and increasing to maximum 40mg/kg/day PO as tolerated) *versus* no deferasirox therapy. Deferasirox was commenced once ferritin increased to >500µg/L. The primary endpoint was ferritin concentration at completion of chemotherapy.

**Results**

The study was terminated prematurely after 16 patients were enrolled (deferasirox arm n=10; control arm n=6) due to excess grade 2-4 GIT (frequency 1.26 vs 0.75 events/chemotherapy cycle respectively; p=0.04) and infectious toxicity (frequency 2.22 vs 1.38 events/chemotherapy cycle respectively; p=0.05) experienced in the deferasirox arm. Deferasirox was poorly tolerated; 4/10 patients unable to continue the drug predominantly due to GIT toxicity. Post-treatment ferritin, % change in ferritin and number of transfusions received were no different between arms. CR rate was lower in the deferasirox arm (60% vs. 100%; p=0.234), and all treatment-related deaths (n=3; all infectious-related) occurred in the deferasirox arm. Median OS was similar in both arms (median 450 vs. 476 days respectively; p=0.72).

**Conclusions**

Use of deferasirox to prevent iatrogenic iron overload in AML patients undertaking induction / consolidation is ineffective, poorly tolerated, and associated with excess GIT and infectious toxicity.

**Keywords** iron chelation, acute myeloid leukemia, deferasirox

**Conflict of interest** No conflict of interest to declare.

Monday 29 October

HSANZ Free Communications 1: AML - Clinical Interventions  
O004

0830-1000

Plenary 2 (Auditorium)

0915-0930

# **Azacitidine in Combination with the Oral mTOR Inhibitor Everolimus (RAD001) in Relapsed/Refractory FLT3-ITD AML**

Peter Tan<sup>1</sup>, Patricia Walker<sup>1</sup>, John Catalano<sup>2</sup>, Anthony Schwarer<sup>1</sup>, Sharon Avery<sup>1</sup>, Sushrut Patil<sup>1</sup>, Stephen Opat<sup>1</sup>, Andrew Spencer<sup>1</sup>, Andrew Wei<sup>1</sup>

<sup>1</sup>Department of Haematology, The Alfred Hospital, Monash University, Melbourne, Australia <sup>2</sup>Department of Haematology, Frankston Hospital, Frankston, Australia

## **Background**

The outcome in chemo-resistant FLT3-ITD AML is dismal and although FLT3 inhibitors are effective, emergent drug resistance is common (8/8 patients; Smith *et al*, *Nature* 2012). The response rate (CR/CRi+PR) for azacitidine in relapsed and refractory AML is 11-19% (ASH 2009 #1054&2044). Azacitidine with Sorafenib has activity in advanced FLT3-ITD AML (ASCO 2012 #6558); CR/CRi of 50% (11/22) and acquired FLT3-TKD mutations in 6/8. Targeting pathways downstream of FLT3 represents an alternative strategy in the presence of inhibitor resistant mutations.

## **Aim**

We sought to determine the preliminary efficacy of combining azacitidine with the mTOR inhibitor everolimus in AML, focusing on outcomes in patients with FLT3-ITD.

## **Methods**

A phase Ib/II open label dose-escalation study of azacitidine 75 mg/m<sup>2</sup> sc daily days 1-5 and 8-9 in 28-day cycles with 2.5, 5 or 10 mg everolimus orally on days 5-21.

## **Results**

40 patients, median age 65 years (range 17-78) received 2.5 mg (n=6), 5 mg (n=12) or 10 mg (n=22) everolimus with azacitidine. 27 (68%) had relapsed (6 post-alloSCT), 11 (28%) had primary refractory and 2 (5%) untreated AML. 11/40 (28%) had poor risk karyotype and 7/37 (19%) FLT3-ITD. Clinical response in FLT3-ITD AML was 43% (3 PR), 0/3 with FLT-TKD and 30% (2 CR, 7 PR) in other patients. Mean absolute marrow blast change was -37% in FLT3-ITD, -10% in FLT3-TKD and -6% in the others. Response duration was 3, 4 and 10 months in the 3 responding patients with FLT3-ITD; 2 proceeded to Cy-TBI alloSCT, with CR documented post-SCT but relapse occurred in both (2 and 5 months). Correlative studies examining other molecular lesions (e.g. RAS), serum FLT3 ligand, everolimus levels and plasma inhibition of phospho-FLT3 and phospho-p70S6K will be presented.

## **Conclusion**

Azacitidine with the mTOR inhibitor everolimus has interesting activity in a subset of patients with chemo-resistant FLT3-ITD AML and should be examined further.

**Keywords** AML, azacitidine, mTOR, FLT3

**Conflict of interest** This research was supported by Novartis and Celgene. The companies had no role in data analysis or abstract preparation.

**Monday 29 October**

**HSANZ Free Communications 1: AML - Clinical Interventions  
O005**

**0830-1000**

**Plenary 2 (Auditorium)**

**0930-0945**

**Outcomes and Prognostic Factors for Patients with Acute Myeloid  
Leukaemia Admitted to the Intensive Care Unit**

Kathryn Jackson<sup>1,2</sup>, Peter Mollee<sup>1</sup>, Kirk Morris<sup>2</sup>, Dwane Jackson<sup>2</sup>, Peter Kruger<sup>1</sup>,  
Kerenaftali Klein<sup>3</sup>, Glen Kennedy<sup>2</sup>

<sup>1</sup>Princess Alexandra Hospital, Brisbane, Queensland, Australia; <sup>2</sup>Royal Brisbane  
and Women's Hospital, Brisbane, Queensland, Australia; <sup>3</sup>School of Population  
Health, The University of Queensland, Brisbane, Queensland, Australia

**Aim**

Patients with acute myeloid leukaemia (AML) receiving intensive chemotherapy commonly experience life-threatening complications requiring intensive care unit (ICU) support. Limited information is available regarding which patients are likely to benefit from an ICU admission and their subsequent outcomes.

**Methods**

This is a retrospective study of 505 patients with newly-diagnosed AML who were treated at the Royal Brisbane Hospital or Princess Alexandra Hospital with intensive chemotherapy between 1<sup>st</sup> January 1999 and 31<sup>st</sup> December 2010. Statistical analyses were undertaken to identify risk factors for ICU admission, short- and long-term outcomes, and prognostic factors predicting survival following ICU admission.

**Results**

Eighty-three patients (16%) were identified who had required 92 ICU admissions in the setting of induction or consolidation chemotherapy. The primary indication for ICU admission was haemodynamic instability in 43 patients (47%) and respiratory impairment in 37 patients (40%), most commonly as the result of infection (75%). Vasopressors were required in 67% of admissions, mechanical ventilation in 59%, and haemodialysis in 15%. Survival to ICU discharge, hospital discharge, 6 months, and 12 months were 67%, 61%, 49%, and 39%, respectively. Patients admitted to ICU had worse overall survival than patients not requiring ICU admission (median OS = 0.7 years vs 3.5 years,  $P < 0.0001$ ). Multivariate analysis identified independent prognostic factors predicting mortality prior to hospital discharge to be mechanical ventilation use and higher fibrinogen, and mortality at 12 months to be associated with mechanical ventilation use and AML cytogenetic risk group.

**Conclusions**

Patients with AML undergoing intensive chemotherapy who develop severe acute complications requiring ICU admission have the potential to experience long-term survival. Prognostic factors exist which identify those more likely to recover, but are insufficient to identify a subgroup in whom ICU admission is futile.

**Keywords** Acute myeloid leukaemia, Intensive care

**Conflict of interest** No

Monday 29 October

HSANZ Free Communications 1: AML - Clinical Interventions

O006

0830-1000

Plenary 2 (Auditorium)

0945-1000

## Use of High-dose Cytarabine Induction in Adult *de novo* AML is Not Associated with Improved Survival

Kathryn Jackson<sup>2</sup>, Kirk Morris<sup>1</sup>, Simon Durrant<sup>1</sup>, Devinder Gill<sup>2</sup>, Peter Mollee<sup>2</sup>, Paula Marltan<sup>2</sup>, Glen Kennedy<sup>1</sup>

<sup>1</sup> Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.

<sup>2</sup> Princess Alexandra Hospital, Brisbane, Queensland, Australia

### Aim

High-dose cytarabine (HiDAC) in the treatment of adult AML is associated with improved disease-free survival (DFS). However, there is limited data on the relative benefits of incorporating HiDAC into induction versus consolidation chemotherapy. We aimed to determine the outcomes of *de novo* AML treated with standard-dose cytarabine (SDAC) versus HiDAC-based induction strategies at our institutions.

### Methods

All patients with *de novo* AML (excluding APML) aged 15-59 years treated with induction chemotherapy at our institutions between February 1999 and July 2011 were retrospectively identified from institutional databases. Information on cytogenetic risk (Intergroup criteria), chemotherapy protocols, responses, DFS, and OS were then determined retrospectively by review of individual medical records.

### Results

In total, 211 patients had been treated, including 100 patients (47%) treated with SDAC regimens, with 95 receiving 7+3 with idarubicin, and 5 receiving 7+3+7 with etoposide; and 111 patients (53%) treated with HiDAC-based induction, including 76 treated with "Big ICE", 26 with "HiDAC 7+3", and 4 with FLAG. Median age was 45 yrs (range 15-59 yrs). Based on cytogenetic profile, 18% of patients had favourable-risk, 60% had intermediate-risk and 16% had adverse-risk disease. Overall CR rate was similar between the two induction strategies (93% vs. 91% for SDAC vs HiDAC, respectively,  $P = 0.62$ ). A majority of patients who received SDAC induction subsequently received HiDAC-based consolidation (85%); conversely, most patients induced with HiDAC received SDAC in consolidation (72%,  $P < 0.0001$ ). A similar proportion of patients in each group underwent allogeneic transplantation in CR1 (31% vs. 24% for SDAC vs HiDAC, respectively,  $P = 0.26$ ). At a median follow-up of survivors of 46 months (range 1-152 months), HiDAC-based induction was not associated with an improvement in DFS or OS when compared to SDAC (DFS  $P = 0.38$ , OS  $P = 0.55$ ). Even when stratified into specific cytogenetic risk groups, no improvement in either DFS or OS was demonstrable with HiDAC-based induction.

### Conclusion

HiDAC-based induction for *de novo* AML in adults <60 years is not associated with improvement in CR rate, DFS, or OS in comparison to the use of SDAC induction.

**Keywords** Acute myeloid leukaemia, Cytarabine. **Conflict of interest:** No

Monday 29 October

HSANZ Free Communications 2: Molecular Genetics of Leukaemia

O007

0830-1000

Room 216

0830-0845

# **Global Proteome Analysis of Acute Lymphoblastic Leukemia Cells Resistant to mTOR Inhibition by Everolimus**

Jordan Basnett<sup>1</sup>, Kenneth Bradstock<sup>2</sup>, Linda Bendall<sup>1</sup>

<sup>1</sup> *Westmead Institute for Cancer Research, Westmead Millennium Institute, NSW, Australia.* <sup>2</sup> *Haematology Department, Westmead Hospital, NSW, Australia.*

Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer. The inability to further intensify current treatments in high risk and relapse patients due to dose limiting toxicities of chemotherapeutic agents, demands the development of new agents. One exciting new treatment currently in clinical trial, is the mTOR inhibitor everolimus. Preclinical studies demonstrated its effectiveness in treating ALL, however, as was seen with other kinase inhibitors such as imatinib, resistance emerged in a subset of the human ALL xenografts following prolonged treatment in vivo. Aberrations to the proteome of the resistant cells are the most likely mechanism of acquired resistance to the mTOR inhibitor.

Protein was isolated from cells sensitive and resistant to the mTOR inhibitor everolimus. Proteins were separated using 2D electrophoresis, quantified using DIGE and identified using MALDI/TOF mass spectrometry. 51 proteins were significantly differentially expressed in the resistant cells compared to parental cells which are sensitive to everolimus, 2 of which were upregulated and the remaining down regulated. 7 proteins have been successfully identified to date WDR1, AN32E, PRS7, ENOA, TBCA, HBA, HBB all of which are >1.8 fold downregulated in resistant cells, apart from WDR1 which is increased 1.6 fold. HBA, PRS7, TBCA, ANP32E and ENOA have a corresponding decreased genetic expression is observed (>1.5 fold) while the remaining proteins do not have a significant change in gene expression. Decreased PSMC2, a subunit of the 26S protease, may lead to an increase in the activity of pro survival proteins such as PKC and cyclinD1 and proto-oncogenes p53 and c-rel.

Global proteomic analysis of cells resistant to mTOR inhibition by everolimus provides insight into cellular activity and highlights possible means by which cells could become resistant to mTOR inhibition.

**Keywords** Leukemia, Everolimus, Resistance

**Conflict of interest** Everolimus was supplied by Novartis. The company had no role in analysing the data.

Monday 29 October

HSANZ Free Communications 2: Molecular Genetics of Leukaemia

O008

0830-1000

Room 216

0845-0900

## Cytogenetic Assessment of Acute Lymphoblastic Leukaemia is Enhanced by the Addition of DNA Microarray Testing

Meaghan Wall,<sup>1</sup> Adrian Zordan,<sup>1</sup> Ruth N. MacKinnon,<sup>1,2</sup> Lynda J Campbell<sup>1,2</sup>

<sup>1</sup> Victorian Cancer Cytogenetics Service, St Vincent's Hospital Melbourne, Fitzroy, Victoria, Australia <sup>2</sup> Department of Medicine (St Vincent's Hospital Melbourne), Melbourne University, Fitzroy, Victoria, Australia

### Aim

Karyotyping has prognostic significance in acute lymphoblastic leukaemia (ALL). Yet cytogenetic assessment may be hampered by the low mitotic index and poor chromosome morphology intrinsic to many ALL samples. DNA microarrays provide information about copy number aberrations (CNA) without the need to obtain metaphases and overcome some limitations of metaphase cytogenetics (MC). This study aimed to test the utility of DNA microarray testing in ALL.

### Method

Samples from patients with a diagnosis of new or relapsed ALL were tested (n=11). MC and FISH were performed according to standard techniques. DNA for microarray testing was extracted from bone marrow surplus to diagnostic requirements.

### Results

Eight patients had a new diagnosis of B-ALL (age 1-28 years). Three patients had a diagnosis of T-ALL (age 9-66 years); 2 had relapsed disease. Favourable (n=1), intermediate (n=6) and poor (n=4) cytogenetic risk groups were represented. MC plus FISH detected an abnormality in 9/11 (82%); DNA microarray testing was abnormal in all cases. DNA microarray detected additional CNA in 8/11 (73%) cases. Biologically relevant genes affected by CNA included *CDKN2A*, *PAX5*, *IKZF1* and *BTG1*. Copy neutral loss of heterozygosity was identified in 2 cases (both T-ALL); the third T-ALL case demonstrated chromothripsis involving the short arm of chromosome 1. Three patients had balanced translocations that were only detected by MC and a case with *iAMP21* could only be classified by FISH.

### Conclusion

DNA microarrays provide complementary information to MC and FISH. All three modalities may be required in order to completely characterise the leukaemic clone.

**Keywords** ALL, Cytogenetics, DNA microarray

**Conflict of interest** No conflict of interest to disclose



Monday 29 October

HSANZ Free Communications 2: Molecular Genetics of Leukaemia

O009

0830-1000

Room 216

0900-0915

**A Report of a Duplication of a Known Copy Number Variation Region That Had Previously Only Been Reported to be Present in 0, 1 or 2 Copy Numbers and its Implication in Familial Chronic Lymphocytic Leukaemia**

Elizabeth Mary Tegg<sup>1,2</sup>, Patrick Danoy<sup>3</sup>, Matthew Brown<sup>3</sup>, Russell Thompson<sup>4</sup>, Katherine Anne Marsden<sup>1,2</sup>, Ray Michael Lowenthal<sup>1,2,4</sup>, Joanne Dickinson<sup>4</sup>  
<sup>1</sup>Royal Hobart Hospital, Hobart, Tasmania. <sup>2</sup>School of Medicine, University of Tasmania. <sup>3</sup>The University of Queensland Diamantina Institute. <sup>4</sup>Menzies Research Institute Tasmania, University of Tasmania, Hobart, Tas, Australia

**Aim**

To compare the copy number variations (CNV) of 3 subjects with familial chronic lymphocytic leukaemia (CLL).

**Methods**

A genome wide Illumina 610 Quad SNP array with CNV analysis performed using PennCNV software was undertaken on 117 subjects from 13 families who have a dense aggregation of haematological malignancies.

**Results**

Three subjects who had CLL at the time of peripheral blood DNA extraction were found to have 2 CNVs in common. One CNV was an acquired deletion of chromosome 13q14. The second CNV they shared was a duplication (3 copies) of chromosome 4q13.2 from base position 69064675 to 69087412 (NCBI36/hg18 version of the human genome). This region on chromosome 4 is a known region of CNV, but had previously only been reported that 0, 1 or 2 copies are present. Deletions (1 or 0 copies) of this region have the highest frequency in south-east Asia where CLL has the lowest incidence. This region of the genome contains the gene *UGT2B17*. A total of 35 subjects (from 12 of the 13 families) in our familial dataset have 3 copies of this region.

**Conclusion**

This region on chromosome 4q13.2 has previously been reported to be a site of a predisposition gene in CLL. It is intriguing to hypothesise that this CNV may play a role in the genetic predisposition to familial CLL

**Keywords:** Familial CLL, Genomic copy number variation.

**Conflict of interest:** No

Monday 29 October

HSANZ Free Communications 2: Molecular Genetics of Leukaemia

O010

0830-1000

Room 216

0915-0930

# **Next Generation Sequencing of Cells Resistant to mTOR Inhibition by Everolimus in Acute Lymphoblastic Leukemia**

Jordan Basnett<sup>1</sup>, Kenneth Bradstock<sup>2</sup>, Linda Bendall<sup>1</sup>

<sup>1</sup> *Westmead Institute for Cancer Research, Westmead Millennium institute, NSW, Australia.*

<sup>2</sup> *Haematology Department, Westmead Hospital, NSW, Australia*

Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer. The inability to further intensify current treatments in high risk and relapse patients due to dose limiting toxicities of chemotherapeutic agents, demands the development of new agents. One exciting new treatment currently in clinical trial, is the mTOR inhibitor everolimus. Preclinical studies demonstrated its effectiveness in treating ALL, however, as was seen with other kinase inhibitors such as imatinib, resistance emerged in a subset of the human ALL xenografts following prolonged treatment in vivo. Mutation and/or changes to gene expression are potential mechanisms of resistance to everolimus.

There were 74 novel single nucleotide polymorphisms (SNPs) and 29 insertion/deletion mutations present in the resistant but not the sensitive cells. Mutations predicted to alter protein function in genes that regulate cell proliferation and survival were detected and included mutations in DYRK1a, CHEK1, ATG5, PKN2 and MLL5. The mutation in DYRK1a is predicted to result in a protein truncated at amino acid 105 with the loss of all protein binding and kinase domains. DYRK1A is a dual-specificity yak-related kinase and is related to cyclin-dependent kinases and MAPK. It has a role in G0 entry and Ras-induced senescence and is mutated in breast, lung and ovarian cancers. The gene dose of DYRK1A is important with DYRK1A (located on chromosome 21) contributing to the neurological phenotype in trisomy 21. The loss of DYRK1A has the potential to overcome the anti-proliferative effects of everolimus by preventing G0 entry. Furthermore, analysis of gene expression revealed AP1 to be the most highly upregulated transcription factor in the everolimus resistant cells. DYRK1a has the potential to negatively regulate AP1 via direct effects on Sirtuin 1. These data suggest a novel mechanism by which cells can remain in cell cycle and thereby overcome the anti-proliferative effects of RAD001.

**Keywords** Leukemia, Everolimus, Resistance

**Conflict of interest** Everolimus was supplied by Novartis. The company had no role in analysing the data.

Monday 29 October

HSANZ Free Communications 2: Molecular Genetics of Leukaemia

O011

0830-1000

Room 216

0930-0945

# **Analysis of MicroRNAs in Normal Human Promyelocytes and in Acute Promyelocytic Leukaemia (APL) Demonstrates Abnormal up Regulation of a Large MicroRNA Gene Cluster on Chromosome 14q32 in APL**

David Agapiou, Christina Brown, Shihong Yang, Alberto Catalano, Harry Iland  
*Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia*

## **Aim**

Deregulated microRNA (miRNA) expression occurs in solid organ and haematological malignancies suggesting miRNAs have tumour suppressor or oncogenic potential. We have previously identified co-ordinated up regulation of a large miRNA cluster on chromosome 14q32 in 22/32 (68.5%) APL patients relative to other subtypes of acute myeloid leukaemia (AML). The expression of 14q32 miRNAs in normal human promyelocytes however is not known. To explore this deregulation of 14q32 miRNAs in APL we have purified normal promyelocytes and compared their 14q32 miRNA expression with that seen in APL and other AMLs.

## **Methods**

Unstimulated bone marrow or G-CSF stimulated peripheral stem cell collections were obtained from three healthy haematopoietic stem cell donors including a paired bone marrow and peripheral stem cell sample from the same individual who donated twice. Promyelocytes were purified by flow cytometry utilising CD45/side scatter characteristics to identify granulocytic cells and sorting the CD16<sup>neg</sup>, CD11b<sup>neg</sup> population. Morphological assessment confirmed that sorted cells were promyelocytes. The expression levels of four microRNAs from the 14q32 cluster (miR-127; miR-337-3p; miR-299; miR-495) were determined in the four normal promyelocyte samples and compared to 32 diagnostic APL and 10 non-APL AML bone marrow samples using TaqMan RT-PCR.

## **Results**

We were able to reproducibly isolate promyelocytes from DMSO-cryopreserved normal human samples. In normal promyelocytes, expression of miR-229-5p, miR-337-3p, and miR-127 were significantly lower than high 14q32 expressing APL samples ( $p < 0.05$ ) but were not different to other AMLs or the 10/32 APL samples with low 14q32 expression. There was a trend of lower expression for miR-495 in normal promyelocytes compared to high 14q32 expressing APL but this difference did not reach statistical significance ( $p = 0.11$ ). G-CSF stimulation did not appear to alter microRNAs expression levels in the paired samples from the same donor.

## **Conclusion**

Normal human promyelocytes express low levels of 14q32 cluster miRNAs confirming that up regulation of this cluster in APL is abnormal which suggests that 14q32 miRNA deregulation may play a role in the pathogenesis of APL.

**Keywords:** MicroRNAs, APL, chromosome 14q32 **Conflict of Interest:** No

Monday 29 October

HSANZ Free Communications 2: Molecular Genetics of Leukaemia

O012

0830-1000

Room 216

0945-1000

# **Improved Detection of Karyotypic Abnormalities in Myelodysplastic Syndromes by Combined Use of Single Nucleotide Polymorphism Arrays and Metaphase Cytogenetic Testing**

Meaghan Wall,<sup>1</sup> Ruth N. MacKinnon,<sup>1,2</sup> Adrian Zordan,<sup>1</sup> Kathleen C. Rayeroux,<sup>1</sup> Lynda J Campbell<sup>1,2</sup>

*1 Victorian Cancer Cytogenetics Service, St Vincent's Hospital Melbourne, Fitzroy, Victoria, Australia. 2 Department of Medicine (St Vincent's Hospital Melbourne), Melbourne University, Fitzroy, Victoria, Australia*

## **Aim**

Cytogenetic testing plays a key role in the diagnosis and risk stratification of myelodysplastic syndromes (MDS). Molecular karyotyping using single nucleotide polymorphism arrays (SNP-A) identifies copy number changes at higher resolution than metaphase cytogenetics (MC). SNP-A also detects acquired uniparental disomy (aUPD), a feature of MDS clones that goes undetected by MC. We hypothesised that MC+SNP-A would improve the characterisation of MDS and aimed to test the utility of incorporating SNP-A into the cytogenetic assessment.

## **Methods**

Bone marrow from MDS patients (n=30) was processed for MC according to standard techniques. For SNP-A testing, DNA was extracted and hybridised to CytoSNP-12 BeadChip arrays (Illumina, San Diego, CA).

## **Results**

Median patient age was 77 years (range 20-94) and 18 (60%) cases were male. The MDS subtypes represented were: RA (n=2), RARS (n=2), RCMD (n=17), RAEB-1 (n=1), RAEB-2 (n=5), MDS with 5q- (n=1) and t-MDS (n=2). The abnormality rate for MC was 13/30 (43%) for MC and 16/30 (53%) for SNP-A. Sixteen abnormalities were detected by MC versus 21 by SNP-A. Recurrent abnormalities detected by MC were: -Y (n=4), -7 (n=2) and +8 (n=2); two cases had a cytogenetically cryptic deletion of 12p identified by SNP-A. The SNP-A and MC results were discordant in 8 (27%) cases. In two cases, a +8 and a +21 clone, present in 36% and 30% metaphases respectively, were identified only by MC. Regions of copy number loss on 5q, 8p, and 12p, 1.3-13 Mb in size, were identified by SNP-A only. SNP-A also delineated regions of aUPD on 1p, 3q, 7q, 11q, 12q and 19q. The addition of SNP-A testing changed the karyotype from normal to abnormal in 3 cases and increased the complexity score in the remaining 5 cases.

## **Conclusion**

SNP-A testing is feasible and improves the precision of karyotyping in MDS.

**Keywords** Myelodysplasia, cytogenetics, DNA microarray

**Conflict of interest** No conflict of interest to disclose.

**Monday 29 October 0830-1000**

HSANZ Free Communications 3: StemCell Transplantation - Early Interventions Room 217  
**O013** **0830-0845**

**Iodine-131 Rituximab Radioimmunotherapy with BEAM Conditioning and Autologous Stem Cell Transplant for Mantle Cell Lymphoma**

Paul C Kruger<sup>1</sup>, Julian P Cooney<sup>2</sup>, J Harvey Turner<sup>3</sup>

<sup>1</sup>Fremantle Hospital Haematology Department, <sup>2</sup>Royal Perth Hospital Haematology Department, <sup>3</sup>The University of Western Australia, Fremantle Hospital Nuclear Medicine Department, Perth, WA, Australia

**Aim**

To study the effect of adding concomitant <sup>131</sup>I-rituximab radioimmunotherapy (RIT) to carmustine, etoposide, cytarabine, melphalan (BEAM) conditioning prior to autologous stem cell transplantation (ASCT) for mantle cell lymphoma.

**Method**

A phase II physician-sponsored study of aggressive recrudescent mantle cell lymphoma in five consecutive patients. Prospective personalized dosimetry performed in each patient limited whole body radiation absorbed dose to 0.75Gy. Predose rituximab and <sup>131</sup>I-rituximab was administered on day -15 prior to ASCT, with BEAM conditioning. Response was evaluated by <sup>18</sup>F-Fluorodeoxyglucose Positron Emission Tomography at day +100, overall and progression free survival (PFS). Engraftment times for neutrophils and platelets, and toxicity were also defined.

**Results**

Six RIT-ASCT procedures were performed in five patients. The engraftment rate was 100%. The complete response rate was 100% at three months. Overall survival is 4/5 (80%) at a median follow up of 36 months (range 5-110). Median PFS was 24 months (range 5-82). Three patients relapsed after RIT-ASCT; one relapsed at 82 months, was treated with a second RIT-ASCT and is in renewed complete remission after 24 months. Another was treated for localised inguinal relapse with radiotherapy and remains in subsequent remission. The other patient who relapsed died 12 months post RIT-ASCT. Toxicity comprised grade 3 mucositis in two patients, systemic cytomegalovirus infection in one patient and hypothyroidism in one patient.

**Conclusion**

The addition of <sup>131</sup>I-rituximab RIT to BEAM conditioning prior to ASCT for mantle cell lymphoma may improve tumour eradication, does not appear to significantly increase toxicity, and can be repeated upon relapse.

**Keywords** Radioimmunotherapy, bone marrow transplant, lymphoma.

**Conflict of interest** No conflict of interest to declare.

Monday 29 October

0830-1000

HSANZ Free Communications 3: StemCell Transplantation - Early Interventions Room 217  
O014

0845-0900

## Reduced Intensity Transplants Using G-CSF-Mobilized Hemopoietic Cells From Haploidentical Related Donors

Ken Bradstock, John Kwan, Ken Mickelthwaite, David Gottlieb, Emily Blyth, Gillian Huang, Stephanie Deren, Mark Hertzberg  
*Blood and Marrow Transplant Service, Westmead Hospital, NSW, Australia*

### Aim

To determine the safety and feasibility of using G-CSF-mobilized peripheral blood progenitor cells from haploidentical related donors for transplantation in patients with poor-risk hematological malignancies receiving reduced intensity conditioning therapy and post-transplant cyclophosphamide as GVHD prophylaxis.

### Methods

Eight patients aged 33 to 65 years (median 48) with poor prognosis hematological malignancies (3 AML, 1 MDS, 2 Ph+ ALL, 1 DLBCL, 1 ALL) who lacked HLA-matched related or unrelated donors underwent transplantation using G-CSF-mobilized PBSC from haploidentical relatives, after receiving reduced intensity conditioning therapy with fludarabine, cyclophosphamide, and single fraction TBI 200 cGy. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg IV daily on days +3 and +4, followed by daily oral tacrolimus and mycophenolate. Median follow-up is 8 months (1-11). Results were compared with a previous cohort of 12 patients receiving unmanipulated haploidentical bone marrow (IMJ 2012, in press).

### Results

Neutrophil and platelet recovery (ANC >1.0 median day 18, range 11-45 ; platelets >20 median day 23, 1-45) was comparable with 15 and 17 days respectively, for bone marrow. Six patients had neutropenic fevers, but there was no mucositis, use of TPN, or early transplant-related death. Five of 7 assessable patients had complete donor chimerism in blood T cells and granulocytes at day +28, while 2 had only host DNA ; one patient with MDS with high-titre anti-donor HLA antibodies, and one patient with Ph+ ALL. This is comparable to 2 of 12 patients with graft rejection receiving bone marrow. No acute or chronic GVHD and no disease relapse has occurred in 5 patients with complete donor chimerism.

### Conclusion

The use of unmanipulated G-CSF-mobilized HPC collected from haploidentical relatives appears feasible for patients receiving reduced intensity conditioning and high-dose cyclophosphamide as GVHD prophylaxis, with rates of engraftment, graft rejection and GVHD comparable to that seen with haploidentical bone marrow. This protocol offers an allogeneic transplant option with low cost and toxicity for patients without a HLA-identical donor.

**Keywords** bone marrow transplant, haploidentical donors, graft rejection

**Conflict of interest** None

Monday 29 October

0830-1000

HSANZ Free Communications 3: StemCell Transplantation - Early Interventions Room 217  
O015

0900-0915

**Comparison of Pre-transplant Thymoglobulin and Post-transplant Prednisolone as Graft-versus-Host Disease (GVHD) Prophylaxis in Matched Unrelated Donor (MUD) Peripheral Blood Stem Cell Transplants (PBSCT)**

David Ritchie, Andrew Lim, Emily Li, Kate Mason, Rosemary Hoyt, Simon He, Ashish Bajel, Andrew Roberts, Andrew Grigg, Jeff Szer  
*Royal Melbourne Hospital, Parkville, Vic, Australia*

**Aim**

To compare the impact of three GVHD prophylaxis regimens – cyclosporin plus methotrexate (CM); CM plus post-transplant prednisolone day +14 to day +84 (CMP), and CM plus pre-transplant Thymoglobulin 4.5 mg/kg total dose (CMT) – on acute and chronic GVHD (aGVHD, cGVHD), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) reactivation, and relapse-free and overall survival (RFS, OS).

**Results**

We analysed three sequential cohorts (CM n=24, CMP n=47, CMT n=62, total n=133) of MUD PBSCT recipients. Respectively, median ages were 45, 45 and 43; with high risk disease in 83%, 74% and 68%; reduced intensity conditioning in 12%, 12% and 24%; and median follow-up of 103, 94 and 33 months respectively. 5 year relapse-free survival (RFS) and OS were not significantly different between the three cohorts (CM 63% and 65%, CMP 55% and 56%, CMT 63% and 69%. 5 year OS in high vs low risk disease was CM 52% vs ND, CMP 48% vs 73% and CMT 65% vs 77%. Grade 2-4 aGVHD rates were CM 88%, CMP 69% and CMT 42% (p=0.0001). The incidence of any cGVHD at 12 and 36 months was CM 90% and 90%, CMP 90% and 90%, and CMT 24% and 60% (p=0.0001). Incidence of extensive cGVHD at 12 and 36 months was CM 25% and 67%, CMP 36% and 67%, and CMT 12% and 21% (p=0.0003). By weekly polymerase chain reaction monitoring, CMV reactivation at any time prior to day +100 was highest in CMP 69% compared to CM 48% and CMT 40%. EBV reactivation occurred in 28% of CMT group with no cases of post-transplant lymphoproliferative disorder in any of the three cohorts.

**Conclusion**

The use of pre-transplant Thymoglobulin (but not post-transplant prednisolone) substantially reduces aGVHD and cGVHD incidence in recipients of MUD PSCT without adversely affecting viral reactivation or disease relapse even in high risk disease.

**Keywords** graft-versus-host disease, allogeneic transplantation, Thymoglobulin

**Conflict of interest** No conflict of interest to disclose.

Monday 29 October

0830-1000

HSANZ Free Communications 3: StemCell Transplantation - Early Interventions Room 217  
O016

0915-0930

# **Non-myeloablative (NMA) Allogeneic Stem Cell Transplantation (alloSCT) for Multiple Myeloma and Older Patients with AML is Safe and Feasible**

Patricia Walker<sup>1,2</sup>, David Kipp<sup>1</sup>, Sharon Avery<sup>1</sup>, Sush Patil<sup>1</sup>, Andrew Wei<sup>1</sup>, David Curtis<sup>1,2</sup>, Anthony Schwarer<sup>1</sup>, Jenny Muirhead<sup>1</sup>, Andrew Spencer<sup>1,2</sup>

<sup>1</sup> Malignant Haematology and Stem Cell transplantation Service, The Alfred

<sup>2</sup> Dept Clinical Haematology, Monash University, Melbourne, Vic, Australia

## **Aim**

We report a single institution experience of NMA alloSCT for otherwise incurable older patients with AML and high risk multiple myeloma (MM).

## **Methods**

All patients received conditioning with fludarabine 48mg/m<sup>2</sup> oral on D-4 to -2 and 2Gy TBI on D0. GVHD prophylaxis consisted of cyclosporin and mycophenolate mofetil.

## **Results**

Since May 2008, 45 patients have undergone NMA alloSCT. Twenty AML - median age 60yrs (range 42-66) and 85% in CR1. Cytogenetic risk was intermediate for 15 and poor for 4. Four patients were FLT3+. Twenty five MM - median age of 51yrs (range 41-61). Indication for SCT was de novo high risk disease (poor risk cytogenetics or ISS III or high LDH or refractory to novel agent induction) in 15 and relapse in 10. Donors were sibling 47% or unrelated 53% with a median CD34 dose of 6.14x10<sup>6</sup>/kg and CD3 of 2.2x10<sup>8</sup>/kg. Thirty nine were performed as an outpatient procedure with a D30 unplanned readmission rate of 18%. All patients engrafted with 26 (58%) having grade IV neutropenia (median nadir 0.42x10<sup>9</sup>/L) and 7 febrile neutropenia. Median D90 chimerism was CD3 94% and CD33 100% with 3 patients receiving DLI for mixed chimerism. Acute GvHD occurred in 52% (grade I-II 36%; III-IV 16%) and chronic GvHD 49% (limited in 8 and extensive in 13). At a median follow up of 570 days, 9 (45%) patients with AML had relapsed and 4 died from AML. For MM patients, 12 (48%) relapsed with 100% response to salvage therapy and 2 subsequently died from MM. There were 6 treatment related deaths with TRM AML 20% vs MM 8% (2 infection, 3 GVHD and 1 cardiac).

## **Conclusion**

NMA alloSCT for older patients with AML and high risk MM is feasible with low treatment related mortality.

**Keywords** AML, myeloma, transplant

**Conflict of interest** No



Monday 29 October

0830-1000

HSANZ Free Communications 3: StemCell Transplantation - Early Interventions Room 217  
O017

0930-0945

# **Characterization of TPO Kinetics within Day 60 after Allogeneic Hematopoietic Stem Cell Transplantation and its Correlation with Megakaryocytes Ploidy Distribution**

Haixia Fu<sup>1</sup>, Xiaohui Zhang<sup>1\*</sup>, Lanping Xu, Daihong Liu, Kaiyan Liu, Xiaojun Huang\*

<sup>1</sup>Haixia Fu and Xiaohui Zhang share the first authorship

\* Xiaohui Zhang and Xiaojun Huang share the corresponding authorship

*Peking University People's Hospital Institute of Hematology, Beijing, China*

## **Aim**

To evaluate the characterization of the TPO kinetics and its correlation with megakaryocytes ploidy distribution pattern within day 60 after allo-HSCT.

## **Results**

All patients achieved white blood cell engraftment with a median time of 14 days. Forty-one patients achieved platelet engraftment at a median of 15 days. The multivariate analysis showed endogenous TPO levels and the number of transplanted CD34+ cells were significant risk factors for rapid platelet engraftment ( $P=0.006, 0.011$ , respectively). In the early period (day +15 and day+30), no statistical correlation was observed between absolute platelet count and TPO level, while a strong inversed correlation was observed between the TPO levels and the platelet counts in the late period post allo-HSCT (day +45 and day +60) ( $r=-0.467$ ,  $P=0.001$ ). Furthermore, the analysis showed a reduction of ploidy and more immature MKs in the TP+ group on day 60 after allo-HSCT. In addition, on day 60, the group with lower TPO levels ( $\leq 250$  pg/ml) was associated with a significantly better 3-yr OS ( $78.1 \pm 50.0\%$  vs.  $50.0 \pm 14.4\%$ ;  $P=0.045$ ) and lower TRM ( $P=0.038$ ).

## **Conclusion**

Endogenous TPO levels were associated with the delayed platelet recovery and thrombocytopenia after allo-HSCT, especially during the late period post allo-HSCT. The treatment of exogenous rhTPO in the early period post allo-HSCT, the improvement of TPO ability and the promotion of the MK maturation process in the late period post allo-HSCT should be considered as therapy strategies for delayed platelet recovery and thrombocytopenia after allo-HSCT.

**Keywords** hematopoietic stem cell transplantation; thrombocytopenia; thrombopoietin

**Conflict of interest** No conflict of interest to disclose.

Monday 29 October

0830-1000

HSANZ Free Communications 3: StemCell Transplantation - Early Interventions Room 217  
O018

0945-1000

# **Safe and Effective Prophylaxis of CMV Disease in Recipients of Allogeneic Haemopoietic Stem Cell Transplants Using Adoptive T cell Transfer – Long Term Follow-up of 50 Patients**

E Blyth<sup>1,4</sup>, L Clancy<sup>4</sup>, R Simms<sup>2,3</sup>, J Burgess<sup>2,3</sup>, CKK Ma<sup>2,3</sup>, P Shaw<sup>5</sup>, K Micklethwaite<sup>1,4</sup>, DJ Gottlieb<sup>1,4</sup>

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Laboratory & <sup>5</sup> The Children's Hospital at Westmead; Westmead, NSW, Australia

## **Aim**

To investigate the use of donor-derived cytomegalovirus (CMV) specific cytotoxic T cells (CTL) administered early post transplant to prevent CMV disease.

## **Methods**

CMV CTL were generated from peripheral blood or G-CSF mobilised stem cell harvest product from CMV seropositive stem cell donors. A single cell infusion of  $2 \times 10^7$  cells/m<sup>2</sup> was administered to transplant recipients from day 28. Demographics, treatment and results were compared with a control cohort of patients treated at the trial centres over the time frame that the trial was conducted.

## **Results**

We infused 50 patients with CMV CTL between 2003 and 2011. 52% of patients reactivated CMV within 100 days post transplant. When compared to controls, there was no difference in the rate of reactivation but patients treated on the study had reduced requirements for intravenous antiviral therapy (17 v 35% ; p=0.02) and reduced number of days of treatment (3.4 v 8.7 days per patient in the cohort, p=0.04). Immune monitoring showed greater expansion of CMV specific T cells in patients with CMV reactivation compared with those without (median peak level of CMV directed immunity 557 v 94; p=0.01). One patient who received T cells developed CMV disease and died of pneumonitis despite treatment with ganciclovir. Acute and chronic GVHD and overall and progression free survival were similar in the CTL and control groups.

## **Conclusion**

In this non randomised study, adoptive transfer of CMV specific CTLs was safe and reduced the requirement for CMV specific pharmacotherapy with ganciclovir or foscarnet and the total number of days of therapy without affecting the rate of CMV reactivation. A low rate of CMV disease was observed. We postulate that infused cells are able to expand in response to viral antigen, limit viral replication and prevent progression to tissue infection and may be particularly useful in controlling late CMV disease.

**Keywords** CMV, adoptive immunotherapy, haemopoietic stem cell transplant

**Conflict of interest** No conflict of interest to disclose.

Monday 29 October

HSANZ Free Communications 4: The Immunology of Haematology

O019

0830-1000

Room 218

0830-0845

## **Panobinostat and Azacitidine Therapy Reduces the Frequency of TNFR2+ Regulatory T cells in MDS/AML Patients**

Chindu Govindaraj<sup>1</sup>, Peter Tan<sup>2</sup>, Andrew Wei<sup>2</sup>, Andrew Spencer<sup>2</sup>, Magdalena Plebanski<sup>1</sup>

<sup>1</sup>Department of Immunology, Central Clinical School, Monash University, Melbourne, VIC, Australia. <sup>2</sup>Department of Clinical Haematology, The Alfred Hospital, Monash University, Melbourne, VIC, Australia

### **Background**

Regulatory T cells (Tregs) contribute to the immuno-suppressive AML microenvironment and enable the survival of malignant haematopoietic cells. In AML patients, there are increased Treg percentages in PBMCs at diagnosis compared to healthy controls, and higher frequencies predict poor response to induction chemotherapy. Therefore, Tregs are an attractive target to enhance the immune system's anti-leukemic effect. Tregs that express Tumour Necrosis Factor Receptor 2 (TNFR2) are associated with maximal immune suppressor activity.

### **Aim**

To investigate the effect of the pan-deacetylase inhibitor panobinostat (LBH589) and azacitidine (Aza) on T cell subsets, including CD4 and CD8 effector T cells (Teffs) and TNFR2+ Tregs in newly diagnosed MDS/AML patients.

### **Methods**

MDS/AML patients were treated with Aza subcutaneously on days 1-5 for each 28-day cycle combined with total of 7 doses of LBH589 orally on days 5-19 (M/W/F). The levels and function of T cell subsets within PBMCs were assessed via flow cytometry, at baseline and at the end of cycle 1 and 3.

### **Results**

TNFR2+ Tregs were present at higher levels in MDS/AML patients (pre-treatment) than in healthy controls. Aza/LBH589 therapy significantly reduced the frequency of TNFR2+ Tregs at the end of cycle 1 and this effect persisted to the end of cycle 3. Interestingly, the ratio of CD4 and CD8 Teffs to TNFR2+ Tregs was significantly enhanced by three treatment cycles. CD8 Teffs from patients produced significantly lower levels of IL-2 than healthy controls and therapy did not alter these IL-2 levels.

### **Conclusion**

Aza/LBH589 therapy reduced TNFR2+ Treg frequencies, which suggests potential enhancement of anti-leukemic immunity. Although the ratios of both CD4 and CD8 Teffs to TNFR2+ Tregs were increased within 3 cycles of treatment, CD8 T cells from AML patients did not produce normal levels of IL-2. The correlation between these immunological changes and clinical outcome is ongoing.

**Keywords** Regulatory T cells, Azacitidine, Panobinostat

**Conflict of interest** No

Monday 29 October

HSANZ Free Communications 4: The Immunology of Haematology

O020

0830-1000

Room 218

0845-0900

## Multi-virus Specific T Cells Expanded from Excess Mobilised Peripheral Blood Stem Cell Harvests for Prophylactic Adoptive Immunotherapy

Renee Simms<sup>1</sup>, Jane Burgess<sup>1</sup>, Leighton Clancy<sup>1,2</sup>, Emily Blyth<sup>1,3</sup>, Rebecca Brown<sup>1</sup>, Chun Kei Kris Ma<sup>1</sup>, David Gottlieb<sup>1,2,3</sup>

<sup>1</sup>Westmead Millennium Institute, Sydney, Australia, <sup>2</sup>Sydney Cellular Therapies Laboratory, Westmead Hospital, Sydney, Australia, <sup>3</sup>University of Sydney, Australia

### Introduction

There is a high risk of infection following haemopoietic stem cell transplantation (HSCT). Reactivation post transplant of Cytomegalovirus (CMV), Epstein-Barr Virus (EBV) and Varicella Zoster (VZV) and adenoviral infection in paediatrics causes significant morbidity and mortality. Adoptive transfer of *ex vivo* generated virus specific cytotoxic T lymphocytes (CTL) can restore immunity and prevent infection.

### Aim

To characterise donor-derived multi-virus specific CTL products generated from stem cell harvests in a Phase I/II trial of prophylactic infusion following HSCT.

### Methods

Multi-virus specific CTL products were generated from 3-4 ml of mobilised apheresis collections. Monocytes differentiated into dendritic cells (DC) were transfected with an adenoviral vector encoding CMV pp65, EBV (EBNA-1, LMP-1 and LMP-2) and the VZV vaccine, Varivax. Transfected DC were co-cultured with PBMC to stimulate expansion of virus specific CTL. Cultures were re-stimulated once and cultured for 14 days with IL-2 every 2-3 days. Cultures were assessed for alloreactivity and specificity by Cr<sup>51</sup> cytotoxicity assay and interferon- $\gamma$  production by ELISpot.

### Results

Of 18 CTL cultures six failed to achieve the target cell dose. In the remaining cultures, there was a mean 15 fold increase in cell number (range 7.8-42.1) consisting of memory T cells (mean 97%) with a higher percentage of CD8 (mean 52.8%, 11-91.5%) compared to CD4 T cells (mean 37.6%, 5.6-75.7%). No culture exhibited alloreactivity against recipient derived targets (0-2% specific lysis at E:T ratio of 20:1). Nine of 10 cultures showed killing of targets loaded with CMV pp65 (Mean 53.3% specific lysis at E:T 20:1, 4.2-88.2%). Specificity towards each virus targeted in CTL production could be detected by interferon- $\gamma$  production by ELISpot for 4 cultures tested. Nine products have been infused and follow up continues.

### Conclusions

Mobilised apheresis products can be used as a source of starting material to generate virus specific T cells. Collection of stem cell products is already incorporated into quality systems of transplant centres allowing for more rapid inclusion of T cell products into established quality controlled processing pathways.

**Keywords:** Adoptive transfer, T cell, viral infection **Conflict of interest:** No

Monday 29 October

HSANZ Free Communications 4: The Immunology of Haematology

O021

0830-1000

Room 218

0900-0915

# **Combination Agonist Therapy to Enhance the Antigen Presenting Capacity of CLL B Cells to NKT and T cells as a Step Towards Autologous Immunotherapy**

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<sup>1</sup>HITRL, Cancer Immunology Program, Peter MacCallum Cancer Centre, East Melbourne, Vic. <sup>2</sup> Dept of Microbiology and Immunology, University of Melbourne, Parkville, Vic, Australia

## **Introduction and Aim**

Chronic lymphocytic leukaemia (CLL) is potentially amenable to immunological control as indicated by its sensitivity to allogeneic stem cell transplantation (SCT). Most patients with CLL are unable to undergo SCT. Autologous immunotherapy therefore represents a potential means of inducing immunological control over CLL. We examined the effects of a range of B cell agonists including anti-CD40, CpG, aGalCer and lenalidomide to the improve antigen presenting cell (APC) function of primary CLL samples from untreated patients (n=10).

## **Results**

We identified that even untreated primary CLL B cells are capable of acting as APC for allogeneic T cell responses. Autologous T cell or NKT cell responses however required activation the CLL B cells with one or more agonistic stimuli. The combination of the agonistic anti-CD40 antibody CP-870,893 in combination with the TLR9 agonist CpG resulted in the most potently activated APC phenotype and function. When these activated CLL cells were used to present aGalCer, they were able to expand autologous NKT cells by 2.5 to 30 fold. Furthermore, the addition of lenalidomide to the CLL APC significantly enhanced both the proliferation and cytokine production by autologous T cells consistent with its recently described effects in the immunological synapse.

## **Conclusion**

CLL B cells can, under the correct activating signals, become effective and potent APC capable of inducing autologous T cell and NKT cell activation, which in turn show anti-CLL efficacy. These cells may form the basis of anti-CLL immunotherapy.

**Keywords** Chronic lymphocytic leukemia, NKT cells, immunotherapy.

**Conflict of interest** No conflict.

Monday 29 October

HSANZ Free Communications 4: The Immunology of Haematology

O022

0830-1000

Room 218

0915-0930

## Assessment of Specific Antifungal Immune Responses in Peripheral Blood Cells Derived from Healthy Donors

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### Aim

Invasive (filamentous) fungal infections (IFIs) are common in patients with haematological malignancy, affecting 15-35% of children and 7-25% of adults. Azole prophylaxis has significantly reduced *Candida* related IFIs, however aspergillosis and drug-resistant IFIs such as mucormycosis, fusariosis and scedosporiosis are increasing in frequency, causing mortalities from 50% to almost 100%. Specific anti-fungal immune deficiency persists up to a year following stem cell transplant. Adoptive transfer of *in vitro* expanded anti-fungal T cells derived from healthy allogeneic donors may aid in the prophylaxis and treatment of IFIs in high-risk patients. To determine a baseline immune response against which patient responses could be measured, immune responses to common filamentous fungi by peripheral blood mononuclear cells (PBMC) of healthy donors were determined by proliferation and cytokine production assays.

### Results

Water-soluble lysates derived from pure cultures of *A. fumigatus*, *A. flavus*, *A. terreus*, *F. solani*, *F. oxysporum*, *R. oryzae* and *S. prolificans* induced specific proliferation of PBMC from healthy donors (n=2). In both cultures, maximal proliferation were observed with 10 or 20µg/ml of each lysate. The extracellular levels of T helper (Th)-type 1, Th-2 and chemotactic cytokines were assessed following stimulation with 10µg/ml of the above and *C. albicans* (n=4). All lysates induced significant upregulation of MIP-1β and MCP-1 in all cultures. *F. oxysporum* and *C. albicans* induced significant upregulation of RANTES while IL-17 and IFNγ were significantly upregulated by *A. fumigatus* and *C. albicans*. In contrast, no increase in IL-4 levels was observed following stimulation with any fungal lysate tested. The cell of origin of each product is to be determined.

### Conclusion

Antifungal responses in healthy individuals are characterized by specific lymphoproliferative responses and production of Th1 and innate effector cytokines, but not the Th2 cytokine, IL-4. These preliminary data suggest the presence of antifungal reactive cells in peripheral blood and raise the possibility that PBMC from healthy allogeneic donors may be suitable for *in vitro* expansion of anti-fungal cells for immune reconstitution in high-risk patients.

**Keywords** anti-fungal immunity, adoptive cell therapy, invasive fungal infections

**Conflict of interest** No conflict of interest to disclose

Monday 29 October

HSANZ Free Communications 4: The Immunology of Haematology

O023

0830-1000

Room 218

0930-0945

## Split Anergy of Prominent Clonal T Cell Expansions is a Hallmark of Myeloma and a Model for Tumour-Induced Suppression in Cancer

Hayley Suen<sup>1</sup>, Ross Brown<sup>1</sup>, Shihong Yang<sup>1</sup>, Christian Bryant<sup>2</sup>, James Favaloro<sup>2</sup>, John Gibson<sup>1</sup>, P Joy Ho<sup>1</sup>, Phillip Fromm<sup>2</sup>, Derek Hart<sup>2</sup>, Douglas Joshua<sup>1</sup>

<sup>1</sup>*Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia;*

<sup>2</sup>*DC Biology & Therapeutics, ANZAC Research Institute, Sydney, NSW, Australia*

### Aim

Clonal cytotoxic CD8+ T cell expansions are found in the peripheral blood of patients with many malignancies including multiple myeloma (MM). Our aim was to study the cause of clonal T cell anergy in patients with MM and investigate potential mechanisms to reverse anergy.

### Method

A Betamark kit was used for identification of CD3+CD8+CD57+TCRVβ+ T cell expansions. Microarrays were performed using an Affymetrix GeneChip Human Genome U133 plus 2.0. Proliferation assays used CFSE and MACSiBead particles. Flow sorting and analysis were conducted on a BD ARIA II.

### Results

51% of MM patients (n=264) had expanded T cell clones detected by TCRVβ analysis (14.3% of CD3 cells, range 4-49%). These were confirmed by IgH CDR3 sequencing. Restimulating these cells with antiCD2, CD3, CD28 beads failed to induce significant proliferation, demonstrating the anergic nature of these cells (median 6% proliferation) compared with other CD8 cells (70%). Geneset analysis of mRNA microarrays identified genes associated with anergy and long term survival, with upregulated *RAS*, *CSK* and *TOB* and suppressed *ERK* pathways. Functional studies suggest these non-proliferating clones display split anergy as interferon-γ production is normal *ex vivo*. Importantly, the T cell clones in 10 year survivors were only partially anergic (median 62% proliferation). While T cell clones in large granular lymphocytic leukaemia (LGL) may be associated with *STAT3* mutations, our microarray data showed that *STAT3* is not upregulated in MM T cell clones. Preliminary studies with agents thought to overcome anergy (IL-12, IL-15, IL-21, anti-CD137 and anti-CD134) had little effect on severely anergic T cell clones.

### Conclusion

Clonal cytotoxic CD8+ T cell expansions are frequently observed in MM patients. They exhibit split anergy and are not associated with upregulated *STAT3*. Overcoming anergy of these T cells has the potential to restore immunological control of MM and other cancers. The partially anergic T cell clones of 10 year survivors offer a unique cohort for future studies.

**Keywords** Multiple myeloma, anergy, T cell clones

**Conflict of interest** No

Monday 29 October

HSANZ Free Communications 4: The Immunology of Haematology

O024

0830-1000

Room 218

0945-1000

## Mechanisms of Immune Suppression in Multiple Myeloma

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<sup>1</sup>Peter MacCallum Cancer Center, East Melbourne, Victoria, Australia

<sup>2</sup>The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia

### Aim

To identify novel inhibitory receptors on tumour infiltrating lymphocytes in multiple myeloma (MM) patients for the development for targeted therapy recent

### Methods

All MM patient samples were processed and stored at Peter Mac under informed consent. 12 colour multiparameter FACS was used for phenotype studies and student T tests and ANOVA used for statistical analysis.

### Results

Multiple myeloma (MM) is an incurable plasma cell malignancy of the bone marrow (BM). Recent studies have shown that both T and natural killer (NK) cells are intricately involved in the immunosurveillance of MM. In the absence of the immunosuppressive tumour microenvironment these effectors can lyse autologous MM cells. Here we report that both therapy and MM pathophysiology contribute to immune suppression in MM. From a therapy point of view, the use of corticosteroids significantly reduces the expression of the major cytotoxic receptor, NKG2D, on NK cells. Furthermore, corticosteroid therapy is antagonistic to now commonly used immune stimulatory drug lenalidomide. We explored mechanisms of MM-induced immune suppression and found killer-immunoglobulin like receptors (KIRs) of the 2DL family could be induced on effectors cells by MM. Inhibitory 2DL KIRs bind to HLA-C molecules and inhibit NK and T cell function. Analysis of matched MM patient peripheral blood and BM samples revealed an increase in the number of KIR+ NK and T cells in the BM tumour site, not seen in aged matched healthy controls. This also correlated with an increased level of CD57+ cells, suggesting immune exhaustion at the tumour site.

### Conclusions

Immune suppression in MM presents a major barrier for total disease control. We report a specific family of inhibitory receptors (KIR2DLs) that are up-regulated on tumour infiltrating lymphocytes in the BM of MM patients. With recent clinical success of anti-CTLA, anti-PD1 and anti-PDL1 therapy in solid tumours, we believe that targeting inhibitory receptors in MM may be of potential via attenuation of immune suppression.

**Keywords** Multiple myeloma, natural killer cell, immune suppression

**Conflict of interest** No



Monday 29 October  
ANZSBT Presidential Symposium  
O025

0830-1000  
Room 219/220  
0830-0848

## Effects of Short Term Temperature Excursions on Irradiated Platelet Concentrates

Kelly Winter, Lacey Johnson, Matthew Kwok, Tanja Hartkopf-Theis, Samantha Reid, Denese Marks  
*Research and Development, Australian Red Cross Blood Service, Sydney, NSW, Australia*

### Aim

Platelets stored outside the acceptable temperature range (20-24 °C) for any length of time during processing, transportation or storage are usually discarded. However, there is currently limited published data on the effects of short excursions outside this range on platelet quality. The aim of this study was to evaluate the *in vitro* quality of irradiated platelets following temperature excursions below or above their optimal storage temperature.

### Methods

Whole blood derived pooled platelet concentrates prepared in 28 % plasma/SSP+ were gamma-irradiated with 25-50 Gy on day 1 and then exposed to temperatures of either 18 °C or 28 °C for 3 hours without agitation (n=10) on day 2. Before and after the temperature excursions, platelets were stored under standard conditions at 22 °C with agitation. Platelet concentrates were sampled on day 1 prior to the temperature excursion, day 2 after the excursion, then on days 5 and 7. Platelets were tested using an array of *in vitro* assays designed to test platelet quality and functionality. Irradiated platelet concentrates stored at 22 °C with agitation served as a reference.

### Results

Following a 3 hour excursion at 18 °C there were no differences in the measured *in vitro* quality parameters compared with reference irradiated platelet concentrates ( $p>0.05$ ). In contrast, irradiated platelet concentrates stored at 28 °C for 3 hours displayed increased glucose consumption and lactate production immediately following the temperature excursion on day 2 ( $p<0.001$  and  $p=0.002$  respectively). However, there were no changes in ADP and collagen aggregation, hypotonic shock response, annexin V and viability ( $p>0.05$ ).

### Conclusion

Short term temperature excursions for 3 hours at 18 or 28 °C had little effect on *in vitro* platelet quality. This information may be of use when deciding the fate of a specially matched (e.g. HLA-matched) platelet component that has been stored outside the recommended temperature range for short periods of time.

**Keywords** platelets, temperature, irradiated

**Conflict of interest** No conflict of interest to disclose

Monday 29 October  
ANZSBT Presidential Symposium  
O025

0830-1000  
Room 219/220  
0848-0906

# **Glucose-containing Additive Solution Supports Platelet Recovery and *In Vitro* Quality Parameters Following Cryopreservation**

Lacey Johnson, Kelly M. Winter, Diana Vidovic, Shereen Tan, Denese C. Marks  
*Research and Development, Australian Red Cross Blood Service, Sydney, Australia*

## **Aim**

Platelets for transfusion are typically stored at 20-24 °C for up to 5 days. However, the shelf-life can be extended to 2 years when frozen at -80 °C in 5-6% dimethylsulfoxide (DMSO). Platelets are frozen in a hyperconcentrated state to minimise residual DMSO concentration in the transfused product. Consequently, these platelets require reconstitution upon thawing, which is typically carried out using a unit of plasma. However, reducing the plasma content in platelet products may provide both operational and clinical benefits. As such, the aim of this study was to determine whether the use of a glucose-rich additive solution (PAS-G) could replace plasma in the preparation and reconstitution of a cryopreserved platelet product, whilst maintaining *in vitro* platelet quality.

## **Methods**

DMSO (5% final concentration) was added to buffy coat-derived pooled leukoreduced platelet concentrates on day 2 following whole blood collection. The platelets were then hyperconcentrated by centrifugation (1250 x g) and frozen at -80 °C. The cryopreserved platelet units (n=12) were thawed at 37 °C, reconstituted in a unit of PAS-G (Pall Corporation) and stored at 22 °C with agitation. Platelet recovery and *in vitro* quality were examined prior to freezing, immediately after thawing and at 6 and 24 hours post-thaw.

## **Results**

The platelet recovery after thawing was greater than 70%, with each unit containing an average of  $252.5 \pm 35.9 \times 10^9$  platelets. The frozen platelets displayed a reduction in the surface expression of several key platelet receptors, including GPIb $\alpha$  and GPIIb. Further, freeze/thawing induced the expression of the platelet activation markers CD62P, CD63, annexin V and increased the secretion of multiple cytokines. Importantly, platelets were still capable of *in vitro* aggregation in response to both collagen and ADP, despite a measurable loss in activity.

## **Conclusion**

Preparation and reconstitution of cryopreserved platelets in PAS-G maintains post-thaw platelet recovery and *in vitro* quality for up to 24 hours, without the need for supplementation with plasma. This data supports a novel use for a new generation platelet additive solution and may lead to future improvements to cryopreservation techniques.

**Keywords** Cryopreservation, platelet concentrate, dimethylsulfoxide

**Conflict of interest** No conflict of interest to disclose

Monday 29 October  
ANZSBT Presidential Symposium  
O027

0830-1000  
Room 219/220  
0906-0924

## Identification of Potential Dendritic Cell and Monocyte Inflammatory Biomarkers to Predict Patient Outcomes in the Transfusion Setting

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<sup>1</sup>Research and Development, Australian Red Cross Blood Service, Brisbane QLD Australia, <sup>2</sup>Queensland University of Technology, Brisbane QLD Australia

### Aim

The basis for poor outcomes in some patients post transfusion remains largely unknown. In addition, there is evidence that the age of blood components transfused significantly affects patient outcomes. An in vitro whole blood model of transfusion was utilised to investigate potential myeloid dendritic cell (DC) and monocyte inflammatory markers that may predict patient outcomes in the transfusion setting.

### Methods

ABO-compatible leukodepleted packed red blood cells (PRBC) were cultured with freshly collected "recipient" whole blood at 25% blood-replacement-volume for 6hrs. PRBC were stored at 4°C and utilised in the transfusion model at various time points during storage until expiry (Day (D) 2, 14, 28 and 42). In parallel, LPS or Zymosan (Zy) were added to model infection. Recipients were maintained for the duration of each PRBC time course (2 recipients, 4 PRBC units, n=8). Recipient DC and monocyte production of IL-6, IL-10, IL-12, TNF-α, IL-1α, IL-8, IP-10, MIP-1α, MIP-1β, MCP-1) were determined via flow cytometry. Changes in immune response were calculated by comparison to a parallel "no transfusion" control (Wilcoxin matched pairs, 95% CI). Influence of storage age was calculated using ANOVA.

### Results

Exposure to PRBC resulted in significant suppression of DC and monocyte inflammatory responses. In particular DC and monocyte production of MIP-1α and IL-1α were significantly reduced, regardless of PRBC storage. Storage-independent PRBC-mediated suppression of DC and monocyte IL-1α was also evident in cultures co-stimulated with Zy. In addition, a significant reduction in both DC and monocyte TNF-α, IL-6, MIP-1α, MIP-1β and IP-10 were evident following PRBC exposure in co-culture with either LPS or Zy as models of infection. PRBC storage attenuated monocyte TNF-α production when co-cultured with LPS (P<0.01).

### Conclusions

The complexity of the transfusion context was reflected in the whole blood approach utilised. Modulation of DC and monocyte inflammatory response was largely independent of PRBC storage. Significant suppression of DC and monocyte immune regulators may contribute to poor patient outcomes. We propose TNF-α, IL-1α, IL-6 and MIP-1α as potential biomarkers of patient outcomes post-transfusion.

**Keywords** Dendritic Cell, Monocyte, Immune Suppression, Blood Transfusion

**Conflict of interest** No conflict of interest to disclose

Monday 29 October  
ANZSBT Presidential Symposium  
O028

0830-1000  
Room 219/220  
0924-0942

**Variation in Transfusion Practice in Cardiac Surgery: A Report from the Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) Cardiac Surgery Database**

Zoe McQuilten<sup>1,2,3</sup>, Nick Andrianopoulos<sup>1</sup>, Erica Wood<sup>1,2,3</sup>, Merrole Cole-Sinclair<sup>4</sup>  
John McNeil<sup>1</sup>, Peter Cameron<sup>1</sup>, Christopher Reid<sup>1</sup>, Julian Smith<sup>1,3</sup> and Louise Phillips<sup>1</sup> on behalf of the Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) Steering Committee.

<sup>1</sup>Monash University, <sup>2</sup>Australian Red Cross Blood Service, <sup>3</sup>Monash Medical Centre and <sup>4</sup>St Vincent's Hospital, all in Melbourne, Victoria, Australia

**Aim**

To measure variation in transfusion of red blood cells (RBC), platelets (PLT), fresh frozen plasma (FFP) and cryoprecipitate (CRYO) in cardiac surgery.

**Methods**

Procedures on all adult patients who underwent cardiac surgery from January 2005 – December 2011 at 25 Australian hospitals as recorded in the ANZSCTS database were included. Summary statistics and multiple logistic regression were used to examine variation and possible association of patient and hospital level factors with the following transfusion outcomes: one or more RBC, PLT, FFP and CRYO and 5 or more RBC from surgery until hospital discharge.

**Results**

In 43482 recorded procedures 55% were isolated coronary artery bypass graft (CABG), 17% were isolated valve, 11% were CABG and valve, and 17% were other procedures. Rates of transfusion following adjustment for patient and procedure factors varied across hospitals for one or more RBC from 22% to 67%, 5 or more RBC 5% to 25%, one or two RBC 10% to 34%, one or more PLT 11% to 39%, one or more FFP 11% to 48% and one or more cryoprecipitate 1% to 20%. The difference in transfusion rates was not accounted for by hospital level factors (odds ratio, 95% confidence interval) including for one or more RBC: private vs. public (0.7, 0.2 – 2.4), non-teaching vs. teaching (1.3, 0.4 – 4.5), or state (1.5, 0.9 – 2.7)

**Conclusions**

Substantial variation in transfusion of all blood components and large volume RBC transfusion was identified and remained even after adjustment for patient-level factors. Hospital-level factors examined did not account for the observed differences between institutions. Further work to elucidate factors contributing to this variation and how it may influence patient outcomes is warranted.

**Keywords** Transfusion, Cardiac surgery

**Conflict of interest** No

**Monday 29 October**  
**ANZSBT Presidential Symposium**  
**O029**

**0830-1000**  
**Room 219/220**  
**0942-1000**

**Clinical Coding Data to Describe Critical Bleeding - Lottery or Mother Lode?**

AJ Zatta<sup>1</sup>, Z McQuilten<sup>1,2</sup>, N Aoki<sup>1</sup>, L Stevenson<sup>3</sup>, K Badami<sup>4</sup>, K Davis<sup>5</sup>, N Andrianopoulos<sup>1</sup>, P Cameron<sup>1</sup>, J Isbister<sup>6</sup>, L Phillips<sup>1</sup>, E Wood<sup>1</sup> *on behalf of the Massive Transfusion Registry Steering Committee*

<sup>1</sup>Monash University<sup>2</sup>Australian Red Cross Blood Service and <sup>3</sup>Blood Matters Program, Victorian Department of Health, all in Melbourne, VIC <sup>4</sup>New Zealand Blood Service, Auckland <sup>5</sup>Royal Adelaide Hospital, Adelaide, SA <sup>6</sup>University of Sydney, Sydney, NSW, Australia.

**Background**

Clinical coding data, collected primarily for hospital funding, are increasingly being studied to understand blood utilisation according to clinical demand.

**Aim**

To examine the validity of using clinical coding data to determine types of critical bleeding events (CBE) requiring massive transfusion (MT)

**Results**

Data regarding MT indications were collected from medical records for 282 patients from 33 hospitals across Australia & New Zealand. Data collectors categorised types of CBEs into defined bleeding contexts (Table 1). Admission DRG and ICD10 codes were extracted separately. ICD10 coding logic, using diagnosis and procedure codes, was developed to assign patients a bleeding context. The DRGs were similarly grouped. Both ICD10 and DRG were compared against the data collectors' interpretation. Excellent agreement was found between the bleeding context assigned by data collectors and ICD coding logic. In contrast, poorer agreement was achieved between data collectors and DRG.

Bleeding context derived from medical record (n)	Observed agreement with the medical record	
	of ICD10 coding logic	of DRG classifications
Trauma (46)	91.3%	56.5%
Cardiac surgery (34)	88.2%	52.9%
Obstetric haemorrhage (43)	97.7%	95.3%
Vascular surgery (43)	69.8%	62.8%
Liver surgery (6)	50.0%	0.0%
Gastrointestinal haemorrhage (64)	82.8%	8.8%
<b>% Agreement (kappa value)</b>	<b>82.6% (0.79)</b>	<b>53.7% (0.48)</b>

Table 1: Agreement between bleeding context identified from different data sources

**Conclusions**

ICD10 coding logic developed for the Massive Transfusion Registry is reliable and sufficiently accurate to broadly categorise CBEs.

**Keywords** Critical Bleeding, Massive Transfusion, Clinical Coding Data

**Conflict of interest** This research was partially supported by CSL Biotherapies. The company had no role in analysing the data or preparing the abstract.

Monday 29 October

APSTH/ASTH Free Communications 1: Coagulation Science

O030

0830-1000

Room 210

0830-0845

## Discoid Platelet Aggregations Visualized by in vivo Molecular Imaging, and Contribution of Inflammatory Cytokines

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### Aim

To elucidate the underlying mechanisms of cardiovascular diseases based on chronic inflammation, it is vital to examine the multi-cellular kinetics in living animals, and the thrombosis mechanism without endothelial cell (EC) disruption remains unclear. Therefore, we developed in vivo imaging technique based on single- and multi-photon microscopy, and we assessed dynamic cellular interplay in thrombosis models (Fig 1, 2008 JCI, 2012 Blood). We visualized that rapidly developing thrombi composed of discoid platelets without EC disruption was triggered by ROS (Fig 2).

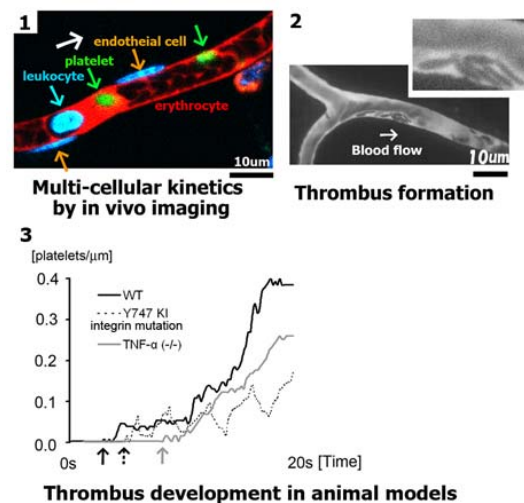
### Results

Using this technique, we elucidated that Lnk (adapter protein) regulates integrin signaling leading to stabilization of developing thrombus (2010 JCI). In addition, we established the culture system of human iPS-derived platelets, and we confirmed their functional role in vivo (2010 JEM). We also elucidated the contribution of inflammatory cytokines, ROS, and integrin signaling to our thrombosis models (2011 Blood). The inflammatory cytokines including TNF- $\alpha$  and IL-1 could be key components of the EC response (Fig 3). Thrombus formation was then initiated by the binding of platelet GPIb- $\alpha$  to endothelial vWF. Integrin activation was required for late phase thrombus stability.

### Conclusion

In sum, our imaging system can be a powerful tool to analyze thrombus formation. We clarified the mechanism of discoid platelet aggregations on undisrupted endothelium. The initial platelet aggregation subsequently leads to irreversible integrin- and actin-dependent thrombus development. Inflammatory cytokine signaling in ECs also played pivotal role.

**Keywords** In vivo imaging, platelet, inflammation. **Conflict of interest** No



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0834-0900

### 14-3-3 $\zeta$ is Essential for the PAR4-Dependent Calcium Flux Triggering the Procoagulant Function of Murine Platelets

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#### Aim

The 14-3-3 family of adaptor proteins regulate diverse cellular functions including cell signalling, proliferation and apoptosis. The in platelet abundant 14-3-3 $\zeta$  isoform has previously been implicated in regulating adhesion and signalling by Glycoprotein (GP)Ib $\alpha$ . The aim of this study is to identify novel roles for 14-3-3 $\zeta$  in regulating haemostasis and thrombosis *in vivo*.

#### Method

Here, by using 14-3-3 $\zeta$ <sup>-/-</sup> mice, we measured platelet adhesion, aggregation, thrombin generation, calcium flux, P-selectin expression, GPIIb/IIIa activation and phosphatidylserine (PS) exposure *in vitro*. Collagen+epinephrine were injected I.V. to induce pulmonary embolism *in vivo*.

#### Results

Surprisingly, 14-3-3 $\zeta$ <sup>-/-</sup> platelets have no defect in GPIb $\alpha$ -adhesion or shear-dependent thrombus formation *in vitro*, however they exhibit a specific defect in thrombin-induced platelet activation. 14-3-3 $\zeta$ <sup>-/-</sup> platelets have diminished thrombin-dependent cytosolic calcium flux. This calcium signalling defect was associated with defective platelet aggregation, P-selectin expression and GPIIb/IIIa activation by threshold dose of thrombin or PAR-4 peptide. High-dose thrombin+CRP resulted in reduced PS-exposure and procoagulant function in 14-3-3 $\zeta$ <sup>-/-</sup> platelets. *In vivo*, thrombin generation and emboli were decreased in the pulmonary embolism model in 14-3-3 $\zeta$ <sup>-/-</sup> mice.

#### Conclusion

These studies define a novel and pathophysiologically role for 14-3-3 $\zeta$  in platelets regulating cytosolic calcium flux and signalling downstream of the PAR-4 receptor.

**Keywords** 14-3-3, platelets, thrombin

**Conflict of interest** No

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0900-0915

## The Effect of Factor Xa, Thrombin and New Anticoagulants on Human Platelet Glycoprotein VI Expression and GPVI-mediated Coagulation

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### Aim

Human platelet glycoprotein VI (GPVI) plays a critical role in platelet activation and procoagulant response. Changes in platelet GPVI receptor density, via ectodomain shedding, may alter thrombotic tendency. The aims of our research are to:

- determine if coagulation induces GPVI ectodomain shedding
- delineate if coagulation-induced GPVI shedding can be modulated by anticoagulants with different therapeutic targets, including thrombin and FXa

### Method

Coagulation was induced by recalcification of PRP with 20 mM CaCl<sub>2</sub> for 75 min. Subsequent flow cytometric analysis of platelet GPVI was performed on treated PRP from 11 healthy donors using a mAb directed against the human GPVI ectodomain, 4B8. Upon recalcification, samples were also centrifuged to yield supernatant for ELISA measurement of the shed GPVI fragment, sGPVI. Thrombin generation was assessed using the calibrated automated thrombogram (CAT).

### Results

Recalcification resulted in a mean loss of platelet GPVI of 68.3%, measured by flow cytometry, compared to untreated samples. This loss was inhibited by the FXa inhibitor, enoxaparin, in a dose dependent manner, but not by the thrombin inhibitor, lepirudin. Similarly, thrombin generation by CAT was completely suppressed by both the thrombin inhibitor, dabigatran and the FXa inhibitor, rivaroxaban, but only pre-incubation with rivaroxaban resulted in significant inhibition of sGPVI generation upon recalcification.

### Conclusion

Coagulation induces GPVI ectodomain shedding, shown by loss of platelet GPVI by flow cytometry, and a corresponding rise in sGPVI measured by ELISA. FXa inhibitors reduce coagulation-induced GPVI shedding, whereas thrombin inhibitors do not. Coagulation-induced GPVI ectodomain shedding is a potentially important homeostatic mechanism against further thrombus growth, and may be modulated by new anticoagulants via "off-target" effects on platelet function, potentially contributing to the efficacy and/or safety profile of these medications.

**Keywords** platelet glycoprotein VI, thrombin, Factor Xa

**Conflict of interest** No conflict of interest to disclose



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0915-0930

### miR-494 Downregulates Protein S Expression

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#### Background

Increased oestrogen levels from oral contraceptives, oestrogen replacement therapy, or pregnancy have been associated with acquired Protein S (PS) deficiency and increased risk of thrombosis. However, the mechanism of hormonal regulation of PS expression has not been fully elucidated. As oestrogen signalling is known to regulate microRNA (miR) production as an indirect means of modulating target gene expression, we investigated the regulation of Protein S expression by miR-494.

#### Methods

*In silico* analysis of *PROS1* 3' untranslated region (UTR) identified two binding sites for miR-494, and direct targeting of *PROS1* by miR-494 was determined by dual luciferase reporter assays in HuH-7 and HeLa cells transiently transfected with a firefly luciferase reporter containing the *PROS1*-3'UTR sequence, a *Renilla* luciferase control vector, pRL-SV40, and increasing concentrations of miR-494 or negative control miR precursors. Reporter vectors containing mutated miR-494 binding sites were also generated (*PROS1* 3'UTR-Mut1 and *PROS1* 3'UTR-Mut2) and analysed by dual luciferase reporter assays. Effects of oestrogen on miR-494 and *PROS1* mRNA levels in HuH-7 cells were determined by quantitative PCR, and ongoing work is characterising oestrogen-mediated changes to secreted PS levels in culture supernatant of HuH7 cells.

#### Results

Cotransfection of miR-494 inhibited the relative luciferase activity of *PROS1* 3'UTR in a dose-dependent manner by up to 40% compared to controls in HuH7 and HeLa cells, and luciferase activities of *PROS1* 3'UTR-Mut1 showed a lower level of inhibition compared to that of unmutated *PROS1* 3'UTR. Western blotting for secreted PS from HuH7 conditioned culture medium showed that miR-494 reduced PS levels, and endogenous levels of miR-494 in HuH7 cells were significantly ( $p=0.04$ ) increased following treatment with 17 $\beta$ -estradiol.

#### Conclusions

The results in this study indicate that miR-494 interacts with the 3'UTR of *PROS1* at multiple binding sites and continues to exert its inhibitory effects via its undisrupted binding site. Our findings also provide evidence for posttranscriptional regulation of PS expression by miR-494 via oestrogen signalling.

**Keywords:** Protein S, microRNA, oestrogen

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0930-0945

## Cell Death Imager-1 Ligands in Apoptotic Platelets

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### Background and Aim

Cell Death Imager-1 (CDI-1) is a tri-peptide trivalent arsenical compound that when conjugated with reporter groups is able to distinguish between activated and apoptotic platelet populations *in vitro*. It enters the cell during mid to late phases of apoptosis where there is loss of plasma membrane integrity and is retained in the cytosol via the interaction of the trivalent arsenical moiety with closely spaced thiol proteins. Fluorescently tagged CDI-1 binds to distinct proteins in apoptotic platelets. Our aim was to identify these platelet ligands.

### Method

Freshly washed human platelets were prepared from whole blood and treated with 30  $\mu$ M of ABT 737 at 37°C for two hours. Apoptosis in the treated population was confirmed by flow cytometry using Annexin V and CDI-1 binding. Apoptotic platelets and resting control platelets were incubated with biotinylated CDI-1 or control compound that lacks the CDI-1 reactive arsenical moiety, then washed three times. Platelet lysates were prepared and binding proteins pulled out on streptavidin-agarose. The binding of CDI-1 to protein is mediated by covalent bonding to closely spaced protein dithiols. Protein ligands were competitively eluted from CDI-1 using 2 mM of the small dithiol, 2,3 dimercaptopropanol, and separated on gel electrophoresis. The identity of the major binding proteins in the apoptotic group were determined by mass spectrometry.

### Results

The major binding CDI-1 binding proteins in apoptotic platelets are the intra-cellular proteins, clathrin-1 and hexokinase-1.

### Conclusion

CDI-1 targets distinct protein ligands in apoptotic platelets of which clathrin 1 and hexokinase-1 are prominent constituents. Clathrin-1 plays a role in vesicle formation during endocytosis and hexokinase-1 is involved in the mitochondrial production and exchange of energy. These findings may have implications for these processes during apoptosis.

**Keywords :** Platelet Apoptosis, Cell Death Imager-1, Protein Ligand

**Conflict of interest:** No

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O035

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### Increased Thrombin Generation in a Mouse Model of Cancer Cachexia

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#### Aim

Patients with malignancies often suffer from cachexia and thromboembolism. No direct link between cachexia and hypercoagulability has been documented, although both are associated with high levels of inflammatory markers. Therefore we examined thrombin generation in Colon 26 mice, a tumour model of cancer cachexia characterised by inflammatory markers including tumour-derived IL-6.

#### Methods

Four groups of immuno-competent mice were studied: i) Cachectic mice engrafted subcutaneously with murine Colon 26 (C26) carcinoma cells (n=12); ii) Non-tumour bearing controls (n=10); iii) Mice engrafted with a non-cachectic C26 variant (n=5); iv) Non-tumour bearing pair-fed group with food intake matched to cachectic mice (n=6). Citrated blood was collected after 14 days and a modified calibrated automated thrombogram assay performed to measure mean and peak thrombin generation potential. Plasma IL-6 levels, platelet count and erythrocyte sedimentation rate (ESR) were also measured. Hepatic expression of coagulation factors was examined by microarray transcript profiling.

#### Results

Cachectic C26 mice had a mean weight loss at harvest of 16% by day 14. Pair-fed mice had an equivalent weight loss. Cachectic mice had a mean endogenous thrombin potential of  $522.1 \pm \text{SD } 65.8$  arbitrary units (AU) which was significantly higher ( $p < 0.001$ ) than non-tumour bearing control mice [ $197.1 \pm 16.1$  AU], and peak thrombin was also elevated in cachectic relative to control mice ( $p < 0.001$ ). Surprisingly, lagtime to thrombin generation was also increased in cachectic mice ( $p < 0.001$ ). No significant differences were observed between non-cachectic C26 tumour-bearing, mock-engrafted and pair-fed groups. Circulating IL-6 levels were markedly elevated in cachectic mice at 216 pg/mL, being barely detectable in the other groups. Platelet counts were elevated ( $p < 0.001$ ) and ESR markedly increased in cachectic mice with prominent rouleaux. Altered expression of many genes associated with coagulation was evident in livers of C26 mice.

#### Conclusion

Cachectic C26 mice, characterised by IL-6 production and reduced food intake, exhibit increased thrombin generation potential compared with non-cachectic tumour-bearing mice, mock-engrafted controls and pair-fed mice. Processes specific to cachexia may contribute to cancer-related thrombogenicity.

**Keywords.** Hypercoagulability; Inflammation; Cancer **Conflict of interest** No

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APSTH/ASTH Free Communications 2: Bleeding Disorders

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0830-1000

Room 211

0830-0845

**Autoantibody Against GPIIb/IX is an Independent Predictive Factor for Poor Response to Intravenous Immunoglobulin G Therapy in Adults with Severe Immune Thrombocytopenia**

Jun Peng, Shi-hui Ma, Yu Hou, Xiao-yuan Dong, Jing Liu, Yuan-yuan Zhu, Cheng-shan Guo, Xi-min Liu, Rui-rong Xu, Ting Niu, Xiao-min Wang, Yan Shi, Yuan Yu, Wen Wang, Ping Qin, Heyu Ni, Ming Hou

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**Aim**

Immune thrombocytopenia (ITP) is a common autoimmune bleeding disorder, in which platelet surface GPIIb/IIIa and GPIb/IX are the two most commonly targeted autoantigens. Our previous studies in animal models of ITP demonstrated that intravenous immunoglobulin G (IVIG) could protect against anti-GPIIb/IIIa-mediated thrombocytopenia but failed to ameliorate ITP induced by most anti-GPIb/IX antibodies. This multi-center human study was to evaluate the association between the specificity of anti-platelet autoantibodies and response to IVIG treatment in a cohort of 138 previously untreated adults with severe ITP.

**Method**

Patients received 0.4g of IVIG/kg daily for 5 consecutive days. The criteria for an initial response were platelet counts  $\geq 30 \times 10^9/L$  and doubling of baseline counts within 7 days of dosing, and absence of bleeding.

**Results**

Among the 55 patients who had anti-GPIb/IX antibodies, only 23 (41.8%) achieved a response, as compared with 66 of 83 patients (79.5%) who were negative for anti-GPIb/IX antibodies ( $P < 0.001$ ). No significant difference in response was observed between patients who had autoantibodies to GPIIb/IIIa (62.3%) and those without anti-GPIIb/IIIa antibodies (66.7%;  $P = 0.59$ ). Logistic regressions including main effects and the interaction between these two antibodies showed that there was no influence of anti-GPIIb/IIIa antibodies on the effects of anti-GPIb/IX antibodies with regards to their association with IVIG response.

**Conclusion**

In adults with severe ITP, the presence of autoantibodies to GPIb/IX is an independent predictive factor for poor response to IVIG treatment. Identifying these non-responders is important to avoid this ineffective treatment and decrease unnecessary IVIG consumption.

**Key Words:** Immune Thrombocytopenia; Intravenous Immunoglobulin G; Autoantibody

**Conflict of interest** No

Monday 29 October

APSTH/ASTH Free Communications 2: Bleeding Disorders

O037

0830-1000

Room 211

0845-0900

### Laboratory Investigation of TTP: Data from the Australian Registry

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#### Aim

Advances in the understanding of the pathophysiology of thrombotic thrombocytopenic purpura (TTP) have led to testing using ADAMTS13 assays. ADAMTS13 <10% has been shown to be highly specific for idiopathic TTP and predicts a higher relapse rate. Patients with thrombotic microangiopathy due to other causes have worse outcomes than idiopathic cases. Here we present laboratory data from the Australian TTP registry.

#### Results

60 patients were registered from 14 sites; 3 were excluded for incomplete data, leaving 64 episodes in 57 pts. Median age was 40yrs (14-81y), 57% female, 17% relapses, 41% idiopathic & 59% with ≥1 identified precipitant. ADAMTS13 levels were available in 36 cases (16 idiopathic, 20 secondary). The proportion of idiopathic cases with ADAMTS13 <10% was higher than secondary cases (69% vs. 30%; p=0.02). The secondary cases with ADAMTS13 <10% included infection (15%) and autoimmune (15%). LDH at presentation was lower in the idiopathic vs. secondary group (569 vs. 814; p=0.01), but Hb (median 106.5 vs. 89.5), plt (median 21 vs. 29) and creatinine (median 0.1 vs. 0.14) were not significantly different (all p≥0.1). Gastro-renal presentations were less common in cases with ADAMTS13 <10% (29%) than >10% (79%) (p=0.003), but there was no significant difference in neurological presentations (65% vs. 53%; p=0.46). Patients with ADAMTS13 <10% had better outcomes (complete remission without impairment (CR) 69%; death 0%) than >10% (CR 39%, death 28%).

#### Conclusion

Apart from LDH, laboratory parameters were similar in idiopathic and secondary cases. ADAMTS13 was much more likely to be <10% in the idiopathic group, although some secondary cases had levels <10%. There was no difference in neurological presentations but gastro-renal presentations were more common in those with ADAMTS13 >10%. Outcomes were worse in ADAMTS13 >10%, consistent with the underlying secondary causes.

**Keywords** thrombotic thrombocytopenic purpura, registry, ADAMTS13

**Conflict of interest:** The registry receives partial support from Alexion Pharmaceuticals Australasia. The company had no role in data analysis or preparing the abstract.

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**Novel Mutations of the von Willebrand Factor Gene in Patients with von Willebrand Disease seen in Taiwan**

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**Introduction**

VonWillebrand disease (vWD) is considered to be the commonest hereditary bleeding disorder, it is probably also very common in Taiwan but has not been well studied. We aim to present clinical, laboratory and genetic studies of vWD seen in Taiwan.

**Methods**

A revised classification of vWD described by Salder JE (1994) and criteria for the diagnosis of vWD and its subtypes given by Federici AB (1998) were used. Exons and its junction of von Willebrand factor (vWF) gene were amplified and sequenced. Informed consent was obtained from patients and family members.

**Results**

We have seen 54 patients (53 families) of type 1, 55 patients (25 families) of type 2 and 10 patients (9 families) of type 3 vWD from 1990 to 2012. Their mean ages (ranges) (years) of diagnosis and sex were 28.6 (8-56), M/F 19/35 in type 1, 26.4 (1-67) and M/F 21/34 in type 2 and 13.2 (2-30) and M/F 3/7 in type 3 vWD. Most associated symptoms were bleeding from the wound (66.7% in type 1, 83.6% type 2 and 100% type 3), bleeding after dental extraction (70.2% type 1, 81% type 2 and 100% type 3), epistaxis (35.2% type 1, 78.2% type 2 and 77.8% type 3), gum bleeding (24.1% type 1, 72.7% type 2 and 88.9% type 3) and menorrhagia (50% type 1, 84.5% type 2, 100% type 3), the latter might lead to a higher incidence of female patients. Six different mutations from 6 index patients with type 1, 15 different mutations from 19 index patients with type 2 and one homozygous mutation from one patient with type 3 vWD were identified. Among them, 8047-8051 delGAGTA, InsCCCT, Lys2621Stop, Cys2533Phe, 1817-1818 delG and Arg2287Gln identified in type 1; Leu1696del, Glu1519del, Leu1276Gly and IVS30 -1~-2 delCT in type 2 and Tyr812Stop in type 3 vWD were found to be novel.

**Conclusion**

The phenotypic and genetic characteristics of our patients with vWD are not different from those observed in Western countries. Novel mutations were identified in 5, 4 and 1 in type 1, type 2 and type 3 vWD, respectively.

**Keywords** vWD, vWF gene mutation, Taiwan

**Conflict of interest** No

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0915-0930

# **Diversity of Glanzmann Thrombasthenia in China: Seven Novel Mutations in $\alpha$ IIb and $\beta$ <sub>3</sub> genes Identified Among 21 Unrelated Patients**

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## **Aim**

To investigate the phenotypes and genotypes in 21 Chinese pedigrees with GT, and identify the molecular mechanism of GT.

## **Results**

All the probands had normal platelets count and coagulation profiles, but their BT prolonged and platelets response to various aggregation inducers impaired except for ristocetin. Flow cytometry showed that platelet surface expression of  $\alpha$ IIb $\beta$ <sub>3</sub> was < 5%, 5% - 25%, and > 50% of Controls in 15, four, and two patients, respectively. Gene analysis revealed 15 mutations located in  $\alpha$ IIb gene, eight point mutations (F331L, D560A, E48X, Q747P, L721R, P126H, Q860X and R553X), four splicing mutations (IVS20+2T>C, IVS20-1G>A, IVS29+1G>A and IVS15(-1)delG) and three deletion mutations (69-79del, 2930del and 1631-1732del). There were five mutations in  $\beta$ <sub>3</sub> gene including three point mutations (C374Y, C437F and Q272X) and two deletion mutations (2930delG and 1748delC). Interestingly, one patient had four novel homozygous mutation (1528□1529insT, 1530A>C, 1531G>C and 1532A>T) in exon 15 of  $\alpha$ IIb simultaneously. To analyse the effects of these mutations on  $\alpha$ IIb $\beta$ <sub>3</sub> surface expression, the wild type or mutant  $\alpha$ IIb cDNAs were transfected into CHO cells together with a wild type  $\beta$ <sub>3</sub> cDNA. The F331L mutation may affect platelet activation and fibrinogen binding to  $\alpha$ IIb $\beta$ <sub>3</sub> causing the Type III GT phenotype with D560A.

## **Conclusion**

Four homozygous mutation (1529\_1530insT, 1531A>C, 1532G>C and 1533A>T) simultaneously in one patient and 7 novel mutations above are the gene defects of GT. While six novel candidate disease-causing mutations, (F331L, D560A, IVS20+2T>C, 1631-1732del) in  $\alpha$ IIb and (Q272X and 1748delC) in  $\beta$ <sub>3</sub> gene, had never described before.

**Keywords** Glanzmann thrombasthenia□ Gene mutation□ Integrin  $\alpha$ IIb $\beta$ <sub>3</sub>□

**Conflict of interest** No conflict of interest to disclose

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0930-0945

**Molecular Characterisation of Five Mutations Causing Factor X Deficiency in Three Chinese Pedigrees**

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**Aim**

In the present study, we investigated the clinical manifestations, laboratory phenotype and genotype in three probands of unrelated Chinese families and their heterozygous relatives to identify the molecular mechanism of Factor X deficiency.

**Results**

The genetic characterisation revealed five different missense mutations: p.Ser425Pro (proband 1, homozygous), p.Ala-29Pro, p.Phe324Leu (proband 2, heterozygous), p.Ala235Thr, p.Arg347Cys (proband 3, heterozygous). Besides p.Arg347Cys, the other five were all proved to be novel. Sitedirected mutagenesis of FX cDNA was used to introduce the five missense mutations, and wild-type as well as mutant FX proteins were expressed by transient transfection in 293T cells. The mutant FX antigen levels were nearly normal compared with the wild type in cell lysates except the mutant p.Ala-29Pro, which was 74.9±1.93% of the wild-type. However, except for the mutant p.Arg347Cys, the decreased antigen level of FX in the conditioned medium suggested that there was either secretion dysfunction or increased intracellular degradation caused by the missense mutations. The antigen level of the mutant p. Ala-29Pro and p.Phe324Leu in the conditioned medium was moderately decreased, which was 63.5±1.74% and 61.2±1.70% of the wild type, respectively. Evaluation of the coagulant activity of the wild-type and the mutant FX proteins demonstrated that all the mutant proteins have dramatically decreased activity.

**Conclusion**

The mutation p.Ser425Pro and p.Ala235Thr could cause type I factor X deficiency (CRM<sup>-</sup>), while the mutation p.Arg347Cys was associated with type II factor X deficiency (CRM<sup>+</sup>). At the same time, the mutation p.Ala-29Pro and p.Phe324Leu was in agreement with type III factor X deficiency (CRM<sup>Red</sup>).

**Keywords** novel mutation, FX deficiency, molecular mechanism, phenotype-genotype

**Conflict of interest** No conflict of interest to disclose



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# **Analysis of Urgent Reversal of Vitamin K Antagonist Anticoagulation with Prothrombin Complex Concentrates – Implications for Novel Oral Anticoagulants**

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## **Aim**

There is increasing use of systemic anticoagulation in Australia, with both vitamin K antagonists (VKA) and novel oral anticoagulants (NOAC). We sought to determine the INR at which urgent reversal of VKA with prothrombin complex concentrates (PCC) was initiated for both acute bleeding and for prophylaxis prior to invasive procedures or patients with high bleeding risk. Secondary aims include the indication for anticoagulation, presence of acute renal failure, blood product use and mechanism/site of bleeding.

## **Methods**

Single centre retrospective audit. Data extraction of pathology laboratory system with correlation with medical record coding database.

## **Results**

393 patients received 433 PCC doses over a two year period. The median age was 74.2 years (5.5 to 99 years). Atrial fibrillation was the most common indication for anticoagulation (65%). Acute renal failure (eGFR <60mL/min) was present in 43% of patients at the time of administration of Prothrombinex-VF. A total of 212 patients (53%, 232 infusions) received PCC for acute bleeding events. Intracranial bleeding was the most common bleeding site (43.5%). According to indication for anticoagulation and initial INR at the time of PCC administration, most patients were not over-anticoagulated (sub-therapeutic 16.8%, therapeutic 40.5%, supra-therapeutic 42.7%). There was a trend towards higher red cell usage in bleeding patients with supra-therapeutic INRs, but no statistically significant difference in 30 day mortality between the therapeutic and supra-therapeutic INRs.

## **Conclusion**

The majority of patients at our institution receive Prothrombinex-VF for therapeutic or sub-therapeutic INRs. Acute renal failure is present in a significant number of these patients which may prolong the clinical effects of NOAC. There is no difference in 30 day mortality in acutely bleeding patients stratified by the INR at time of PCC administration.

**Keywords** Warfarin, Prothombin complex concentrate, warfarin reversal

**Conflict of interest** No conflict of interest to disclose

**Monday 29 October**

**Nurses Free Communications 1: Hot Issues in Clinical Practice**

**O042**

**0830-1000**

**Room 212**

**0830-0845**

**Minimal Space, No Extra Nurses But Lots of Patients – Finding a New Way to Increase Capacity**

Lucy Burns

*St George Hospital, Kogarah, NSW, Australia*

**Aim**

To increase the capacity of the Haematology Oncology Day Centre (HODC) and reduce the time between referral and chemotherapy

**Results**

An acuity scale was introduced into HODC in May 2011 and demonstrated an immediate impact. Within a few months the waiting time had reduced from 13 -14 days on average to below 10 days. Additionally, the number of patients who were ready to treat but breached the 14 day benchmark had been consistently in double figures but, with the introduction of the scale this number came down rapidly and is now settled around five per month. This is a significant reduction on the baseline data.

**Conclusion**

Use of an acuity based booking system in ambulatory chemotherapy units is an effective way to increase capacity and reduce waiting times. It is also cost neutral and easily transferable to other centres.

**Keywords** Acuity scale; increased capacity; decreased waiting time for chemotherapy

**Conflict of interest** No

Monday 29 October

Nurses Free Communications 1: Hot Issues in Clinical Practice

O043

0830-1000

Room 212

0845-0900

**Outpatient based Haplo-identical Peripheral Blood Stem Cell Transplantation: A Single-centre Experience**

Stephanie Deren

*Blood and Marrow Transplant Service, Westmead Hospital, Sydney, NSW, Australia***Aim**

To review the experience of Westmead Hospital in commencing a Haplo-identical reduced-intensity peripheral blood stem cell transplantation service in an outpatient based setting. We present a case series of 8 patients treated to date, their outcomes and challenges.

**Results**

The use of GCSF mobilised peripheral blood stem cells from haplo-identical related donors presents an alternative for patients with poor prognosis haematological malignancies who have no HLA-identical match. When utilising a reduced-intensity conditioning regimen, these patients can be successfully managed in the outpatient setting, with admission to an inpatient unit only if clinically indicated.

For those patients in the case study, median time to neutrophil engraftment was day +18. 2 of the 8 patients required no admission from conditioning to day +100. The remaining 6 patients were admitted with neutropaenic fever for variable durations in the post transplant phase.

We found that nursing the neutropaenic post transplant patient in the outpatient setting can present challenges in the co-ordination and management of care, and ensuring the appropriate assessment of the patient. It is important to have educated and skilled nursing staff experienced in transplant nursing to guarantee the patient is correctly treated and unnecessary admissions are avoided.

**Conclusion**

The use of GCSF mobilised peripheral blood cells from haplo-identical related donors presents a viable alternative for patients with no HLA -identical match. Our experience has shown that those patients receiving haplo-identical transplants with reduced-intensity conditioning therapy can be successfully managed in an outpatient based setting by experienced nursing staff.

**Keywords** Haplo-identical, stem cell transplantation, nursing**Conflict of interest** No conflict of interest to disclose

**Monday 29 October**

**Nurses Free Communications 1: Hot Issues in Clinical Practice**

**O044**

**0830-1000**

**Room 212**

**0900-0915**

**Clinical Networks: Challenges for the Successful Development of a Network Focusing on Malignant Haematology**

Tracy Clarke

*BMT Network, Agency for Clinical Innovation (ACI), Chatswood, NSW, Australia*

**Aim**

The goal of this project is to provide recommendations for the expansion of the ACI Blood and Marrow Transplant (BMT) Network into malignant haematology and develop a model of care for haematological malignancies commencing with AML.

**Method**

Consultation with haematologists, nurses, allied health staff and patients across NSW was undertaken to identify the challenges they face, the goals they see as important and their key priorities for enhancing services and ensuring positive outcomes for patients in an expanded BMT Network. The typical haematology patients' journey was mapped for rural and metropolitan patients and a literature review of similar models was undertaken.

**Result**

Key themes within each discipline consulted are identifiable across sites within NSW providing care to patients with a haematological malignancy. These emerging themes will provide the framework for developing of a Model of Care for patients with AML and priority areas for development within the network.

The themes include; improved education regarding haematological illnesses disease processes for nurses and allied health staff, reducing the fragmentation of molecular and cytogenetic testing in NSW and support for a uniformed data collection process that will provide relevant and recent outcome measures.

**Conclusion**

The NSW BMT Network has been highly successful and provides direction and support for the coordination of BMT planning and service delivery within a framework promoting access to, safety and efficiency of services in NSW. The success of a clinician led network for malignant haematology is reliant on clinician buy-in and engagement. Strategies for success are based on ensuring that clinicians feel their concerns relating to patient care are addressed and consultation is inclusive, transparent and collaborative and that discussions and activities are relevant and important to the clinicians.

**Key words:** Clinical networks, patient outcomes

**Conflict of interest** No

**Monday 29 October**

**Nurses Free Communications 1: Hot Issues in Clinical Practice**

**O045**

**0830-1000**

**Room 212**

**0915-0930**

**Establishing Best Practice in Nursing Interventions Post Bone Marrow Aspirate and Trephine**

Robyn Matthews, Erin Downs, Emily Russell

*Cancer Care Services, Royal Brisbane & Women's Hospital, Brisbane, Queensland, Australia*

**Aim**

The Oncology Procedure Unit at the Royal Brisbane and Women's Hospital performs approximately 1800 Bone Marrow Aspirate and Trephines (BMAT) per annum. It has been identified that there are no local unit guidelines available for nursing management of patients who attend for a BMAT. This has resulted in inconsistencies of nursing monitoring and management of these patients - in particular vital sign monitoring required for the patient post BMAT. Investigations were undertaken to establish best practice of nursing management for patients who undergo this procedure. Benchmarking was conducted in private and public hospitals and independent collection centres throughout Australia. The aim was to ascertain what pathways or protocols are currently utilised, what sedation is administered and frequency of vital signs completed post the BMAT. A literature search of available studies was undertaken searching the Cochrane Database of Systematic Reviews, The Cochrane Library Issue 6, 2012; Ovid MEDLINE 2002 to June Week 1 2012. Additionally, other local and nationally recognised guidelines and resources were reviewed.

**Results**

One review of UK data in 2004 documented 15 adverse events among 20223 procedures. The main complication recorded was haemorrhage. Based on these results the BMAT procedure is considered to be a low risk procedure. However, the procedural risk increases when conscious sedation is administered. Hypoxia and bradypnea are common side effects of concern requiring nurses to closely monitor the patients' sedation state, respiration rate and oxygen saturation. Benchmarking revealed that the monitoring of vital signs varies considerably in frequency and extent for patients receiving conscious sedation for BMAT.

**Conclusion**

This review has identified critical areas for improvement of nursing management for patients undergoing BMAT. The Oncology Procedure Unit has commenced the development of an interdisciplinary clinical pathway for the management of this patient population. Additionally an electronic resource is under development in the form of a Power Point Presentation as an educational tool for current and future nurses in the unit.

**Keywords** nursing management, BMAT, haemorrhage

**Conflict of interest** No

Monday 29 October

Nurses Free Communications 1: Hot Issues in Clinical Practice

O046

0830-1000

Room 212

0930-0945

## Why Make That Call? - An Evaluation of the 1800 MYELOMA Help Line

Hayley King<sup>1</sup>, Tracy King<sup>2,3</sup>

1 Myeloma Foundation Australia, Melbourne, Vic, Australia

2 Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

3 Sydney Nursing School, Cancer Nursing Research Unit, Sydney, NSW, Australia

### Introduction and Aim

Despite improvements in survival outcomes, myeloma remains an incurable malignancy with people living longer in a chronic state of relapse. Although their own health professionals, particularly doctors, are considered authoritative information sources, there is evidence that patients also seek information elsewhere. Nurse-led help lines are often implemented to provide reliable health-related information. The Myeloma Foundation of Australia (MFA) have maintained a national database of their nurse-led help line since September 2010. This project aims to evaluate the volume and nature of helpline calls to better understand patient and carer information needs and to direct future information resources.

### Method

All calls from a 19 month period were analysed (n= 403). Data included caller role, primary and secondary reasons for calling and call length. A basic measure of success was assigned by the nurse at the time and graded as met, almost met or not met. Only calls with reasons provided were considered evaluable. Valid percentages were used when data were missing.

### Results

340 calls (84%) were evaluated. 68% were from patients and 33% from family members; average length of call was 22 minutes (range: 5-90 mins). Quality ratings were given for 214 calls. Caller needs were met or almost met in 98% of cases. Over 50 separate reasons for calling were listed. The most common reason for calling was myeloma the disease (cited 75 times; of which 24% related to being newly diagnosed), followed by transplant (cited 25 times), pain (cited 21 times) and peripheral neuropathy (cited 19 times) respectively.

### Conclusion

The help line is utilised by those directly and indirectly affected by myeloma, and while a broad range of information is required, information about myeloma the disease is most frequently requested. Thus, the helpline appears to be an opportunity to supplement and reinforce information given by primary health providers. A new, more comprehensive myeloma patient guide being produced by MFA will address the focus on disease specific information being requested by callers.

**Keywords** Myeloma, information, support **Conflict of interest** No

**Monday 29 October**

**Nurses Free Communications 1: Hot Issues in Clinical Practice**

**O047**

**0830-1000**

**Room 212**

**0945-1000**

**Difficult Decisions: A Multidisciplinary Approach to Genetic Counselling for Haemoglobinopathies in an Antenatal Setting**

Fiona Cunningham<sup>1</sup>, Kerry Weekes<sup>2</sup>, Joanne Shaw<sup>1</sup>, Mary Tassigianakis<sup>1</sup>, Donald K Bowden<sup>1,2</sup>, SantRayn Pasricha<sup>1,2</sup>

<sup>1</sup>*Medical Therapy Unit, Monash Medical Centre, Clayton, Vic, Australia*

<sup>2</sup>*Clinical Genetics Laboratory, Monash Medical Centre, Clayton, Vic, Australia*

We conducted an audit of prenatal diagnostic testing for haemoglobinopathies conducted in Victoria from 1996 to 2011. Diagnostic testing became centralised to the Clinical Genetics Laboratory at Southern Health in 1996 with 25 diagnostic prenatal tests being performed. Since then, the numbers of diagnostic tests conducted have fluctuated around a mean of 31.95 per annum, with 25 in 2001, and 30 in both 2006 and 2011. 2007 and 2008 both had 40 CVSs for haemoglobinopathy performed. These reflect an ongoing need for genetic counselling for prenatal testing in haemoglobinopathies.

The need for life-long treatments for transfusion-dependent patients present challenges for parents considering antenatal testing and continuation or termination of an affected pregnancy, both medically and socially. However, improvements in treatments and increased public awareness of these conditions have led to a changing patient perspective on what was once considered a devastating diagnosis. For some couples, this has made the decision as to whether to test, or to continue an affected pregnancy more difficult. A multidisciplinary approach involving clinical, social-work and counselling staff provides a framework to best promote informed decision making for people contemplating testing.

This poster considers two cases highlighting the difficult decisions faced by some prospective parents that involve not just medical considerations, but often complex social situations that become significant with the chronic nature of haemoglobinopathies; one involving a woman with beta-sickle cell disease who underwent prenatal diagnosis and opted to continue with an affected pregnancy, and a second case involving an immigrant with little English and a young child with beta thalassaemia major. She presented late in pregnancy for counselling and had to balance her religious views, with the ongoing demands of possibly caring for two children with thalassaemia in a difficult social framework.

**Keywords** Genetic counselling, haemoglobinopathy, prenatal diagnosis

**Conflict of interest** None

Monday 29 October

HSANZ Free Communications 5: Multiple Myeloma

O048

1030-1130

Plenary 2 (Auditorium)

1030-1045

**Second Autologous Stem Cell Transplantation (ASCT) in Multiple Myeloma (MM): A Single Centre Experience**

Lijun Bai, Cassandra Reid, Wei Xia, Kelly Wong, Ian Kerridge, Chris Ward, Matthew Greenwood

*Cellular Therapeutic Laboratory, Department of Haematology, Royal North Shore Hospital, Sydney, Australia*

**Aim**

Despite the introduction of novel agents such as Thalidomide, Lenalidomide and Bortezomib, ASCT following induction chemotherapy remains the standard of care for consolidating remission in MM. The successful use of these novel agents in remission maintenance post-transplant or in salvage therapy upon relapse has provided a possible alternative to the 'default' option of a second ASCT. The purpose of this study was to assess the benefits and necessity of using a second ASCT strategy for patients with myeloma.

**Results**

The data from 177 patients (Median age 61, male/female= 108/69) who diagnosed with MM in our center between 2001 and 2011 has been assessed. All patients underwent PBSC mobilization. 160 patients (Median age 61, male/female= 98/62) underwent ASCT. An average of 2 (1~7) stem cell harvests were required to achieve  $CD34 \geq 4 \times 10^6/kg$  for two transplants. In 22.5% (40/177) of the patients insufficient CD34 cells were collected for two transplants. Only 11% (18/160) proceeded to second transplant, with 17% (14/84) patients proceeding after relapsed disease. The median age at 2<sup>nd</sup> transplant was 62 (53~70) yrs. For patients undergoing second ASCT, disease free survival (DFS) at 3 yrs was not significantly different than those undergoing single ASCT, but DFS at 5 yrs was significantly poorer in the second ASCT group ( $P=0.011$ ) compare with the patients had single transplant. Overall survival at 3 and 5 yrs was no different between the two groups ( $p=0.705$  and  $0.489$  respectively).

**Conclusion**

In this retrospective single centre study, the use of second ASCT in relapsed MM was associated with inferior DFS at 5 yrs and similar OS vs single ASCT in the era of novel therapies. Few patients (11%) proceeded to second transplant despite provision for a second ASCT being made in the majority of pts at time of collection. Our data suggests that routinely harvesting sufficient HPC for second ASCT in MM should be reconsidered when access novel therapies is routine.

**Keywords** Myeloma, second, transplant

**Conflict of interest** No



Monday 29 October

HSANZ Free Communications 5: Multiple Myeloma

O049

1030-1130

Plenary 2 (Auditorium)

1045-1100

**Blockade of Hedgehog Signalling with the Novel Agent NVP-LDE225 Induces Differentiation, Non-proliferation and Killing of Myeloma Stem Cells *In Vitro***

Shihong Yang, Ross Brown, Celine Loh, Hayley Suen, James Favaloro, John Gibson, P Joy Ho, Douglas Joshua

*Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW Australia*

**Aim**

A small reservoir of chemotherapy-resistant cancer stem cells is a major cause of relapse. The hedgehog (Hh) signalling pathway is now considered to be a key regulator of stem cells. Our aim was to determine the effect that NVP-LDE225, a novel Hh pathway inhibitor, has on myeloma (MM) stem cells.

**Method**

NVP-LDE225 was kindly supplied by Novartis. MM stem cells were defined as side population (SP) cells detected after Hoechst 33342 staining using a BD ARIA II flow cytometer, which was also used for sorting SP cells. Proliferation was monitored with CFSE tracking and viability with propidium iodide (PI). MM cell lines RPMI8226, KMS-11, OPM2, U266 and primary MM cells were included. Expression of Hh pathway proteins PTCH, Smo and Gli1 was determined by flow cytometry.

**Results**

SP cells were identified in all 4 MM cell lines and 86% (18/ 21) of MM BM samples with the percentage of SP cells ranging from 0 to 4.9% of CD38++ cells. There was no significant difference in %SP during therapy (n=11/20). PTCH expression was significantly higher on SP than non-SP cells (80.5% vs 51.7%). *In vitro* dose response curves demonstrated non-specific killing of HS-5 stromal cells above 5µM NVP-LDE225. During culture flow-sorted SP+ plasma cells initially reverted to non-SP cells but the SP phenotype returned, confirming the state of flux in the stem cell compartment. NVP-LDE225 inhibited non-SP returning to SP and induced additional differentiation of SP to non-SP. Proliferation studies using CFSE tracking of sorted SP cells demonstrated a 34% reduction in proliferation after exposure to NVP-LDE225. There was no significant change in Gli1 expression on RPMI8226 cells after exposure to NVP-LDE225.

**Conclusion**

The studies confirm there is flux, to and from the MM stem cell compartment. Our preliminary observations suggest that NVP-LDE225 can induce SP differentiation, inhibit SP proliferation and at high concentrations is toxic to MM stem cells.

**Keywords** multiple myeloma, hedgehog signalling, myeloma stem cells

**Conflict of interest** No conflict of interest to disclose.

Monday 29 October

HSANZ Free Communications 5: Multiple Myeloma

O050

1030-1130

Plenary 2 (Auditorium)

1100-1115

**What Factors are Associated with Stem Cell Transplant (SCT) Among Multiple Myeloma (MM) Patients: Findings from a Population Based Study of Treatment Practice in Victoria 2008/09**

S Harrison<sup>1</sup>, VM White<sup>2</sup>, M Coory<sup>3</sup>, N Wong Doo<sup>1</sup>, M Prince<sup>1</sup>, M Dimech<sup>4</sup>, GG Giles<sup>4</sup>  
<sup>1</sup> Department of Haematology, Peter MacCallum Cancer Institute, Vic. <sup>2</sup> Centre for Behavioural Research in Cancer, The Cancer Council Victoria. <sup>3</sup> Murdoch Children's Research Institute. <sup>4</sup> Cancer Epidemiology Centre, The Cancer Council Victoria

**Aims**

To determine which MM cases receive SCT and to examine disease range of factors associated with this outcome.

**Methods**

Retrospective data collection for all 747 incident MM cases registered in 2008-9 by the Victorian Cancer Registry. Trained staff collated data from medical records and pathology reports including data concerning patients (age, residential location, comorbidities, symptoms), disease (stage, related organ or tissue impairment [ROTI], cytogenetics), treatment (induction therapy, SCT), clinical trials and clinicians (specialty referred to, multidisciplinary care). Associations between patient, disease and clinician factors, with SCT as the outcome, were assessed using Chi-square statistics and logistic regression and  $p < .05$  taken as significant.

**Results**

Data were obtained for 618 (83%) cases. Median age at diagnosis was 70 years. Most (59%) cases were men were symptomatic at diagnosis (83%) and 25%, 26% and 30% had stage 1, II and stage III disease respectively. Among symptomatic patients, 61% aged <65 years, 43% aged 65-69 years and one aged >70 years received SCTs. Cases receiving SCT were on average younger (mean age 57.6 years vs. 60.5). Among patients under 65, SCT was more likely in patients with fewer co-morbidities (none/low 66%; severe/high 46%,  $p = .03$ ) and initial referral to a haematologist (64% vs. 28%). Increasing stage and having renal ROTI reduced the likelihood of SCT. In multivariate analyses, high comorbidity, lack of early referral to a haematologist and having ROTI Renal were the key predictors of not receiving SCT in patients <65.

**Conclusion**

Despite SCT being recommended for MM aged <65 years, 40% of patients in this age did not receive it. While our findings suggest that best practice treatment was not restricted to metropolitan residents, they also imply it may be less likely if patients are not referred early to a haematologist. Involving haematologists early in the management of MM may ensure all receive best practice care.

**Keywords:** Multiple myeloma, stem cell transplantation, patterns of care

**Conflict of interest:** There are no conflicts of interest to declare

Monday 29 October

HSANZ Free Communications 5: Multiple Myeloma

O051

1030-1130

Plenary 2 (Auditorium)

1115-1130

# **Characterisation of Cardiac Amyloid Using Mass-spectrometry, and Correlation with Clinical Characteristics and Survival in an Australian Cohort**

Barbara Withers<sup>1</sup>, Christopher Hayward<sup>2</sup>, Eugene Kotlyar<sup>2</sup>, Steve Rainer<sup>3</sup>, Ahmet Dogan<sup>4</sup>, John Moore<sup>1</sup>

*Department of Haematology<sup>1</sup>, Cardiology<sup>2</sup>, Anatomical Pathology<sup>3</sup>, St Vincent's Hospital, Sydney, NSW, Australia. <sup>4</sup>Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA*

## **Aim**

The aim of this study was to retrospectively evaluate the amyloid subtype, utilising laser microdissection and tandem mass spectrometry (LMD-MS), in a cohort of Australian patients with biopsy proven cardiac amyloidosis, and compare the demographics, clinical characteristics, and survival between different subtypes.

## **Results**

47 patients with biopsy-proven cardiac amyloidosis were included in the study (AL/AH, n=33 ; ATTRwt, n=12 ; ATTRm, n=2). 39 patients had subtype concluded on the basis of LMD-MS analysis, 8 patients were subtyped on the basis of clinical features and supportive investigations. On the basis of LMD-MS analysis, 5 patients were reclassified, 2 from presumed chemotherapy-treated AL/AH to ATTRwt. Investigation using serum and urine EPG/IEPG, with free light chain assay showed 94% sensitivity for AL/AH diagnosis. Subtypes (AL/AH; ATTRwt ; ATTRm) were significantly different on the basis of : mean age, y (60.33 ; 76.08; 52), male gender % (45; 92; 100), mean interventricular septal width mm (14.91;20.8;16.5), and median survival d (105; 1170; not tested). Further analysis may yield other factors affecting survival such as troponin or involved free light chain concentration.

## **Conclusion**

Given vast differences in treatment and survival, these results illustrate the critical importance of accurate subtyping to facilitate prognostication and therapy choice.

**Keywords** Amyloid, Mass spectrometry, Cardiac

**Conflict of interest** No conflict of interest to disclose

Monday 29 October  
HSANZ Free Communications 6: CML Biology  
O052

1030-1130  
Room 216  
1030-1045

## New TKI Transporter Identified that May Contribute to Nilotinib Resistance *In Vitro*

Laura Eadie<sup>1,2</sup>, Timothy Hughes<sup>1,2</sup>, Deborah White<sup>1,2</sup>

<sup>1</sup>Division of Haematology, Centre for Cancer Biology SA Pathology, South Australia,

<sup>2</sup>Department of Medicine, University of Adelaide, South Australia

### Aim

ABCB1 and ABCG2 have previously been demonstrated to interact with nilotinib, however, to date, other drug transporters have not been widely investigated. In this current study we aimed to assess the impact of the closely related transporter, ABCC6, on the transport of nilotinib.

### Results

Preliminary array data from K562 cells incubated overnight in the absence and presence of nilotinib identified a marked increase in ABCC6 expression suggesting it is a likely candidate for nilotinib transport. Further investigation demonstrated upregulation of ABCC6 mRNA during development of nilotinib resistance in CML cell lines *in vitro*. K562 cells exposed to gradually increasing concentrations of nilotinib to a maximum of 2  $\mu$ M expressed significantly greater levels of ABCC6 mRNA: 25 fold higher when compared with control cells ( $p=0.008$ ). IC50 experiments based on p-CrkI expression were conducted on patient MNCs in the absence and presence of three ABCC6 inhibitors: indomethacin, probenecid and pantoprazole. Results demonstrated that all three inhibitors significantly reduced nilotinib IC50 ( $p<0.001$ ) indicating that ABCC6 is likely to be involved in nilotinib transport. In addition ABCC6 mRNA levels were assessed in chronic phase CML patients at diagnosis with results demonstrating wide variation in expression: 3-65% of BCR. These results will be correlated with patient response once this data becomes available.

### Conclusion

Combined, these studies suggest that nilotinib is a likely substrate of the efflux transporter ABCC6. To our knowledge this is the first report of ABCC6 involvement in TKI transport. In addition, ABCC6 over expression may also contribute to nilotinib resistance *in vitro*. With nilotinib now a front line therapy option in the treatment of CML, concomitant administration of ABCC6 inhibitors may present an attractive option to enhance TKI efficacy.

**Keywords** CML, tyrosine kinase inhibitors, ABC transporters

**Conflict of interest** This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract

Monday 29 October

HSANZ Free Communications 6: CML Biology

O053

1030-1130

Room 216

1045-1100

## Macrolide Antibiotic Clarithromycin Targets TKI-induced Autophagy in CML Cells

Lisa Schafranek<sup>1,2</sup>, Tamara Leclercq, Deborah White<sup>1,2</sup>, Timothy Hughes<sup>1,2</sup>

<sup>1</sup>*Division of Haematology, Centre for Cancer Biology, SA Pathology, South Australia,* <sup>2</sup>*Department of Medicine, University of Adelaide, South Australia*

### Introduction

Recent data has shown that TKI-induced blockade of survival pathways results in activation of autophagy in CML resistant to TKI therapy. Furthermore, inhibition of late stage autophagy by chloroquine (CQ) was shown to restore sensitivity of resistant CML cells to TKI through inhibition of autophagy. Clarithromycin (CAM) is a macrolide antibiotic that has been shown to inhibit cancer cell growth. Most recently, we administered in combination with TKI therapy, CAM induced clinical responses in resistant CML patients. We have previously demonstrated that CAM induces cell death by inhibiting dasatinib-induced autophagy.

### Aim

Here further interrogated the interactions of CAM in combination with Das and also investigate the effects of other TKIs in combination with CAM.

### Results

We investigated the effects of the addition of CAM on cell death and apoptosis using Annexin V and 7AAD staining and we observed that CAM increases the sensitivity of imatinib, nilotinib and dasatinib in the K562, KU812, K562 Dox cell line compared to TKI alone, but not the Bcr-Abl negative HL60 cell line. The changes in autophagic and apoptotic pathways were further interrogated by assessing the markers LC3, Beclin-1, p62 and Bcl-2. We show an increase in LC3-II is induced by CAM alone and is further increase by the combination of CAM with TKI. Cellular morphology was assessed using May-Giemsa staining which demonstrated a concomitant increase in cellular vacuole formation induced by the combination of CAM with TKI, which suggests that CAM is inhibiting TKI-induced autophagy. We have investigated the effects of CAM in comparison with the late-stage autophagy inhibitor CQ and the early-stage autophagy inhibitor 3-methyladenine (3MA) and provide evidence that CAM behaves like late-stage autophagy inhibitor CQ.

### Conclusion

CAM is effective at increasing the sensitivity of CML cells to TKI, whilst having little or no effect on cell death as a sole agent. The mechanism of induction of cell death by CAM combined with TKI appears to be via inhibition of late stage autophagy. These findings highlight CAM as a promising therapeutic option for inhibition of TKI-induced autophagy in TKI-resistant patients or patients with persistent CML.

**Keywords** Chronic Myeloid Leukaemia, Autophagy, Clarithromycin, TKI, Chloroquine

**Conflict of interest** No

Monday 29 October

HSANZ Free Communications 6: CML Biology

O054

1030-1130

Room 216

1100-1115

## Targeting Cytokine-Mediated Resistance in CML Progenitor and Stem Cells Using a Monoclonal Antibody Against CD123

Eva Nievergall<sup>1</sup>, Deborah L White<sup>1,3,4</sup>, Agnes S Yong<sup>1</sup>, Hayley S Ramshaw<sup>2,3</sup>, Samantha J Busfield<sup>5</sup>, Gino Vairo<sup>5</sup>, Angel F Lopez<sup>2,3,4</sup>, Timothy P Hughes<sup>1,3,4</sup>, Devendra K Hiwase<sup>1,3,4</sup>

<sup>1</sup>Department of Haematology and <sup>2</sup>Human Immunology, SA Pathology, Adelaide, SA, Australia, <sup>3</sup>Centre for Cancer Biology, Adelaide, SA, Australia, <sup>4</sup>Department of Medicine, University of Adelaide, Adelaide, SA, Australia and <sup>5</sup>CSL Limited, Parkville, VIC, Australia

### Aim

Despite the remarkable efficacy of tyrosine kinase inhibitors (TKIs), chronic myeloid leukaemia (CML) stem and progenitor cells (LSPCs) persist, which is partly due to cytokine-mediated resistance. IL-3 receptor  $\alpha$  (CD123) is a marker for acute myeloid LSCs; however, there is limited data for CML LSPCs. Here, we investigate the expression of CD123 on CML LSPCs and the suitability of targeting them with CSL362, a humanised  $\alpha$ -CD123 monoclonal antibody acting via both blocking IL-3 signalling and promoting antibody-dependent cell-mediated cytotoxicity (ADCC).

### Methods

CD123 expression in normal vs. CML LSPCs was assessed by flow cytometry while STAT5 phosphorylation, Annexin-V apoptosis, LDH release cytotoxicity, CFU-GM and LTC-IC assays were used to test the effect of CSL362 and TKI on these cells.

### Results

Compared to normal donors, CD123 expression is significantly elevated in CD34<sup>+</sup>/CD38<sup>-</sup> LSPCs of CML patients in both chronic phase (CP) and blast crisis, increasing with disease progression. CD123<sup>+</sup> CP-CML LSPCs show *in vitro* self-renewal capacity and are efficiently eradicated dose-dependently via CSL362-mediated ADCC, not only when using allogeneic NK cells from normal donors but, importantly, also by autologous NK cells, collected from CML patients in major molecular response. Moreover, preliminary results suggest preferential elimination of CML over normal LTC-ICs through CSL362-induced ADCC. In addition, CSL362 prevents cytokine-mediated rescue of TKI-induced cell death, however, only in the presence of IL-3 alone and not in a physiological cytokine milieu.

### Conclusion

CSL362 effectively targets CML LSPCs, primarily by selective ADCC-mediated lysis, *in vitro*. Further evaluation of CSL362/TKI combination therapy in CML is indicated.

**Keywords** CML, CD123, monoclonal antibody

**Conflict of interest** This research was supported by CSL Ltd. The company had no role in analysing the data or preparing the abstract.

Monday 29 October  
HSANZ Free Communications 6: CML Biology  
O055

1030-1130  
Room 216  
1115-1130

## Global DNA Methylation Profiling in a Poor-Risk Subset of CML

Dale B Watkins<sup>1,2</sup>, Chung H Kok<sup>1,2</sup>, Richard J D'Andrea<sup>1,2,3</sup>, Timothy P Hughes<sup>1,2</sup>, Deborah L White<sup>1,2</sup>

<sup>1</sup> Haematology Department, Centre for Cancer Biology, SA Pathology, Adelaide, South Australia;

<sup>2</sup> Department of Medicine, The University of Adelaide, Adelaide, South Australia; <sup>3</sup> Department of Haematology and Oncology, The Queen Elizabeth Hospital, Woodville, South Australia

### Aim

Global DNA methylation in chronic myeloid leukaemia (CML) remains poorly explored, as does the impact of the epigenome on patient response to tyrosine kinase inhibitor (TKI) therapy. We aimed to investigate the global DNA methylation profile in CML, and ascertain whether aberrant epigenetic programming might underlie the response heterogeneity observed with TKI therapy.

### Methods

Total white cells were isolated from the blood of 55 chronic phase-CML (CP-CML) patients at diagnosis and 5 normals. CML patients were classified according to their OCT-1 activity (OA) values, with 29/55 classified as poor-risk (low OA) and 26/55 standard-risk (high OA). Whole genome DNA methylation analysis was performed using the Illumina Infinium® HumanMethylation450 BeadChip.

### Results

Using a cut-off of adjusted  $p < 0.05$ , 25,829 different CpG significantly separated CP-CML from normal. Of these, 3,467 CpG had a beta-value fold-change  $> 4$  (597 CpG were hypo- and 2,870 CpG were hyper-methylated in CP-CML). Pathway analysis revealed significant enrichment for genes involved in AML and APML, indicating similar pathways may be under epigenetic control in CML. A number of polycomb group (BMI1 and EZH2) targets were identified, suggesting the possible involvement of this pathway in CML. Comparing low and high OA CP-CML patients revealed 9,861 different CpG which distinguished these two groups. The majority (48.9%) of these CpG probes were located in CpG islands, more specifically in the gene promoter region (56%). Pathway analysis again identified polycomb group (SUZ12, PRC2 and EZH2) target enrichment in low OA, indicating this pathway may also play a significant role in the response variation of these patients.

### Conclusion

We present a comprehensive global DNA methylation analysis of CML that indicates significant changes to the CML epigenome, compared with normal patients, with emphasis on a poor-risk patient subgroup, defined by low OA and associated poor molecular response. These epigenetic changes may contribute to CML pathogenesis, and influence the response heterogeneity observed with TKI therapy.

**Keywords** CML, Methylation, Poor-risk

**Conflict of interest** No conflict of interest to disclose

Monday 29 October

HSANZ Free Communications 7: Low Grade B Cell Disorders

O056

1030-1130

Room 217

1030-1045

# **The Immunophenotype of Plasma Cells in Waldenström Macroglobulinaemia (WM) Closely Resembles Normal Plasma Cells and is Distinct From Multiple Myeloma (MM)**

Piers Blombery<sup>1</sup>, Shaun Fleming<sup>1</sup>, Ian Davis<sup>1</sup>, Vuong Nguyen<sup>1</sup>, Peter Gambell<sup>1</sup>, David Westerman<sup>1,2</sup>, Neil Came<sup>1,2</sup>

<sup>1</sup> Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

<sup>2</sup> University of Melbourne, Victoria, Australia

## **Aim**

To define and compare the immunophenotype (IPT) of bone marrow plasma cells (PC) in WM to normal PC (NPC) and MM.

## **Method**

PCs from 29 patients with WM were analysed either by 4-colour FACSCalibur (n=25) or 8-colour (n=4) FACSCanto-II instruments between 2007-12. PCs were gated according to European Myeloma Net criteria, then interrogated for expression of CD38, CD138, CD45, CD19, CD20, CD56, CD27, CD28, cyt kappa/lambda (by 4-colours) and additionally CD81, CD117 and CD200 with 8-colour acquisition. This was compared with NPC, MM (n=29) and CD20+ MM (n=13).

## **Results**

(% cases pos) CD19 expression				CD20				CD27			
WM	MM	MM CD20+	NPC	WM	MM	MM CD20+	NPC	WM	MM	MM CD20+	NPC
90%	7%	8%	+	57%	7%	100%	-	96%	62%	38%	+
CD56 expression				CD45 expression				CD28			
WM	MM	MM CD20+	NPC	WM	MM	MM CD20+	NPC	WM	MM	MM CD20+	NPC
7%	69%	15%	-	100%	41%	23%	+	4%	24%	23%	-

89% of WM that had cytoplasmic light chain assessment (26/29) showed restriction which was always of the same isotype as the lymphoid cells and paraprotein. The general IPT of WM PC was CD19+, CD45+, CD56-, CD20+, CD27+ and CD28-.

## **Conclusion**

The IPT of the PC compartment in WM shows multiple quantitative and qualitative differences to that of MM. The immunophenotypic characteristics of WM observed in our cohort are similar to that of NPC which supports the hypothesis that the monoclonal PC compartment arises from the malignant B-lymphocyte clone in keeping with normal differentiation patterns rather than de novo or clonal evolution of a distinct population of PCs. In addition, these differences may allow for the immunophenotypic distinction of PC populations in the two disorders, and impact on antibody choice in future minimal residual disease evaluation of the PC compartment in WM.

**Keywords** Flow cytometry, Waldenström, Plasma Cell **Conflict of interest** No



Monday 29 October

HSANZ Free Communications 7: Low Grade B Cell Disorders

O057

1030-1130

Room 217

1045-1100

# **An Optimised Single Tube Ten Colour Flow Cytometric Assay for Detection of Minimal Residual Disease in CLL based on the International Standardised Approach Methodology**

Mary Sartor, David Gottlieb

*Westmead Millennium Institute, Westmead Hospital, Sydney, Australia*

## **Introduction**

The eradication of minimal residual disease (MRD) in CLL predicts for improved outcome. More recently, an international standardised approach (ISA) to the assessment of MRD in CLL that utilises a 4 tube test system and permits a sensitivity of 0.01% (Rawstron et al,) was published.

## **Aim**

To develop a single tube 10 colour flow cytometric assay based on the ISA methodology to be used in a centralised flow cytometry laboratory providing results for a multicenter clinical trial of lenalidomide treatment for MRD following FCR chemotherapy. The method was designed to minimise the requirement for cell numbers in patients with relative leucopenia following chemotherapy.

## **Method**

Peripheral blood (n=51) and bone marrow (n=20) was collected from patients at various time points post treatment for CLL. Monoclonal antibodies were used either according to the ISA methodology or in a single tube incorporating all of the following monoclonal antibodies: CD3 ECD, CD5 PercP5.5, CD19 eFluor, CD20 PE CY7, CD81 FITC, CD22 PE, CD43 APCCY7, CD79b APC, CD38 A700, and CD45 KO. Results from the two methods were compared.

## **Results**

Levels of residual disease in the 71 samples analysed varied from <0.01 to 22%. Twenty-four samples showed residual disease of <1%. Analysing all samples showed an excellent correlation between the two methods slope = 0.989, intercept =0.1 and R (2) =0.992. There was also excellent correlation for disease levels below 1.0%, (median 0.03 range <0.01-0.63%, n=24) slope = 1.15, intercept =0.007 and R (2) =0.994. Bland Altman analysis showed a mean of 0.008 +/- 0.058 (2SD) for values below 1%.

## **Conclusion**

The single tube ten colour flow cytometric assay for detection of MRD in CLL gives equivalent results to the ISA. There is a potential for improved sensitivity resulting from the removal of CD19+/CD3+ contaminating events and by increasing the total number of events acquired since there is no need to divide the sample into multiple tubes, particularly post-treatment when cell numbers are frequently limiting. The single tube assay is also simple, rapid and cost effective.

**Keywords** CLL, MRD, Flow Cytometry **Conflict of interest** No

Monday 29 October

HSANZ Free Communications 7: Low Grade B Cell Disorders

O058

1030-1130

Room 217

1100-1115

**Differential Response of the Bone Marrow (BM) B-lymphocyte and Plasma Cell (PC) Compartments to Fludarabine (F) and or Rituximab (R) Based Treatment (Tx) in Waldenström Macroglobulinemia (WM)**

Azhar Munas<sup>1</sup>, Sumita Ratnasingam<sup>2</sup>, Surender Juneja<sup>2,3</sup>, David Westerman<sup>1,3</sup>, Neil Came<sup>1,2,3</sup>

1 Peter MacCallum Cancer Centre, East Melbourne, Vic, Australia. 2 The Royal Melbourne Hospital, Parkville, Vic, Australia. 3 University of Melbourne, Parkville, Vic, Australia

**Aim**

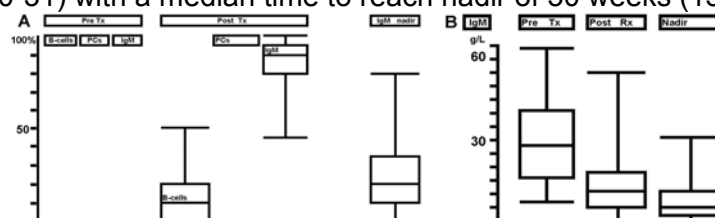
We aimed to confirm anecdotal observations that BMPCs persist in WM despite otherwise effective fludarabine (F) and/or rituximab (R) with or without cyclophosphamide (C) based chemotherapy, which would account for the persistent paraprotein in the majority of cases.

**Method**

Medical records, pharmacy and laboratory information systems from both hospitals identified patients treated for WM between 1999-2011. BM B-lymphocytes and PCs were quantified morphologically as a percentage of total cellularity by immunohistochemistry using CD20, PAX-5, CD138 and MUM1 pre and post Tx.

**Results**

22 patients; 17 males; median age 59 (37-77) underwent 26 full Tx episodes: FCR N=17, FC N=5, FC-allograft N=1, FR N=1, CHOP-R N=1, CR+dexamethasone N=1 (median cycles N=4; range 3-6). The percent reduction of B-lymphocytes, PCs and paraprotein (IgM) from baseline (taken as 100%), and IgM in g/L are summarised in figures A and B. Despite substantial depth of response in B-lymphocytes in all cases, no reduction in PCs was observed in 19/22 evaluable episodes. Nevertheless, the paraprotein continued to fall from a median of 28g/L (7-64) to median 5g/L (0-31) with a median time to reach nadir of 50 weeks (15-207).



**Conclusion**

Following F/R based Tx there is a relatively rapid and substantial reduction in the B-lymphocyte component of WM followed by a more gradual diminution in paraprotein without reduction in BMPC in the majority of cases. This suggests the possibility of the presence of resistant PC clone/s and may provide rationale for optimising future therapy in WM.

**Keywords** Waldenström, Bone Marrow, Plasma Cell. **Conflict of interest:** No

Monday 29 October

1030-1130

HSANZ Free Communications 8: Non-malignant Haematology - Clinical Outcomes Room 218  
**O059**

1030-1045

## Effective Home-administration of Romiplostim in Australian Patients with Primary Immune Thrombocytopenia (ITP)

Ross Baker,<sup>1</sup> Bronwyn Williams,<sup>2</sup> Beng Chong,<sup>3</sup> Kun Nie,<sup>4</sup> Dyfed Evans<sup>5</sup>

<sup>1</sup>Centre for Thrombosis and Haemophilia, Murdoch University, Royal Perth Hospital, WA, Australia; <sup>2</sup>Royal Children's Hospital, Herston, Queensland, Australia; <sup>3</sup>St George Hospital, Kogarah, NSW, Australia; <sup>4</sup>Amgen Ltd, Thousand Oaks, USA; <sup>5</sup>Amgen Australia Pty Ltd, Sydney, Australia

### Aim

Evaluate the efficacy and safety of home-administered romiplostim in Australian ITP patients.

### Methods

Australian patients completing a previous romiplostim ITP study could enrol into an international, single-arm extension study (Bussel et al. *Blood* 2009;113:2161). Romiplostim was initiated at 1 µg/kg/week (patients who received their last dose more than 24 weeks previously, or who previously received placebo) or continued at the last dose from the previous study (all other patients), and adjusted to maintain platelet counts of 50–250 × 10<sup>9</sup>/L. Patients with a stable dose for 3 weeks were permitted to administer romiplostim at home with regular follow-up and blood testing. We report data from the time before and after home-administration initiation.

### Results

Sixteen of 17 (95%) Australian patients home-administered romiplostim for a median of 62.0 (range, 3–81) weeks; only 1 patient discontinued home-administration (administrative decision). Median age, time since ITP diagnosis and baseline platelet count of patients who home-administered were: 45.5 ([Q1, Q3], [30.0, 65.5]) years, 4.2 (range, 0.9–14.3) years and 88.5 ([Q1, Q3], [26.0, 183.0]) × 10<sup>9</sup>/L, respectively. One (6%) patient had undergone splenectomy; 11 (69%) were female. Romiplostim dose, platelet counts and adverse event rates were similar before and after home-administration initiation (Table). No serious thromboembolic events were reported at any time during the study.

### Conclusion

These data suggest that the efficacy and safety of romiplostim are similar when patients receiving a stable dose change to home-administration; home-administration may offer a more convenient treatment option for some ITP patients.

	Average weekly dose (µg/kg), Median (Q1, Q3)	Percentage of weeks with a platelet count, mean (SD)		AEs <sup>a</sup> r (n)	SAEs <sup>a</sup> r (n)
		>50 × 10 <sup>9</sup> /L	>400 × 10 <sup>9</sup> /L		
Weeks –3 to –1, N=13	4.0 (4.0, 8.7)	82.1% (25.9%)	0 % (0%)	20.25 (8)	0 (0)
Weeks ≥1, N=16	4.5 (3.0, 7.9)	78.9% (22.3%)	4% (10.4%)	18.87 (180)	1.00 (10)

First week of home-administration defined as week 1; AE, adverse event; SAE, serious adverse event.

<sup>a</sup>n = number of adverse events; r = study-duration adjusted event rate per 100 patient-weeks.

**Keywords** romiplostim; primary immune thrombocytopenia; thrombopoietin; home-administration

**Conflict of interest** This research was supported by Amgen Inc. The company analysed the data and funded external editorial assistance for abstract preparation.

Monday 29 October

1030-1130

HSANZ Free Communications 8: Non-malignant Haematology - Clinical Outcomes Room 218  
O060

1045-1100

# **Final Australian Results From An International, Multi-center, Single-arm Study Evaluating the Safety and Efficacy of Romiplostim in Adults With Primary Immune Thrombocytopenia (ITP)**

Robert Bird<sup>1</sup>, Tim Brighton<sup>2</sup>, Ross Baker<sup>3</sup>, Kerry Dillingham<sup>4</sup>, Dyfed Evans<sup>5</sup>

<sup>1</sup>Princess Alexandra Hospital, Queensland, Australia; <sup>2</sup>Prince of Wales Hospital, Sydney, NSW, Australia; <sup>3</sup>Centre for Thrombosis and Haemophilia, Murdoch University, Royal Perth Hospital, Perth, Australia; <sup>4</sup>Amgen Limited, Cambridge, UK; <sup>5</sup>Amgen Australia Pty Ltd, Sydney, Australia.

## **Aim**

Describe the safety and efficacy of romiplostim in Australian adult ITP patients.

## **Methods**

Eligibility criteria were broad:  $\geq 18$  years-old; prior ITP therapies ( $\geq 1$  or  $\geq 3$ , dependent on protocol amendment); low platelet counts ( $\leq 10$ , 20 or  $30 \times 10^9/L$ , dependent on protocol amendment) or uncontrolled bleeding; no history of myeloproliferative neoplasms, MDS or bone marrow stem cell disorder. Romiplostim was initiated at  $1 \mu g/kg/week$ , and adjusted to maintain platelets  $\geq 50 \times 10^9/L$ . Rescue medications were allowed at any time; concurrent ITP therapies were reduced when platelets were  $> 50 \times 10^9/L$ . Primary endpoint was incidence of adverse events (AEs) and antibody formation. Secondary endpoint was platelet response: (a) doubling of baseline count and  $\geq 50 \times 10^9/L$  or (b)  $\geq 20 \times 10^9/L$  increase from baseline.

## **Results**

Of the 39 Australian subjects enrolled, 32 (82%) completed the study and 7 (18%) withdrew. Two patients died on study (pneumonia, ischemic stroke; neither considered related to romiplostim). Median (Q1, Q3) time from ITP diagnosis was 4.8 (1.1, 16.0) years; 23 (59%) patients were splenectomised, 20 (51%) were receiving concurrent ITP therapies. Median (range) baseline platelet count was  $14.0 (2.0-29.0) \times 10^9/L$ . Median (range) treatment duration was 75.0 (11-144) weeks. Incidence and type of AEs were consistent with the overall study population (Janssens et al. *Blood* 2011;118:3279); 37 (95%) patients reported AEs, 11 (28%) reported serious AEs (1 event of cerebrovascular accident considered treatment-related), 20 (51%) reported treatment-related AEs. No antibodies to romiplostim were reported; 2 patients (5%) reported thrombotic/thromboembolic events. Almost all patients (37 [95%]) achieved each platelet response definition; median (Q1, Q3) time to response was 1 [(a): (1.0, 4.0); (b): (1.0, 2.0)] week for both.

## **Conclusion**

Romiplostim safely induced a rapid platelet response in adult ITP patients with low platelet counts or bleeding symptoms. Romiplostim is an important, well-tolerated, treatment option, which significantly increases and maintains platelet counts.

**Keywords:** ITP, thrombocytopenia, thrombopoietin

**Conflict of interest:** This research was supported by Amgen. The company funded the study, performed the data analysis and provided medical writing support.

Monday 29 October

1030-1130

HSANZ Free Communications 8: Non-malignant Haematology - Clinical Outcomes Room 218  
O061

1100-1115

## Iron Deficiency Anaemia in Pregnancy and Postpartum: Effect of Oral versus Intravenous Iron Therapy

Alhossain A Khalafallah<sup>1,2</sup>, Amanda Dennis<sup>1,2</sup>

<sup>1</sup>Launceston General Hospital; <sup>2</sup>School of Human Life Sciences, UTAS, Australia

### Background

Nutritional iron deficiency anaemia (IDA) is the most common disorder in the world, affecting more than two billion people. The WHO global database on anaemia has estimated a prevalence of 14% based on a regression-based analysis. Recent data showed that the prevalence of IDA in pregnant women in industrialized countries is 17.4% while the incidence of IDA in developing countries increases significantly up to 56%. Although oral iron supplementation is widely used for the treatment of IDA, not all patients respond adequately to oral iron therapy. This is due to several factors including the side effects of oral iron which lead to poor compliance and lack of efficacy. Previously, the use of IV iron had been associated with undesirable and sometimes serious side-effects and therefore was underutilised. However, in recent years, new type II and III iron complexes have been developed, which offer better compliance and toleration as well as high efficacy with a good safety profile.

### Results

We conducted a large randomised perspective trial comparing the effect of IV iron polymaltose versus oral iron sulphate in the treatment of 200 women diagnosed with IDA during pregnancy. The proportion of women with lower than normal ferritin levels was 79% in the oral iron as compared to 4.5% for women who received IV iron ( $p < 0.001$ ). The percentage of women at delivery with Hb level  $< 116$  g/L was 29% in the oral iron group versus 16% in the IV iron group ( $p = 0.04$ ). The IV iron polymaltose was well tolerated by the pregnant women. Several phase 3 randomised trials assessing the effect of IV ferric carboxymaltose versus oral ferrous sulphate in postpartum. Patients assigned to IV ferric carboxymaltose achieved a haemoglobin rise  $> 20$  g/L faster than the oral iron group ( $p < 0.001$ ). The IV iron group significantly achieved a haemoglobin rise  $> 30$  g/L at any time ( $p < 0.001$ ), and were more likely to achieve a haemoglobin  $> 120$  g/L ( $p < 0.001$ ). There were no serious adverse drug reactions in both groups.

### Conclusions

The new IV iron preparations represent a medical revolution in effective, rapid and safe iron repletion in the management of IDA. This simplifies the treatment of IDA in all populations with IV iron treatment with effective repletion of iron stores and improvement of subjective and objective outcomes of the IDA. In summary, IV iron can be used safely for a rapid repletion of iron stores and correction of anaemia during and after pregnancy.

**Key words:** Iron deficiency anaemia, iron therapy, efficacy, outcome, pregnancy.

**Conflict of interest** NO

Monday 29 October

1030-1130

HSANZ Free Communications 8: Non-malignant Haematology - Clinical Outcomes  
O062

1115-1130

## Red Cell Indices for Screening for Alpha Globin Gene Mutations

Danielle Oh<sup>1</sup>, Anastasia Adrahtas<sup>2</sup>, Kerryn Weekes<sup>2</sup>, Michael Wheeler<sup>3</sup>, Louis Meyepa<sup>3</sup>, Sant-Rayn Pasricha<sup>1,2</sup>

<sup>1</sup>Medical Therapy Unit, and <sup>2</sup>Clinical Genetics and <sup>3</sup>Haematology, Southern Cross Pathology; Southern Health, Melbourne, Vic, Australia

### Aim

The Clinical Genetics laboratory at Southern Health is the State centre for haemoglobinopathy genetic testing in Victoria; testing aims to identify carriers and provide couples at risk of Thalassaemia with suitable advice. Selection of samples to undergo alpha testing is based on red cell indices, however the optimal cut-offs remain uncertain.

### Methods

All blood samples received between April 2011 and April 2012 at Southern Cross Pathology that were tested for FBE, Hb electrophoresis and alpha globin gene genetic studies were retrospectively reviewed. Optimal red cell indices for excluding single and two gene alpha deletions were identified among samples with no other haemoglobinopathy identified.

### Results

3318 patient samples were received: 191 samples met criteria for inclusion; mean MCV was 77.2fL and mean MCH was 25.8pg. The AUC<sup>ROC</sup> for Hb, MCV and MCH to detect presence of any alpha mutation was 0.53, 0.66 and 0.68 respectively; AUC<sup>ROC</sup> to detect two gene mutations was 0.53, 0.88, and 0.87 respectively.

MCH (pg)	MCV (fL)	Sensitivity: any $\alpha$	Specificity: any $\alpha$	Sensitivity: 2 gene	Specificity: 2 gene
26		58.4	65.7	90.9	60.4
27		85.4	39.2	100	24.3
28		97.8	23.5	100	15.4
	79	68.5	47.1	95.5	44.4
	80	80.9	41.2	100	34.9
	81	88.8	30.4	100	24.3
	82	97.8	21.6	100	14.2
27 OR 81		95.5	27.5	100	18.9
27 OR 80		92.1	31.4	100	23.1
27 OR 82		98.9	20.6	100	13

### Conclusion

Single alpha deletions were rare where MCV>82fL or MCH>27pg; no two gene deletions were seen where MCV>80fL or MCH>27pg. Cutpoints selected should be validated in a cross-sectional survey in the Australian population.

**Keywords** Thalassaemia, alpha globin gene, red cell indices

**Conflict of interest** No

**Monday 29 October**  
**ANZSBT Symposium 4: Haemovigilance Session**

**1030-1130**  
**Rooms 219/220**

## **Nuts and Bolts of the Dutch Haemovigilance System**

Jo Wiersum  
*The Netherlands*

Abstract not available at time of going to print

Monday 29 October  
ASTH/APSTH Symposium 1: Haemolysis and Thrombosis

1030-1130  
Room 210

## Risk Factors of Thrombosis and Mortality and Treatment Strategy in Paroxysmal Nocturnal Hemoglobinuria (PNH)

Jong Wook Lee

*Division of Hematology, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, South Korea*

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder caused by a somatic mutation of the phosphatidylinositol glycan-complementation class A (PIG-A) gene in hematopoietic stem cells. Using retrospective data from 301 Korean PNH patients in a nationwide registry, we set out to systematically identify the contribution of clinical signs and symptoms commonly observed in patients with PNH to the risk of early mortality and TE. We identified thromboembolism (TE) and impaired renal function (IRF) were detected in 18% and 17% of patients respectively. History of TE (odds ratio [OR] 6.9; 95% confidence interval [CI], 2.9-16.2;  $p < 0.001$ ) and IRF (OR 3.1; 95% CI, 1.2-8.2;  $p = 0.025$ ) were independent risk factors for mortality. Independent risk factors for TE were LDH  $\geq 1.5$  times the upper limit of normal [ULN] at diagnosis (OR 7.0; 95% CI, 1.52-32.30;  $p = 0.013$ ), IRF (OR 2.9; 95% CI, 1.35-6.16;  $p = 0.006$ ), and abdominal pain (OR 2.2; 95% CI, 1.09-4.39;  $p = 0.027$ ). Clone size was not associated with risk of TE ( $p = 0.122$ ) and early mortality ( $p = 0.547$ ). The management of hemolysis of PNH by focusing on treating the anemic symptoms and episodic exacerbations rather than the causes underlying chronic hemolysis, has dramatically changed with the availability of eculizumab. Eculizumab (Soliris®) which is a humanized monoclonal antibody that inhibits the activation of terminal complement components, directly and potently addresses chronic intravascular hemolysis. Treatment with eculizumab resulted in a dramatic reduction of intravascular hemolysis, as measured by LDH. Bone marrow transplantation (BMT) remains the only treatment option for patients who develop severe aplasia in the clinical course of PNH. In our study, patients with reduced intensity conditioning regimen had a better survival than patients with conventional conditioning regimen ( $p = 0.023$ ). These data demonstrate that Asian PNH patients frequently suffer disabling manifestations during the course of PNH disease. IRF, LDH  $\geq 1.5 \times$  ULN at diagnosis and abdominal pain were identified as independent risk factors for TE, and TE and IRF were found to be a risk factor for mortality. The decision to perform a BMT which is a curative therapy should weigh disease prognosis, by incorporating known adverse prognostic factors against the risk of transplant complications. Selection of the appropriate candidate and the right time should be very concerned for the management of PNH patients.

**Keywords:** paroxysmal nocturnal hemoglobinuria, thrombosis, mortality

**Conflict of interest:** No



**Monday 29 October**  
**ASTH/APSTH Symposium 1: Haemolysis and Thrombosis**

**1030-1130**  
**Room 210**

### **PNH: At the Interface Between Haemostasis and Haemopoiesis?**

Paul Coughlin

*Australian Centre for Blood Diseases, Alfred Hospital, Prahran, Vic and Department of Haematology and Oncology, Eastern Health, Box Hill, Vic, Australia*

Paroxysmal nocturnal haemoglobinuria is a rare acquired clonal disorder of haemopoiesis. Affected blood cells fail to express surface proteins linked via the glycosylphatidylinositol (GPI) moiety and this is in turn caused by a mutation in the PIG-A gene which is required for synthesis of GPI anchors. Although PNH is fundamentally a disorder of haemopoiesis its most obvious clinical expression is brisk intravascular haemolysis with anaemia and haemoglobinuria. A variety of other important, and often devastating, events frequently occur including thrombosis, which may arterial or venous, smooth muscle dysfunction (oesophageal spasm, abdominal pain or erectile dysfunction) and pulmonary hypertension. PNH is also associated with other disorders of haemopoiesis including aplastic anemia, myelodysplasia and acute leukaemia. The pathophysiology of PNH will be reviewed with particular focus on mechanistic links between the haemopoietic defect, haemolysis and vascular disturbances. The use of eculizumab, an inhibitor of the terminal complement pathway has alleviated many of the symptoms of PNH. The response to eculizumab has also provided insights into the mechanisms of disease and these will be discussed.

**Keywords** PNH, complement, haemolysis

**Conflict of interest** No

Monday 29 October

ASTH/APSTH Symposium 2: Control of Coagulation Factor Levels

**VWF Genetic Variations: What We Have Not Learned**

1030-1130

Room 211

Jing-fei Dong

*Puget Sound Blood Center and Division of Hematology, Department of Medicine,  
University of Washington, School of Medicine, Seattle, WA, USA*

Von Willebrand factor (VWF) mediates platelet adhesion at sites of vascular injury and protects coagulation factor VIII (FVIII) against proteolysis. Levels of VWF in the circulation vary significantly among normal subjects, ranging from 20%-475% in cohort samples from the Atherosclerosis Risk in Communities (ARIC) study. Environmental and genetic factors contribute to this variation, with a strong genetic influence that includes ABO. Variations in the promoter or coding sequences of the *VWF* gene have been reported to regulate plasma VWF levels, but the impact of intronic variation is less clear. Previous studies are limited by small sample size that is difficult to detect subtle differences associated with intronic variations, to stratify for environmental factor, and to examine ethnic diversities. We have analyzed the ARIC GWAS database of ~15,000 subjects. Among 7,856 European Americans (EA), we identified 17 intronic SNPs that were associated with VWF levels after adjustment for environmental factors and ABO. These positive SNPs are clustered in a 50 kb region, flanking exons encoding for the D2, D' and D3 domains, even though the 78 available SNPs were distributed evenly in the *VWF* gene. ABO contributes to 15.2% of the variability in VWF levels. Further analysis found 13 and 10 SNPs differentially associated with VWF levels in EA males and EA females (N=10,434), respectively. In contrast, RS1063857 was only one associated with VWF levels in African American (AA) females and none in AA males. When analyzing for interactions between VWF and coagulation factor VIII (FVIII), VWF levels were found not in parallel with FVIII activity in ~ 40% of 10,434 ARIC subjects, with ~ 4% highly mis-matched between the two factors. Four intronic FVIII SNPs were associated with FVIII activity and eight with FVIII-VWF ratio in a gender- and race-dependent manner.

VWF variants were also analysed for 1,092 subjects of 14 ethnicities, whose genomes were sequenced by the next generation sequencing technologies (the 1000G Project). We identified 2,722 SNPs and 97 insertions and deletions, with the D' and D2 domains again being the most ethnically diverse. We identified 31 non-synonymous variants that were predicted to be deleterious and 19 associated with von Willebrand disease (VWD). Some of these VWD-associated variants had a minor allele frequency of > 10% in Africans. Together, these data demonstrate that 1) intronic SNPs influence VWF and FVIII expression; 2) there may be gender and ethnic divergence in genetic VWF and FVIII variation; 3) VWF variants may associate with VWD in an ethnic specific manner that is defined by haplotypes. These results highlight the complexity of *VWF* variations for association with bleeding and atherothrombotic diseases in different ethnicities.

**Keywords:** VWF, FVIII, SNP, von Willebrand disease, ABO

**Conflict of interest:** The author claims no relevant conflict of interest.

**Monday 29 October**  
**ASTH/APSTH Symposium 2: Control of Coagulation Factor Levels**

**1030-1130**  
**Room 211**

**Factor Levels and Cardiovascular Risk**

Harshal Nandurkar

Abstract not available at time of going to print

Monday 29 October

Nurses Free Communications 2: Red Flags in Transfusion

O063

1030-1130

Room 212

1030-1045

**The Changing Face of a Nurse Led Transfusion Program**

Jacqui Jagger, Clare Harris

*Central Coast Local Health District, NSW, Australia***Aim**

Evaluation of a nurse led program supporting patients with a chronic transfusion requirement following diagnosis of a haematological disorder.

Background: In 2009 the Haematology Cancer Nurse Coordinators on the Central Coast of NSW started a nurse led transfusion program. The Chronic Transfusion and MDS Nurse Led Clinic (CT & MDS NLC) aimed to provide an individualised approach to transfusion provision for patients requiring blood products every 4 weeks or more frequently. Over the past 12 months the program has accepted other patients groups onto the program whom require close monitoring of blood counts and symptoms (end stage haematological malignancies).

Flexibility was a key aspect of the service, fostering patient centeredness with a focus on early patient assessment and appropriate, timely referral.

**Method**

Descriptive statistics was used in the analysis of 12 month data (June 2011 – June 2012) which looked at patient numbers and diagnosis, chair usage, presentations to the emergency department, hospital admission, referrals and estimated bed days saved.

Patients and clinicians also completed a satisfaction survey.

**Results**

The transfusion program continues to provide individualised blood product support for patients with MDS, MPD's. However, patients with haematological malignancies requiring significant multifaceted support through end of life care have also benefited greatly from a program that is flexible enough to cope with rapidly changing requirements. The nurse led transfusion program has a high level of satisfaction with both consumers and clinicians.

**Conclusion**

The flexible nature of the program allows for efficiency of chair use. The high level of nursing assessment and monitoring has allowed for appropriate, timely care delivery to particularly unstable patient groups

**Keywords** transfusion, nurse led program, patient centered

**Conflict of interest** No

**Monday 29 October**

**Nurses Free Communications 2: Red Flags in Transfusion**

**O064**

**1030-1130**

**Room 212**

**1045-1100**

## **Albumin Traceability – Lessons Learned from the 2012 Albumex Recall**

Carolyn Greely, Christine Michael, Kylie Rushford, Sanjeev Chunilal, Kate Macdermid, Joanne Shaw, Erica Wood.

*Department of Haematology, Southern Health, Monash Medical Centre, Melbourne, Victoria, Australia.*

### **Aim**

In keeping with national standards and the Department of Health requirements, blood products should always be traceable from donor to recipient and the reverse to ensure accuracy of the recall process. Hospital laboratory records should be 100% compatible with patient medical records, reflecting whether the product was administered, discarded or returned to the blood bank. We reviewed the accuracy and completeness of albumin traceability from donor to patient in our university teaching hospital.

### **Results**

Historically, albumin traceability has been an issue at our health service. In 2009, stocks of albumin were stored in 3 ward areas: Intensive Care Unit (ICU), theatre and Neonatal Intensive Care Unit (NICU). An audit of 48 bottles of albumin showed only 79% compliance with the traceability process. Education to relevant staff took place in these areas. In 2010 a further audit of 32 bottles showed 92% compliance. Consequently stocks were removed from ICU and NICU. Theatre was the only remaining area outside of blood bank with a stock of albumin. In 2011, the hospital transfusion team received several reports of non-compliance with the albumin traceability process. Education and additional prompts were put in place to remind staff of the importance of and process for ensuring traceability. After implementation of these changes a follow-up audit of 47 bottles demonstrated only 87% traceability compliance. Stock was consequently removed from theatre. Following the Therapeutic Goods Administration (TGA) Albumex recall in March 2012, memos were sent to all staff informing them that all albumin was quarantined. Seven bottles of albumin were returned from unauthorised storage in ward areas in response to the memo. These bottles had been recorded as being administered to various patients. Albumin that was ordered and not used was being stored in ward areas in case of unforeseen emergency, circumventing the traceability process. One batch of Albumex was recalled by the TGA and 100% traceability was achieved for this batch. An audit in June 2012 of 35 bottles of Albumex has shown 100% compliance with traceability requirements.

### **Conclusion**

The 2012 Albumex recall highlighted the need for ongoing auditing of albumin traceability. Now that compliance has been achieved monitoring will continue to be performed on an annual basis by the Transfusion Nurse Consultants.

**Keywords** albumin, traceability, recall

**Conflict of interest** No conflict of interest to disclose.

Monday 29 October

Nurses Free Communications 2: Red Flags in Transfusion

O065

1030-1130

Room 212

1110-1115

**Anaemia in Surgical Patients Attending a Preoperative Anaesthetic Assessment Clinic**

Christine Michael, Carolyn Greely, Sanjeev Chunilal, Kylie Rushford, Erica Wood  
*Department of Haematology, Southern Health, Monash Medical Centre, Melbourne, Victoria, Australia*

**Aim**

In surgical patients, it is well documented that preoperative anaemia is a risk factor for poor patient outcomes. The aim of this study was to determine the presence of preoperative anaemia in an elective surgical patient population at Southern Health.

**Method**

A retrospective analysis was performed on a group of patients who attended our preoperative anaesthetic review clinic from January 2011 to January 2012. All surgical cases were included, ranging from minor to major.

**Results**

Of the 2573 patients who attended clinic, 357 underwent a full blood examination on the day of their clinic appointment. Anaemia was defined according to the laboratory parameters of haemoglobin (Hb) below 130g/L in males and 120g/L in females. Fifteen percent (52/357) of patients were found to be anaemic, of whom 69% (36/52) were male and 31% (16/52) were female – and in this anaemic group 17% (6/36) males and 50% (8/16) females had Hb  $\leq$  100g/L. Preliminary findings from this study suggest that no patients had their anaemia investigated with tests such as iron studies. Few patients had their preoperative anemia treated with hematinics and at least two patients were transfused to top up their Hb preoperatively. More than 10 patients received potentially unnecessary transfusions intraoperatively and postoperatively for preexisting anaemia.

**Conclusion**

This study has identified a previously unrecognised problem of preoperative anemia and its management at our health service. Dedicated resources are required to address this important issue. Accordingly, a coordinated and systematic process is currently being implemented to manage and improve outcomes for patients undergoing surgery. Further work is also required to identify preoperative anaemia in patients not attending the anaesthetic review clinic or whose haemoglobin was not checked on the day of clinic visit.

**Keywords** anaemia, preoperative, anaesthetic

**Conflict of interest** No conflict of interest to disclose

**Monday 29 October**  
**HSANZ: Carl de Gruchy Oration**

**1130-1230**  
**Plenary 2 (Auditorium)**

### **Accidental Discoveries: An Accidental Career**

Jeffrey Szer  
*The Royal Melbourne Hospital, Vic, Australia*

Increasing attention to and better funding of translational research based on mechanism-driven preclinical discovery and subsequent well conducted clinical research has resulted in numerous advances in the management of haematological diseases, particularly in the past 20 years. Almost as frequently, serendipity, accidents or the misinterpretation of bad science or a combination of these factors has resulted in such steps forward. The parallels between the advancement of clinical medicine and the progress of an individual career may be equally unpredictable. An exploration of such events may help guide career choices and satisfaction as well as improving the outcome of patients with life-threatening haematologic diseases. Such study may also help to explain how a trainee in medical oncology developed a passion for haematology, acquired some expertise in stem cell transplantation, veered off into some unpredictable rare diseases while doing everything else and continues to have an enjoyable and very satisfying career.

Monday 29 October  
HSANZ ASTH Combined Presidential Symposium  
O066

1330-1500  
Plenary 2 (Auditorium)  
1330-1345

**CD45 Negative but not CD45 Positive U266 Human Myeloma Cells Demonstrate an Epithelial-to-Mesenchymal Transition (EMT) Transcriptional Programme**

Cindy Lin, Tiffany Khong, Sridurga Mithraprabhu, Andrew Spencer  
*Myeloma Research Group, Australian Centre for Blood Diseases, Monash University, Alfred Hospital, Melbourne, Vic, Australia*

**Aim**

CD45 is a protein tyrosine phosphatase and published data in multiple myeloma (MM) show that the proportion of CD45<sup>-ve</sup> cells present at diagnosis correlates with poorer patient outcome and increased risk of extra-medullary disease. The aim of this study was to investigate whether the identification of biological differences associated with differing levels of CD45 expression would provide a model of MM disease progression.

**Results**

Gene expression profiling (GEP) utilising the Illumina HT12-V2 array and GeneGo analysis of U266 human MM cells (which comprise both CD45<sup>+ve</sup> and CD45<sup>-ve</sup> populations) identified the hypoxia-induced EMT pathway as being the most differentially expressed pathway between the CD45<sup>-ve</sup> (EMT on) and CD45<sup>+ve</sup> (EMT off) populations. EMT enables vital cellular changes that occur during embryogenesis and wound healing. Furthermore, it has recently been found to be a critical driver of disease progression and metastasis in a range of solid tumours including carcinoma of the breast and lung. Our GEP findings were validated with QRT-PCR demonstrating increased expression of SNAIL (SNAIL), CTGF and HES1 in the CD45<sup>-ve</sup> subset. Consistent with EMT, NOTCH pathway activation was confirmed within the CD45<sup>-ve</sup> population by the demonstration of a high HES1:DTX1 transcriptional ratio and intra-cellular ICDN1 protein. The CD45<sup>+ve</sup> population expressed increased surface CXCR4 and ICAM-1 (both p<0.001) compared to the CD45<sup>-ve</sup> population with enhanced migration towards (1.55-fold, p=0.0037) and adhesion to (1.39-fold, p<0.0001) HS5 stromal cells, both consistent with CD45 negativity conferring greater metastatic potential. Finally, further analysis of the GEP data demonstrated increased expression of a range of tumour suppressor genes prone to promoter hypermethylation within the CD45<sup>+ve</sup> subset while the CD45<sup>-ve</sup> cells preferentially expressed a range of oncogenes known to be associated with a more aggressive clinical phenotype in the context of solid tumour biology.

**Conclusion**

These data support the hypothesis that in some instances MM disease progression characterised by a more malignant and metastatic phenotype may be driven by activation of the EMT pathway. To the best of our knowledge this is the first time that this has been demonstrated in a haematological malignancy.

**Keywords** Epithelial-mesenchymal Transition (EMT); CD45; Multiple myeloma

**Conflict of interest** No conflicts of interest to disclose



Monday 29 October

HSANZ ASTH Combined Presidential Symposium

O067

1330-1500

Plenary 2 (Auditorium)

1345-1400

# **Analysis of Patients With Common Peripheral T-Cell Lymphoma (PTCL) Subtypes From a Phase 2 Study of Romidepsin in Rel/Ref PTCL**

H Miles Prince,<sup>1</sup> Francine Foss,<sup>2</sup> Barbara Pro,<sup>3</sup> H.Lubomir Sokol,<sup>4</sup> Matthew Greenwood,<sup>5</sup> Dolores Caballero,<sup>6</sup> Peter Borchmann,<sup>7</sup> Frank Morschhauser,<sup>8</sup> Martin Wilhelm,<sup>9</sup> Lauren Pinter-Brown,<sup>10</sup> Swaminathan Padmanabhan,<sup>11</sup> Andrei Shustov,<sup>12</sup> Jean Nichols,<sup>13</sup> Susan Carroll,<sup>13</sup> John Balser,<sup>14</sup> Steven Horwitz,<sup>15</sup> Bertrand Coiffier<sup>16</sup>

<sup>1</sup>Peter MacCallum Cancer Centre, East Melbourne, Australia; <sup>2</sup>Yale Cancer Center, New Haven, CT, USA; <sup>3</sup>Fox Chase Cancer Center, Philadelphia, PA, USA; <sup>4</sup>Moffitt Cancer Center, Tampa, FL, USA; <sup>5</sup>Royal North Shore Hospital, Sydney, Australia; <sup>6</sup>Hospital Universitario de Salamanca, Salamanca, Spain; <sup>7</sup>Klinikum der Universität zu Köln, Köln, Germany; <sup>8</sup>Hôpital Claude Huriez, CHU de Lille, Lille, France; <sup>9</sup>Klinikum Nürnberg Nord, Nürnberg, Germany; <sup>10</sup>UCLA Medical Center, Los Angeles, CA, USA; <sup>11</sup>The Methodist Hospital, Houston, TX, USA; <sup>12</sup>University of Washington, Seattle, WA, USA; <sup>13</sup>Celgene Corporation, Cambridge, MA, USA; <sup>14</sup>Veristat, Inc, Holliston, MA, USA; <sup>15</sup>Memorial Sloan-Kettering Cancer Center, New York, NY, USA; <sup>16</sup>Hospices Civils de Lyon, Lyon, France

## **Aim**

This phase 2 registration study demonstrated the clinical benefit and tolerability of romidepsin in pts with Rel/Ref PTCL. This updated analysis examined results in the 3 major subtypes of PTCL: PTCL-NOS, AITL, and ALK-1–negative ALCL.

## **Method**

Pts with Rel/Ref PTCL after  $\geq 1$  prior systematic therapy received romidepsin 14 mg/m<sup>2</sup>, 4-h IV, on days 1, 8, and 15 every 28 days. Primary endpoint was rate of CR/CRu. Efficacy assessments were made by an independent review committee.

## **Result**

117 pts with PTCL-NOS, AITL, or ALK-1–negative ALCL were included; median prior systemic therapies was 2 (range, 1-8). Overall, CR/CRu, overall response ( $\geq$  PR), and disease control (ORR + SD  $\geq$  90 days) rates were 16%, 28%, and 46%, respectively, and were similar across subtypes. Most pts (62%) with CR/CRu had no response to prior therapy. With a median follow-up of 22.3 mo, median DOR was 28 mo, with responses continuing beyond 48 mo. Most common grade  $\geq 3$  AEs were thrombocytopenia (25%), neutropenia (18%), and infection (15%), with similar tolerability across subtypes.

## **Conclusion**

Romidepsin induced durable responses at similar rates in pts with the 3 major PTCL subtypes. Tolerability profiles were similar across disease groups. These data support the use of single-agent romidepsin.

**Keywords:** romidepsin, peripheral T-cell lymphoma, relapsed/refractory

**Conflict of interest:** This research was supported by Celgene Corporation. Celgene employees assisted with data analysis in collaboration with the authors.

Monday 29 October  
HSANZ ASTH Combined Presidential Symposium  
O068

1330-1500  
Plenary 2 (Auditorium)  
1400-1415

## **Dose Intensity is a Significant Factor in the Achievement of Early Molecular Response in CP-CML Patients Treated with Imatinib**

Deborah White<sup>1,2</sup>, Verity Saunders<sup>1</sup>, Amity Frede<sup>1</sup>, David Yeung<sup>1,2</sup>, Timothy Hughes<sup>1,2</sup>

<sup>1</sup> Division of Haematology, Centre for Cancer Biology SA Pathology, Adelaide

<sup>2</sup> Department of Medicine, University of Adelaide, South Australia

### **Aim**

Early response prediction in CML patients treated with imatinib(IM) will facilitate timely therapeutic intervention where needed. Response can be predicted by the BCR-ABL level at 3 months (early molecular response – EMR) with a level of  $\geq 10\%$  (EMR failure) associated with poor outcomes. In the TIDEL II study patients are treated with 600mg IM upfront, and dose escalated or switched to nilotinib (NIL) for failure to achieve specified PK and molecular milestones. The Aims of this study were to, 1). Assess the predictive value of EMR. 2). Assess factors which contribute to EMR. 3) Assess factors that modify the risk profile in patients who achieve EMR

### **Results**

EMR failure was associated with a lower probability of MMR at both 12 and 24 months ( $p < 0.001$ ) and a significantly lower EFS ( $p = 0.024$ ). IM trough drug levels at day 21 were significantly lower in patients with EMR failure median  $x \pm y$  ( $p = 0.004$ ), suggesting early dose intensity impacts EMR. To further assess the impact of dose at the cellular level the combined effect of IM level and OCT-1 activity (OA) was assessed. Patients with IM levels  $< 1000\text{ng/ml}$  and low OA achieved EMR at a significantly lower rate than all other groups ( $p = 0.004$ ). Interestingly, the risk of progression for patients with EMR failure, and those who achieved EMR but have low OA was similar ( $p = 0.280$ ). Importantly, 3/5 patients who transformed fell within this latter group

### **Conclusion**

EMR is predictive of subsequent response in the TIDEL II study despite a pro-active approach of dose escalation and early NIL switch. Early dose intensity is a critical factor in the achievement of EMR, and IM day 21 PK and OA provide strong early predictors of EMR and subsequent molecular response. The simple stratification of patients based on EMR while predictive of response may fail to identify a key group of patients for whom outcomes are equally poor (low OA, EMR achieved). Optimising therapeutic outcomes in high-risk cases may not be achievable even with early intervention, suggesting that a different approach from the outset, based on predictive biomarkers at diagnosis, should be trialled in these cases.

**Keywords** CML, tyrosine kinase inhibitors, response prediction

**Conflict of interest** This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract

Monday 29 October  
HSANZ ASTH Combined Presidential Symposium  
O069

1330-1500  
Plenary 2 (Auditorium)  
1415-1430

## Determination of the Maximum Tolerated Dose of Panobinostat in Combination with Azacitidine in MDS and AML: A Phase Ib/II Study

Peter Tan<sup>1</sup>, Kate Reed<sup>1</sup>, Patricia Walker<sup>1</sup>, Sharon Avery<sup>1</sup>, Sushrut Patil<sup>1</sup>, Andrew Grigg<sup>2</sup>, Peter Mollee<sup>3</sup>, Othon Gervasio<sup>4</sup>, Ivo Winiger<sup>5</sup>, Dirk Hönnemann<sup>6</sup>, Andrew Wei<sup>1</sup>, Andrew Spencer<sup>1</sup>

<sup>1</sup>Dept of Haematology, The Alfred Hospital, Monash University Melbourne, Australia. <sup>2</sup>Dept of Haematology, Austin Hospital, Melbourne, Australia. <sup>3</sup>Div of Cancer Services, Princess Alexandra Hospital, Brisbane, Australia. <sup>4</sup>Novartis Pharmaceuticals Australia, Sydney, Australia. <sup>5</sup>Novartis Pharma AG, Basel, Switzerland. <sup>6</sup>Celgene Pty Ltd, Melbourne, Australia

### Aim

To investigate the safety, tolerability and preliminary efficacy of combining the oral pan-deacetylase inhibitor panobinostat (LBH589) with azacitidine in MDS/AML.

### Methods

Phase Ib/II multi-centre open label dose-escalation/expansion study, for IPSS int-2/high risk MDS or AML, not eligible intensive therapy. Patients received azacitidine 75mg/m<sup>2</sup> SC on days 1-5 in 28-day cycles with either: 10, 20, 30 or 40mg panobinostat orally 3 days per week (M/W/F) for 7 doses from day 5.

### Results

40 patients enrolled, median age 70 years (36-82); 30 AML had intermediate (18) or poor cytogenetic risk (12), 10 MDS had int-2 (7) or high risk (3) IPSS. Patients entered panobinostat cohorts of 10mg (4 patients), 20mg (7), 30mg (6) or 40mg (6); expansion at 30mg (17). All grade non-haematologic adverse events regardless of causality (>10%) were fatigue (50%), injection site reaction (50%), nausea (38%), diarrhoea (33%), anorexia (28%), febrile neutropenia (15%), constipation (13%), dyspnoea (13%), fever (13%), and vomiting (13%). There were no unexpected adverse events. The maximum tolerated dose (MTD) of panobinostat was defined at 30mg and this dose level was expanded. The overall response rate (ORR; CR+CRi+PR) in AML was 27% (3 CR/CRi, 5 PR) and in MDS was 50% (2 CR, 3 PR). After a median follow-up of 10.6mo, the median OS was 8.0mo (0.7-20.5). There was an improved median OS in patients who did not progress (CR+PR+SD) compared to those with progressive disease (13.3 vs 2.8mo, p<0.001). In AML, there was no difference in response (CR+PR+SD=61 vs 67%) or median OS (7.9 vs 7.1mo, p=n.s.) with respect to cytogenetic risk (int. vs poor, respectively).

### Conclusion

In previously untreated MDS/AML azacitidine with panobinostat at 30mg is well tolerated, demonstrates clinical activity and warrants further evaluation.

**Keywords** AML, MDS, epigenetic therapy

**Conflict of interest** This research was supported by Novartis, Celgene and VCA.

Monday 29 October  
HSANZ ASTH Combined Presidential Symposium  
O070

1330-1500  
Plenary 2 (Auditorium)  
1430-1445

## Elucidation of Lineage Potential of Murine Progenitor Populations: Identification of Thrombopoietin Responsive Bipotential Progenitors

Ashley P Ng, Maria Kauppi, Craig D Hyland, Donald Metcalf, Ladina Di Rago, Warren S Alexander.

*The Walter and Eliza Hall Institute of Medical Research, The University of Melbourne, Melbourne, Vic, Australia*

### Aim

The prospective identification of myeloid progenitors by surface immunophenotype allows physiological and aberrant haemopoiesis to be understood. Critically, relating specific progenitor populations to their lineage potential, response to haemopoietic stress and cytokines, elucidates the role of specific progenitors at steady state and when haemopoiesis is perturbed.

### Results

We characterised murine progenitor populations by in vitro and in vivo assays to determine a robust biological schema with which to understand haemopoiesis, with a focus upon erythroid and megakaryocyte lineage formation.

We identified a bipotential erythroid-megakaryocyte progenitor population (BEMP) which was capable of forming erythroid and megakaryocyte lineages in vitro and in vivo, and showed response of cells derived from this population to increased thrombopoietin signalling, and a model of acute thrombocytopenia using anti-platelet serum.

Furthermore, we identified that a population of progenitor cells that hitherto had been related to granulocyte—monocyte lineage formation, was capable of forming all myeloid lineages.

### Conclusion

Taken together, these data allow an improved understanding of the hierarchical relationships between progenitor populations during erythroid and megakaryocyte lineage formation. Importantly, we have identified a BEMP population which appears to play a key role in emergency haemopoietic responses to experimental acute immune thrombocytopenia, which we show is regulated in significant part, by thrombopoietin signalling.

**Keywords** Bipotential-Progenitor, Thrombopoietin, Thrombocytopenia

**Conflict of interest** No

Monday 29 October  
HSANZ ASTH Combined Presidential Symposium  
O071

1330-1500  
Plenary 2 (Auditorium)  
1445-1500

## Directed Intravascular Leukocyte Migration: A Distinct Leukocyte Guidance Mechanism Mediated by Platelet Thrombi

Zane S Kaplan<sup>1\*</sup>, Mehran Ghasemzadeh<sup>1,3\*</sup>, Imala Alwis<sup>1</sup>, Simone M Schoenwaelder<sup>1</sup>, Michael J Hickey<sup>2</sup>, Yuping Yuan<sup>1</sup>, Shaun P Jackson<sup>1</sup>  
<sup>1</sup>Australian Centre for Blood Diseases, <sup>2</sup>Department of Medicine, Monash University, Melbourne, Australia; <sup>3</sup>Iranian Ministry of Health and Medical Education, Tehran, Iran. \*These authors contributed equally

### Aim

Thrombosis stimulates inflammation, leading to organ injury in a broad range of human diseases, however, the mechanisms regulating this phenomenon remains ill-defined. We therefore sought to define the mechanism by which microvascular thrombi guide leukocytes to sites of vascular injury.

### Results

We developed an ischemia reperfusion (I/R) injury model in the mesenteric circulation of mice to investigate leukocyte recruitment by microvascular thrombi. These studies revealed widespread platelet and fibrin-rich thrombi in the mesenteric microcirculation following I/R injury, with recruitment of leukocytes. Real-time intravital microscopy revealed that microvascular thrombi were highly effective at recruiting leukocytes, inducing leukocyte shape change, and promoting directed migration through the body of the thrombus. To investigate the mechanisms regulating leukocyte migration through platelet thrombi, we developed a localised model of endothelial perturbation induced by microinjector needle injury, which led to highly reproducible, localised platelet thrombus formation and the rapid and efficient recruitment of leukocytes to sites of endothelial injury. Leukocyte recruitment in this model was entirely dependent on  $\alpha$ -thrombin and its platelet cellular receptor PAR-4. To identify the platelet-derived proinflammatory molecules inducing leukocyte migration the releasate from platelets was fractionated using ion-exchange and affinity chromatography methods. These studies identified CTAPIII/NAP-2 as the dominant platelet chemokine inducing neutrophil shape change and polarisation. Moreover, *in vivo* immunofluorescence analysis with an anti-NAP-2 antibody demonstrated the presence of a NAP-2 chemokine gradient within the body of the thrombus and inhibition of the neutrophil NAP-2 chemokine receptor CXCR-2, reduced neutrophil migration through thrombi to the site of vascular injury.

### Conclusion

These studies define a key role for thrombin and its platelet receptor PAR-4 in inducing release of the chemokine NAP-2 at sites of vascular injury leading to the development of a chemotactic gradient within the body of platelet thrombi, guiding leukocytes to the damaged vessel wall.

**Keywords** Inflammation, platelets, leukocytes **Conflict of interest** No

**Monday 29 October**  
**ANZSBT Symposium 5: Pathogen Inactivation and Blood Safety**  
**European PI Experience**

**1330-1500**  
**Rooms 219/220**

Emma Castro  
*Centro de Transfusión de Cruz Roja Española en Madrid, Spain*

**Background**

The current paradigm of safety is the implementation of laboratory screening methods and restrictive donor criteria. Pathogen inactivation is a proactive way to manage pathogens before they enter the blood supply because it impedes the replication of a wide range of viruses, bacteria and parasites within plasma, platelets, or red blood cells.

**PI State of the Art**

There are two different technologies that inactivate viruses in plasma (Solvent Detergent -S/D - and Methylene Blue) another two for the inactivation of a wide range of viruses bacteria and parasites in platelets and plasma (INTERCEPT TM from Cerus Corp. and Mirasol from Terumo BCT). Theraflex UVC for platelets from Macopharma is under development. With respect to red cells, Cerus has developed a system based on S-303, that is under clinical trials at this moment and it is committed for the development of a new system for whole blood. Terumo BCT is also developing a new system for the treatment of whole blood.

S/D Plasma: It was introduced in Germany in 1992 and nowadays is in use in 15 EU countries. More than 12,015,969 bags have been used to treat 4,005,323 patients during the last 20 years without relevant side effects.

Methylene Blue plasma: Intended for individual treatment of FFP units. It is in routine use for more than 12 years in 8 EU countries and more than 5.5 million of units have been transfused without remarkable side effects.

INTERCEPT TM for Platelets and Plasma: The system is in routine use in more than >100 centers in 17 countries (12 in UE) and there are ongoing evaluations in 11 countries (incl. Australia and Malaysia). More than 1,300,000 PI platelets and plasma transfusions. Haemovigilance have demonstrated that no relevant side effects occur.

Mirasol-for Platelets and Plasma: CE marked in 2002. It is in use in 50 centers in 16 countries (7 within EU). Nowadays > 24,000 platelets and >34,000 units of FFP have been transfused, without remarkable side effects.

Theraflex UVC for Platelets: The device obtained the CE mark in 2009. Clinical trial Phase II/III is planned for 2013.

**Conclusion**

Pathogen inactivation systems are widely implemented in Europe with thousands of PI platelets and plasma transfusions without remarkable side effects. This means that the goal of «zero risk» is coming ever closer.

**Keywords** Transfusion transmitted infectious diseases; Pathogen Inactivation;

**Conflict of interest** Author is member of the Scientific Advisory Board of Macopharma. The author's Blood Centre has been awarded Centre of Excellence by Cerus Corporation.

**Monday 29 October**

**ANZSBT Symposium 5: Pathogen Inactivation and Blood Safety**

**1330-1500**

**Rooms 219/220**

**Risk Analysis and Risk Management of Blood Safety in Relation to  
Emerging Pathogens**

Cees van der Poel

*The Netherlands*

Abstract not available at time of going to print

Monday 29 October  
ASTH/APSTH Symposium 3: Risk Factors for VTE

1330-1500  
Room 211

## Traditional and Emerging Risk Factors for VTE

Walter Ageno  
*University of Insubria, Varese, Italy*

VTE is a multicausal disease resulting from the complex interaction between congenital and acquired risk factors. Major, traditional risk factors for VTE include cancer, surgery, trauma, immobilization, acute medical illnesses, pregnancy and puerperium, hormonal therapies. The identification of such provoking factors is important for the primary prevention of the disease, as well as for the secondary prevention, since it drives the optimal duration of anticoagulant therapies. However, between 25% and 50% of VTE events remain classified as unprovoked, because none of these major risk factors is identified.

Over the last years, several additional risk factors were found to be also associated with VTE, although their association is weaker. These include traditional cardiovascular risk factors such as obesity and in particular visceral obesity, hypertension, diabetes, dyslipidemia, smoking, and the metabolic syndrome; endocrine disorders; common thrombophilic conditions such as Factor V Leiden mutation or prothrombin gene mutation; non-O blood group; infective diseases and acute and chronic inflammatory disorders. The role of these minor risk factors is biologically plausible since they are all associated with an hypercoagulable state. Their relevance in clinical practice may be substantial because they are common and often co-existing.

**Keywords** Venous thromboembolism, risk factors, hypercoagulability

**Conflict of interest** No



Monday 29 October

ASTH/APSTH Symposium 3: Risk Factors for VTE

**Risk Factors for Developing Venous Thromboembolism (VTE) in Asia**

1330-1500

Room 211

Lai Heng Lee

*Singapore General Hospital, Singapore*

Venous thromboembolism (VTE) was previously thought to be rare in Asia. However, recent data showed a rising trend in the incidence of VTE in hospitalized patients across Asia even although population studies showed a lower VTE rates as compared to the west.

Predominant risk factors identified for developing VTE are immobilization, increasing age, surgery, malignancy and inherited thrombophilia.

Many studies in Asia have shown that VTEs do occur after surgery but differences in sample size and diagnostic criteria resulted in a wide range of results that are not comparable. Subsequently, two large scale Asian multi-centre studies on VTE associated with major orthopaedic surgery were done. The Smart study showed the clinical VTE rate of 1.2% and the Aida study showed a total venographic VTE rate of 43.2%, with a proximal VTE rate of 10.8%. For abdominal surgeries a recent prospective study from Japan reported 20.8% distal DVT and 2.9% proximal DVT. These well conducted prospective studies showed that while the overall VTE rate approaches the range of VTE rates as reported in Caucasian populations, the VTE rates for proximal VTE and hip surgery are noticeably lower. Interestingly, the consistently lower VTE rates in hip surgeries are seen in most other Asian studies. The risk of surgery associated VTE has been shown to be mitigated by appropriate prophylaxis measures.

Active malignancy is the commonest medical condition associated with VTE. The others are stroke, congestive cardiac failure and chronic lung disease. The malignancies most frequently associated with VTE are gynaecologic cancers, colorectal cancers, and lung cancers. Comparing to western studies, lower rates of VTE in early stage pancreatic and stomach cancer are reported, however, in advanced stages, the VTE rates far surpass those reported in Caucasian populations.

For hereditary thrombophilia, Factor V Leiden mutation and Prothrombin gene mutation at position 20210 are particularly rare in East Asians, but Protein C and S deficiencies are more commonly seen in Asian patients compared to Caucasians. However, the relative risks of VTE and predictors of recurrences associated with such hereditary risks are not known.

VTE is a problem in Asia which needs addressing. Except for hereditary risk factors, other major risk categories do not differ from the Caucasian populations, although differences within each risk category are noted. While it appears that incidence of VTE is lower in Asian populations, but when subjected to similar "prothrombotic stress", VTE risk seems likely to be similar between Asians and Western populations.

**Keywords:** Venous Thromboembolism Asia

**Conflict of interests:**

- 1) Served on Advisory Boards by Leo and Bayer.
- 2) Received educational and travel grants from Bayer and Sanofi
- 3) Investigator for Einstein, Recover and Hokusai Studies

Monday 29 October  
ASTH/APSTH Symposium 3: Risk Factors for VTE

1330-1500  
Room 211

## Evaluating Hypercoagulable States With Novel Laboratory Methods

Jennifer Curnow

*Concord Hospital and Vascular Biology Group, ANZAC Research Institute, Sydney, NSW, Australia*

A variety of conditions are associated with hypercoagulability, a predisposition to abnormal clot formation. Traditional thrombotic risk factor investigation has focussed on the diagnosis of an underlying condition. In up to 50% of cases, no underlying cause is found, frustrating both physician and patient. An alternative approach is the laboratory identification of a hypercoagulable profile which correlates with the clinically observed thrombotic phenotype. Simple coagulation assays, such as the activated partial thromboplastin time, provide limited characterisation of individual haemostatic function, and are based on the superseded coagulation cascade model. Assays which incorporate multiple components of the complex haemostatic system and more closely model *in vivo* functions, are necessary, particularly in the diagnosis and management of hypercoagulable states. Thrombin generation assays, the thromboelastogram and fibrin generation and fibrinolysis assays have been utilised in this way. Published clinical correlations for venous thromboembolism, coronary artery disease and malignancy will be described. Recent descriptions of the influence of complement components and inflammatory cells in thrombus formation may provide further novel means of evaluating hypercoagulable states in the laboratory. Optimization of laboratory methods for risk stratification may identify individual variations in the haemostatic phenotype and response to therapies, such that application of modifiers of haemostasis could be tailored to individual circumstances, minimising both thrombotic and bleeding risks, as circumstances change, over time.

**Keywords** Hypercoagulable states, laboratory methods

**Conflict of interest** No

**Monday 29 October**  
**Nurses Symposium 3: Focus on Survivorship**

**1330-1500**  
**Room 212**

**The FHCRC Survivorship Program: Exercise and thrive**

Karen Syrjala

Abstract not available at time of going to print

Monday 29 October  
Nurses Symposium 3: Focus on Survivorship

1330-1500  
Room 212

## Female Sexual Health Post Allograft – A New Zealand Perspective

Catherine Wood  
*Wellington Hospital, Wellington, New Zealand*

### Introduction

Sexuality and fertility issues have become increasingly more important as stem cell transplantation becomes a life extending or curative treatment for increasing numbers of women. Issues such as decreased libido, premature menopause, vaginal changes, genital graft versus host disease and infertility may have a devastating effect on a woman's quality of life post transplant.

A comprehensive approach is required to ensure that sexuality and fertility issues in transplant survivors are assessed and treated proactively. The provision of high quality education and information plus the involvement of a multidisciplinary team is essential in delivering quality care to these patients.

### Aim

The specific aims of the study were:

1. To determine the type of information women have been given about vaginal GVHD, sexuality and fertility before they had their transplants.
2. To discover the kind of information women would like to receive about these issues before and after HSCT.
3. To assess if a gynaecology service designed especially for women undergoing HSCT is helpful with early detection and treatment of vaginal graft versus host disease and assistance with fertility and sexuality issues.

### Results

The results of the study showed that the provision of a gynaecology service for women pre and post HSCT was important in diagnosing and treating genital GVHD and for addressing post HSCT issues around sexuality and fertility. The results also showed that the information and education about genital GVHD, sexuality and fertility currently provided for women needs to be significantly improved.

### Conclusion

Women who were under the care of a structured and comprehensive HSCT related gynaecology programme were more informed and satisfied than women who did not have access to such a programme.

**Keywords** Stem Cell Transplant, Sexuality, Genital GVHD

**Conflict of interest** No

Sunday 28 October  
Nurses Symposium 3: Focus on Survivorship

1330-1500  
Room 212

## **“Get Behind Me Satan – I’ve Got Other Issues Now You Know” – and Other Strategies to Help Survive Steroid Therapy in Those With Multiple Myeloma. Results from a Local Study**

T King<sup>1,2</sup>, K White<sup>1</sup>, L Acret<sup>1</sup>, T Lindsay<sup>2</sup>

*Sydney Nursing School, Cancer Nursing Research Unit, University of Sydney<sup>1</sup>; Institute of Haematology, RPAH<sup>2</sup>; Psycho-Oncology Service, RPAH<sup>2</sup>*

### **Background**

The goals of treatment for multiple myeloma (MM) are to induce remission, prolong survival and maintain quality of life. Corticosteroids (“steroids”) are an important component of treatment regimens but cause a number of significant, poorly understood side effects (SE) which profoundly affect quality of life (QoL).

### **Aim**

Examine the experience of SEs of high dose steroids of MM patients and their carers, including SE profile, severity and overall impact.

### **Method**

A two-phase study with a mixed method design was employed. **Phase one (qualitative)**, Focus group and individual interviews with current MM patients and carers to examine the experience of SEs, information and support needs. Data was analysed with content analysis. **Phase Two (mixed)** Prospective collection using a tailored patient diary recorded over 2 month period to capture subjective assessment of SE, type, frequency, severity and impact. Symptom Assessment Scale and questions prompts with scales and open-ended questions were included. Individual interviews were also undertaken pre and post diary data collection.

### **Results**

47 participants participated in the focus group interviews and 22 participants took part in in-depth individual interviews and diaries. 126 weekly journals were completed. An overriding theme of ‘being constantly attuned to and monitoring of an altered self’ whilst on steroids was impacted by the chronicity of their disease. Sub themes identified were in the domains of ‘side effects experienced and consequences there of’, ‘self management skills’ and ‘coping’. Most commonly reported SEs include mood changes, insomnia, facial flushing and let down effect. The overt nature of mood changes have an adverse impact and adaptive behaviours such as isolating self from others were utilised to manage this SE. Mood and energy changes are common reasons for dose adjustment, and dose reductions due to SEs are frequent.

### **Conclusions**

Themes identified in the interview and journaling confirm that steroids cause a range of SEs and that dose reductions are often applied to minimise the impact. Patients use experience and self management strategies to accommodate steroids effects. Results from this study will inform part 2 of the study and identify ways to improve clinical management of SEs associated with steroid therapy.

**Keywords** Multiple myeloma, steroids, supportive care

**Conflict of interest** No

Monday 29 October  
Nurses Symposium 3: Focus on Survivorship

1330-1500  
Room 212

### Development of the Australian Cancer Survivorship Centre – Implementation in Practice

Michael Jefford

*Australian Cancer Survivorship Centre, A Richard Pratt Legacy, Peter MacCallum  
Cancer Centre, Melbourne, Victoria*

The Australian Cancer Survivorship Centre, A Richard Pratt legacy, received (non-recurrent) funding from The Pratt Foundation and the Victorian Department of Health (DH) in 2009. As a result, ACSC has a dual focus – on improving care for patients at Peter Mac and also for people affected by cancer throughout the state. Funding supports a small staff. ACSC does not provide direct clinical services, but aims to support patients, carers, survivors and health professionals to achieve improved outcomes for cancer survivors. ACSC officially launched, with its website ([www.petermac.org/cancersurvivorship](http://www.petermac.org/cancersurvivorship)) in October 2010.

ACSC supports two targets relevant to survivorship care described within Victoria's Cancer Action Plan 2008-2011. The first is that, by 2011, "we will establish a state-wide program that trials patient-centred models of survivorship care." This is known as the Victorian Cancer Survivorship Program (VCSP). DH has funded 6 pilot projects (total funding ~\$1.7m), which seek to improve care of survivors, particularly around the end of initial cancer treatment. ACSC supports the funded projects and convenes a community of practice (follow the link to VCSP on the ACSC website or go to [www.petermac.org/cancersurvivorship/VictorianCancerSurvivorshipProgram](http://www.petermac.org/cancersurvivorship/VictorianCancerSurvivorshipProgram)). The second reference within VCAP is that "by 2012 we will provide evidence of training of the cancer workforce in survivorship awareness." ACSC is working with key partners, including Cancer Learning ([www.cancerlearning.gov.au](http://www.cancerlearning.gov.au)), to facilitate this outcome.

ACSC has developed information for survivors to support materials produced by Cancer Council and other agencies. ACSC has also developed materials to support health professionals to provide improved care, with an emphasis on the post-treatment phase.

Work at Peter Mac has included trialling survivorship care plans and the development of education sessions for both survivors and health professionals. The presentation will describe the current work plan of ACSC, including work to support the above VCAP goals, and will describe some of the challenges to implementing an improved model of care for cancer survivors.

**Keywords** Survivor, Post-treatment, Rehabilitation

**Conflict of interest** No

**Monday 29 October**

**HSANZ Symposium 5: Haematopoietic Stem Cell Biology**

**1530-1630**

**Plenary 2 (Auditorium)**

**Molecular Biology and Prognostication in MDS**

Speaker to be advised

Abstract not available at time of going to print

Monday 29 October  
HSANZ Symposium 5: Haematopoietic Stem Cell Biology

1530-1630  
Plenary 2 (Auditorium)

## HSC Self-renewal Through Asymmetric Cell Divisions

Stephen Ting

*Alfred Health & Monash University, Department of Haematology & Australian Centre for Blood Diseases, Division of Blood Cancers, Stem Cell Research Group, Melbourne, Vic, Australia*

Haematopoietic stem cells (HSCs) are characterised by their dual ability for self-renewal and multipotentiality that results in the lifelong supply of all blood cellular components. Yet the molecular mechanisms underlying HSC self-renewal remain essentially unknown. From a clinical perspective, this knowledge could herald at least two applications: one being the ability to expand HSCs *ex vivo* for both therapeutic and gene therapy purposes and the other, to study whether leukemia stem cells (LSCs) have corrupted and are therefore, potentially targetable via these aberrant LSC self-renewal processes. A proposed mechanism for the duality of HSC function is asymmetric cell division (ACD) whereby the daughter cells from HSC divisions maintain stemness by the asymmetric segregation of cell fate determinants. The molecular details of ACD have predominantly been studied through invertebrate model systems of the *Drosophila* and *C. Elegans*. Within these respective systems, gene networks involved in cell polarity and mitotic spindle orientation are integral for successful ACD of tissue-specific stem and progenitor cells. In mammalian tissues, stem cells from the brain, skin and muscle have been shown to undergo ACD. Similarly, for mammalian haematopoiesis aspects of ACD have been documented in T cells, B cells and also, HSCs. Importantly, perturbation of ACD has been implicated in both solid tissue and blood cancers. Based on the hypothesis that the molecular network governing ACD in invertebrate systems is conserved in mammalian haematopoiesis, we performed a functional screen on potential cell polarity and cell fate determinants to identify a cluster of genes that enhance *in vivo* HSC function. These genes are involved in cellular processes of endocytosis (*Ap2a2*) and the cytoskeletal network that impacts upon respectively, mitotic spindle orientation (*Gpsm2*), actin polymerisation (*Tmod1*) and microtubule and primary cilia functions (*Kif3a*). Furthermore, the endocytic protein, *AP2A2* was shown to segregate asymmetrically during real-time imaging of HSC divisions, thereby linking enhanced HSC function to a potential asymmetric cell fate determinant.

**Keywords** HSC, Self-renewal, Asymmetric cell division

**Conflict of interest** None



**Monday 29 October**  
**ANZSBT Symposium 6: Transfusion Safety in Practice**

**1530-1630**  
**Rooms 219/220**

**National Safety and Quality Health Service Standards**

Margaret Banks

Abstract not available at time of going to print

**Monday 29 October**  
**ASTH/APSTH Symposium 4: Cerebrovascular Disease**

**1530-1630**  
**Room 210**

## **The Intrinsic Coagulation Pathway in Ischaemic Stroke**

Bernhard Nieswandt

*University Hospital and Rudolf Virchow Center, DFG Research Center for  
Experimental Biomedicine, University of Würzburg, Würzburg, Germany*

### **Aim**

Ischaemic stroke is a leading cause of death and disability worldwide and only one effective treatment exists, namely thrombolysis. Although pathological coagulant is thought to be involved in the pathogenesis of ischaemic stroke, the underlying mechanisms are only poorly understood. Recent studies with factor XII (FXII)-deficient mice revealed that the FXII-induced intrinsic coagulation pathway is essential for pathological thrombus formation but dispensable for hemostasis.

### **Results**

Studies in mice and rats have now shown that deficiency or inhibition of FXII profoundly protects the animals from ischaemic brain injury. After transient middle cerebral artery occlusion, the volume of infarcted brain in FXII-deficient and FXII inhibitor-treated animals was substantially less than in the respective controls, without an increase in infarct-associated hemorrhage. Targeting FXII reduced fibrin formation in ischaemic vessels, and reconstitution of FXII-deficient mice with human FXII restored fibrin deposition. Mice deficient in the FXII substrate factor XI were similarly protected from vessel-occluding fibrin formation, suggesting that FXII contributes to pathologic clotting through the intrinsic pathway.

### **Conclusion**

This presentation will summarise recent developments in understanding the contribution of FXII-dependent contact activation to the development of thrombo-inflammatory neurodegeneration in the setting of ischaemic stroke.

**Keywords**    Stroke, FXII, coagulation

**Conflict of interest**    No

**Monday 29 October**  
**ASTH/APSTH Symposium 4: Cerebrovascular Disease**

**1530-1630**  
**Room 210**

## **Thrombolysis in Stroke**

Chris Bladin

*Department of Neurosciences, Eastern Health (Monash University), Box Hill, Vic, Australia*

Stroke is the one of the leading cause of death, and the largest cause of adult disability in Australia with over 60,000 new strokes each year. Cerebral ischaemia is the main cause of stroke, usually due to embolism to the brain from a known cardio-embolic source eg atrial fibrillation.

This talk will focus on the therapies used for treating acute ischaemia stroke, including stroke thrombolysis and some of the newer new fibrinolytic agents, as well as the new role that brain imaging is playing in the specific selection of patients for treatment.

**Keywords** acute ischaemia stroke, stroke thrombolysis

**Conflict of interest** No

**Monday 29 October**  
**ASTH/APSTH Symposium 5: ITP**

**1530-1630**  
**Room 211**

## **Update on ITP**

Terry Gernsheimer

*University of Washington School of Medicine, Puget Sound Blood Center,*

Autoimmune thrombocytopenia (ITP) is characterized by increased platelet destruction and shortened platelet lifespan. More recently it has been recognized that a defect in platelet production also plays a part, with the bone marrow unable to increase production and compensate for the accelerated destruction. Multiple mechanisms likely play a part and include targeting of megakaryocytes by the antibodies with resultant apoptosis as well as only minimal increases in thrombopoietin. These new insights have led to the development of new therapeutic modalities and a change in our approach to both the patient with new onset ITP as well as refractory disease.

In session we will consider the new model of the pathophysiology of ITP and how this has affected treatment algorithms. The International Consensus on the Evaluation and Management of ITP will be reviewed in this context.

**Monday 29 October**  
**ASTH/APSTH Symposium 5: ITP**

**1530-1630**  
**Room 211**

### **ITP in Chinese Populations**

Raymond SM Wong  
*Prince of Wales Hospital, Hong Kong*

Chronic immune thrombocytopenia (ITP) is an autoimmune disorder characterized by autoantibody-induced platelet destruction and reduced platelet production, leading to a low platelet count ( $<100 \times 10^9/L$ ). Treatment of ITP lacks uniformity and is often based on physician experience and institutional protocols. In this session, the management of Chinese patients with ITP in our institution will be reviewed.

**Keywords** Immune thrombocytopenia, Chinese

**Conflict of interest** Research fund and speaker honorarium from GlaxoSmithKline

Monday 29 October

1530-1630

Nurses Symposium 4: Through the Eyes of the Beholder - Stories from the FrontlineRoom 212

## Life After Transplant

Paul Baden  
*Northcote, Vic*

In August 2005 I was diagnosed with Non Hodgkin's Lymphoma (Mantle Cell), treatment started at The Royal Melbourne Hospital and in Dec 2005 I had a Bone Marrow Transplant (brother was the donor).

My story will include:

- the early days of the transplant – Graft versus Host disease, infections, months in hospital;
- the next stage of recovery, out of hospital at home, with family;
- planning a major trip to Europe with my family 18 months after the transplant which we did;
- setting goals;
- My focus and what I have achieved since 2005, from writing a cabaret show about my journey to working with the Red Cross, the Cancer Council and Consumer Committees with Melbourne Health;
- Getting back to full health and how long it has taken;
- And where I am now.

I will include my journey and share my story.

The questions I asked myself, ie Why me? Why am I one of the lucky ones who survived?

What has changed? Including my values, beliefs etc

**Keywords** Challenges, gratitude, support, taking action, excitement, life balance, giving back, hope and inspiration

**Monday 29 October**

**Nurses Symposium 4: Through the Eyes of the Beholder - Stories from the Frontline**

**1530-1630**

**Room 212**

## **Kicking Up Daisies: A Young Patient's Perspective on Living with Primary Myelofibrosis**

Jacinta Lewin

The aim of this discussion is to share the humour, insights and musings of a patient who has been diagnosed with primary myelofibrosis from 2009.

I will cover the challenging and comical hurdles faced by patients and carers in an effort to draw out some key tips for nurses and specialists. The stories will range from frantically attempting to come to terms with your own mortality at the age of 24 to dealing with unexpected responses to a rare diagnosis.

In my experience truth can most definitely be stranger than fiction. I will focus on the importance of assisting a patient with other rights and may provide some tips on how patients seek to 'manage' their treating haematologists and nurses.

The solid conclusion from this talk will be that living with a chronic illness is about sheer determination, managing a good team and a dark and sometimes abrasive sense of humour.

Monday 29 October

1530-1630

Nurses Symposium 4: Through the Eyes of the Beholder - Stories from the FrontlineRoom 212

**Story from a Carer**

Petra Schell

*Melbourne, VIC, Australia*

Craig Perkins passed away in January 2011. He had lived with non-Hodgkins lymphoma for 14 years, during the last 2 years the lymphoma transformed from a follicular lymphoma to a diffuse large cell lymphoma. When Craig was initially diagnosed with cancer he was treated at St Vincent's Hospital, he was subsequently treated at the Peter MacCallum Cancer Centre. We found out in late December 2010 that his treatment was no longer working and he passed away at home 12 days later.

As his partner I was present at some of the critical points during Craig's treatment and subsequent palliative care at the Peter MacCallum Cancer Centre. Craig and I experienced many positives in relation to our interactions with the centre particularly in relation to Craig's treatment and care from key staff, however we also experienced many shortcomings with hospital systems associated with transitioning from treatment to palliative care.

I will be talking about what I saw as the positive aspects of our interaction with the centre and the shortcomings and will provide suggestions to improve future patient/partner experiences during the transition to palliative care and how that care is delivered will be made.

**Keywords** treatment, palliative care, death**Conflict of interest** No



**Monday 29 October**  
**HSANZ Masterclass: ALL in Adolescents**

**1730-1830**  
**Room 207**

**ALL in Adolescents**

Nicola Goekbuget

Abstract not available at time of going to print

Monday 29 October  
HSANZ Masterclass: MPD

1730-1830  
Room 216

## MPD

Claire Harrison  
*Guy's and St Thomas' Hospitals, London, UK*

The chronic myeloproliferative neoplasms (MPNs) are a group of clonal disorders of a hematopoietic multipotent stem/progenitor cell, initially recognized by William Dameshek in 1951, that are distinguished from chronic myelogenous leukemia (CML) for not expressing the BCR-ABL1 protooncogene (Philadelphia-chromosome and BCR/ABL negative chronic MPNs). Their classification and diagnostic criteria have been revised in 2008 by the World Health Organization following the landmark discovery of the recurrent V617F mutation in *JAK2*. They include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF); PV and ET can progress to myelofibrosis, named as post-PV or post-ET MF, that is clinically indistinguishable from primary MF, and 1% to 20% of cases can transform to acute myeloid leukemia (AML). This masterclass will focus upon real issues in management of patients with these disorders including the roles of novel therapies.

**Keywords** MPD, management

**Conflict of interest** Received speaker fees from Novartis, Shire, Cellgene, Sanofi Avensis; consultancy work for YM Bioscience, S\*Bio, Sanofi Avensis and research funding from Shire and Novartis

**Monday 29 October**  
**HSANZ Masterclass: MDS**

**1730-1830**  
**Room 217**

**MDS**

Speaker to be advised  
Abstract not available at time of going to print

**Monday 29 October**  
**HSANZ Masterclass: AML**

**1730-1830**  
**Room 218**

**AML**

Charles Craddock  
*Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, UK*

Advances in risk stratification, novel drug and antibody based treatment strategies and an individualised approach to allogeneic stem cell transplantation with particular emphasis on strategies to improve outcome post-transplant will be discussed.

**Monday 29 October**

**1730-1830**

**ANZSBT Masterclass: “There were three dames from Quebec ..” Limericks and rare red blood cell phenotypes**

**Room 219**

**“There Were Three Dames from Quebec ..” Limericks and Rare Red Blood Cell Phenotypes**

**Christopher Stowell**

*Massachusetts General Hospital & Harvard Medical School, USA*

**Aim**

The aim of this lecture is to review the H deficient RBC phenotypes from the perspective of the Transfusion Service.

**Results**

The speaker will review the serological work-ups of several patients with H-deficient phenotypes, the molecular basis for these phenotypes, and how such cases can be managed at the level of the transfusion service.

**Conclusion**

“Chance favors only the prepared mind..” Louis Pasteur.

**Keywords** – Bombay, H-deficient RBC

**Conflict of Interest** – none

**Monday 29 October**  
**ASTH/APSTH Masterclass: Thrombosis and Cancer**

**1730-1830**  
**Room 208**

## **Thrombosis and Cancer**

Peter L Gross

*Thrombosis and Atherosclerosis Research Institute, McMaster University, Canada*

Venous thromboembolism (VTE) in the setting of cancer will be reviewed. The incidence will be reviewed including a validated clinical prediction rule for incidence. This justifies trials testing primary prophylaxis against VTE in cancer patients at high risk for developing VTE. Data supporting the use of low molecular weight heparins as the standard of care for treatment of VTE will be reviewed. Challenging cases will highlight an approach to incidental imaging abnormalities, and thrombosis in the settings of central lines, of thrombocytopenia and of brain metastasis.

**Keywords** cancer, venous thrombosis, prophylaxis

**Conflict of interest** PLG has received speaker honoraria in the past from Bayer, Leo, Pfizer and Sanofi, all of which make products that will be discussed in the presentation.

**Monday 29 October**  
**ASTH/APSTH Masterclass: ITP**

**1730-1830**  
**Room 209**

## **ITP**

Terry Gernsheimer  
*Puget Sound Blood Center, University of Washington School of Medicine, Seattle,  
Washington, USA*

At this session the current state of our understanding of the pathophysiology of ITP will be discussed, including new insights regarding platelet production, thrombopoietin and the role of T regulatory cells. With this in mind we will consider the appropriate evaluation of the patient with suspected ITP and how that might affect treatment decisions. Finally, we will discuss treatment algorithms for ITP, when thrombopoietin receptor agonists should be considered and indications for splenectomy. New international guidelines for ITP will be used as a model for decision making.

**Keywords**     autoimmunity; thrombocytopenia; platelets

**Conflict of interest**     Amgen Corporation – consultation, advisory board  
Symphogen corporation – advisory board

**Monday 29 October**

**Nurses Masterclass: Implementation of survivorship services into practice**

**1730-1830**

**Room 212**

### **Implementation of Survivorship Services into Practice**

Karen Syrjala

Abstract not available at time of going to print



HSANZ Young Investigators Symposium

O072

Plenary 2 (Auditorium)

0830-0845

## Identification of Sphingosine Kinases as Therapeutic Targets in B-cell Acute Lymphoblastic Leukaemia

Craig Wallington-Beddoe<sup>1</sup>, Kenneth Bradstock<sup>2</sup>, Linda Bendall<sup>1</sup>

*Westmead Institute for Cancer Research, Westmead Millennium Institute, The University of Sydney, NSW Australia; <sup>2</sup>Haematology Department, Westmead Hospital, Sydney, NSW Australia.*

### Aim

Sphingosine 1-phosphate (S1P) is a bioactive lipid with roles in cell proliferation and survival, produced by the sphingosine kinases, SK1 and SK2. Here we assess the relevance of SKs in B lineage acute lymphoblastic leukaemia (B-ALL).

### Results

Retroviral transduction of wildtype (WT) murine B-cell progenitors with BCR/ABL resulted in aggressive ALL when injected into WT mice. In contrast, fewer WT recipients of SK1<sup>-/-</sup> or SK2<sup>-/-</sup>-transduced cells developed disease ( $p = 0.007$ ) demonstrating a role for SK1 and SK2 in ALL development. SK1 protein is highly expressed in B-ALL cells. The combined SK1/SK2 inhibitor SKI-II, the SK1 inhibitor SK1-I and the SK2 inhibitor ABC294640 all reduced intracellular S1P concentrations. This decreased proliferation in all cell lines tested with IC<sub>50</sub> values of 1 $\mu$ M-7 $\mu$ M, 4 $\mu$ M-10 $\mu$ M and <40 $\mu$ M and viability with IC<sub>50</sub> values of 2 $\mu$ M to >10 $\mu$ M, 12 $\mu$ M-18 $\mu$ M and 50-60 $\mu$ M respectively. SKI-II resulted in caspase-dependent cell death whereas SK1-I and ABC294640 produced caspase-independent cell death. All SK inhibitors synergized with imatinib in BCR/ABL (Ph)<sup>+</sup> ALL cells and ABC294640 synergized with the pan-histone deacetylase inhibitor AR-42 and the proteasome inhibitor bortezomib in Ph<sup>+</sup> and Ph<sup>-</sup> ALL cells. Three weeks of 100mg/kg daily of ABC294640 significantly reduced detectable leukaemia in NOD/SCID IL2 $\gamma$ <sub>c</sub><sup>-/-</sup> mice engrafted with three different human ALL xenografts, including a Ph<sup>+</sup> positive xenograft. ABC294640 also extended the survival of mice with established xenografts (>1% ALL in the blood) by 12 days ( $p = 0.0012$ ). When imatinib was combined with ABC294640 survival was prolonged when compared to either agent used alone ( $p = 0.044$ ).

### Conclusion

Loss of SKs reduces the incidence of ALL in a murine model of BCR/ABL driven disease and the SK2 inhibitor, ABC294640, reduces disease and extends survival in a human xenograft model of ALL. This agent also synergises with a number of potential therapeutic agents and further extends survival in a xenograft model of Ph<sup>+</sup> disease when combined with imatinib. This has potential to translate into a useful anti-leukaemic strategy.

**Keywords** Leukaemia, sphingosine kinase, mouse model

**Conflict of interest** No

**Tuesday 30 October**  
**HSANZ Young Investigators Symposium**  
**O073**

**0830-1000**  
**Plenary 2 (Auditorium)**  
**0845-0900**

**CD300 Molecule Expression and Regulation on Dendritic Cell Subsets**

RE Gasiorowski, GJ Clark, PD Fromm, DNJ Hart  
*Dendritic Cell Biology and Therapeutics Group, ANZAC Research Institute, Sydney*

**Aim**

The human CD300 family consists of six immunoregulatory leucocyte membrane molecules which modulate immune effector cell functions including their differentiation, migration and survival. Four CD300 molecules are expressed primarily in cells of the myeloid lineage, including dendritic cells (DC). Recent data suggested that individual CD300 family members may form heterodimers. Consequently understanding individual CD300 molecule expression in relation to other CD300 family members is critical to understanding how they each modulate immune effector functions. We aimed to clarify the expression and regulation of CD300 molecules on peripheral blood DC.

**Method**

We used multiparameter flow cytometry to analyse cell surface expression of CD300 family members on peripheral blood DC subsets from healthy donors. To correlate surface protein expression and gene expression, DC subsets were immunopurified and quantitative RT-PCR was performed to monitor expression of each CD300 gene normalised to a housekeeping gene. DC subsets were incubated with TLR ligands and retinoic acid to investigate the effect on CD300 expression.

**Results**

The CD300a and CD300a/c monoclonal antibodies (mAb) bound all DC populations. The mAb to CD300d,e&f did not bind to the CD141<sup>+</sup> and pDC populations and the CD300f mAbs also failed to bind to CD1c<sup>+</sup> DC. DC mRNA analysis validated individual CD300 molecule expression and revealed potential heterodimers. Exposure to different activators altered the pattern of CD300a-f expression.

**Conclusion**

CD300 molecules are differentially expressed in DC subsets and their expression varies with stimulation via TLR ligands. The data highlights the potential role of CD300 molecules in regulating DC responses. Given their known immunomodulatory function CD300 molecules represent attractive targets for therapeutic manipulation of DC.

**Keywords** Dendritic cells, CD300, immunomodulation

**Conflict of interest** No conflict of interest to disclose

Tuesday 30 October  
HSANZ Young Investigators Symposium  
O074

0830-1000  
Plenary 2 (Auditorium)  
0900-0915

# **Pre-transplant Low-dose Thymoglobulin and its Impact on the Predictive Power of Risk Factors for Chronic Graft-versus-Host Disease**

Andrew Lim<sup>1</sup>, Ashanka Beligaswatte<sup>2</sup>, Marnie Collins<sup>3</sup>, Kate Mason<sup>1</sup>, Emily Li<sup>1</sup>, James A. Russell<sup>4</sup>, Andrew Daly<sup>4</sup>, Jeff Szer<sup>1</sup>, Ian Lewis<sup>2</sup>, Jan Storek<sup>4</sup>, David Ritchie<sup>1</sup>  
<sup>1</sup>Royal Melbourne Hospital, Parkville, Vic, Australia. <sup>2</sup>Royal Adelaide Hospital, Adelaide, SA, Australia. <sup>3</sup>Centre for Biostatistics and Clinical Trials, East Melbourne, Vic, Australia; <sup>4</sup>University of Calgary, Calgary, Alberta, Canada

## **Aim**

To determine if low-dose (total 4.5 mg/kg) Thymoglobulin (LDThymo), an agent that reduces chronic graft-versus-host disease (cGVHD) incidence by 20-30% when given with conditioning for allogeneic haematopoietic stem cell transplantation (alloHSCT), nullifies the predictive power of traditional risk factors for cGVHD.

## **Results**

Between the years 2004 and 2011, LDThymo was given to 359 alloHSCT recipients (UC n=270, RMH n=67, RAH n=20; median age 48; acute leukaemia n=211, chronic myeloid leukaemia [CML] n=21, other myeloid malignancy n=53, other lymphoid malignancy n=74). Conditioning was myeloablative in 92%. Donors were unrelated in 64%. Median follow-up was 585 days. The primary endpoint was time to initiation of systemic immunosuppression for cGVHD (cGVHD-IS). Patients with death or relapse before day 30, or primary graft failure were excluded from cGVHD analysis. At 1 year, overall survival (OS) was 72.3% (95%CI 67.4-76.7%), cumulative incidence of cGVHD-IS was 30.7% ( $\pm$  2.5%), and cumulative incidence of relapse (CIR) was 15.4%  $\pm$  2.0%. At 3 years, OS was 60.2% (54.2-66.0%), cumulative incidence of cGVHD-IS was 32.4%  $\pm$  2.6% and CIR 25.1%  $\pm$  2.7%. From univariate analysis, no risk factor (higher recipient and donor age, sex mismatch, unrelated donor, 1-2 HLA allele mismatch, diagnosis other than CML, use of peripheral blood stem cells) was significantly associated with time to onset of cGVHD-IS. However, the study was underpowered to conclude equivalence (defined as  $\pm$ 10%) for these variables.

## **Conclusion**

In this multicentre cohort, traditional risk factors for cGVHD were not predictive in the presence of pre-transplant low dose Thymoglobulin. This suggests that in patients undergoing T cell depleted alloHSCT, novel predictors of cGVHD may be needed to stratify risk and assign therapy for cGVHD.

**Keywords** thymoglobulin, chronic graft-versus-host disease, risk factor

**Conflict of interest** No conflict of interest to disclose.

**Tuesday 30 October**  
**HSANZ Young Investigators Symposium**  
**O075**

**0830-1000**  
**Plenary 2 (Auditorium)**  
**0915-0930**

**Prophylactic Infusion of Multi-virus Specific T cells for Management of Viral Reactivation and Infection in Patients Post Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)**

CKK Ma, E Blyth, L Clancy, R Simms, J Burgess, K Micklethwaite, DJ Gottlieb  
*Westmead Millennium Institute, University of Sydney, Westmead, Australia*

**Aim**

We present the preliminary data on a phase I/II clinical trial administering multi-virus specific T cells prophylactically to patients who have undergone HSCT.

**Methods and Results**

Donor derived CMV, EBV, adenoviral and VZV specific T cells were generated according to standard operating procedures of the Sydney Cell and Gene therapy group. HSCT recipients received  $2 \times 10^7/m^2$  virus specific T cells at or after day 28 post transplant and were monitored for evidence of viral reactivation and graft versus host disease. 9 patients who underwent sibling donor HSCT from Feb 2011 to Feb 2012 were recruited to the study. Of the 7 patients who received CMV specific T cells, 3 (42%) patients had CMV reactivation post T cell infusion with a mean peak CMV DNA titre of 995 copies/ml. No patient developed CMV disease but one patient received valganciclovir at the discretion of the treating physician. No clinical EBV, adenoviral and VZV reactivation or disease was seen in trial patients. 3 patients (30%) developed grade II-IV acute graft versus host disease (aGVHD). In comparison, 39% (11 patients) of a contemporaneous control cohort of 28 HSCT recipients developed CMV reactivation with a mean peak CMV DNA titre of 24334 copies/ml. 54% of the control group who reactivated CMV required ganciclovir and/or foscarnet therapy. Adenoviral antigen was detected in 1 patient in the control group with diarrhoea; EBV and adenoviral DNA was detected in the blood and urine of 1 patient in the control group with fever and viral encephalitis. 9 patients (32%) in the control group developed grade II-IV aGVHD.

**Conclusion**

Mutli-virus specific T cell therapy appears safe in the setting of HSCT. There is a trend towards a reduction in the need for antiviral treatment for CMV reactivation. Further study is required to determine the safety and efficacy profile of multivirus specific T cells given prophylactically following HSCT.

**Keywords** immunotherapy, virus specific T cells, allogeneic stem cell transplant

**Conflict of interest** No conflict of interest to disclose.

Tuesday 30 October  
HSANZ Young Investigators Symposium  
O076

0830-1000  
Plenary 2 (Auditorium)  
0930-0945

### Depletion of Jak2V617F MPN Stem Cells by Interferon-alpha in a Murine Model of Polycythemia Vera

Steven Lane<sup>1</sup>, Ann Mullally<sup>2</sup>, Geoff Hill<sup>1</sup>, Ben Ebert<sup>2</sup>

<sup>1</sup>Queensland Institute of Medical Research, Brisbane, Australia.

<sup>2</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

#### Aim

Interferon alpha (IFN $\alpha$ ) is an effective treatment for patients with myeloproliferative neoplasms (MPN). In addition to inducing hematological responses in most MPN patients, IFN $\alpha$  reduces the JAK2V617F allelic burden and can render the JAK2V617F mutant clone undetectable in some patients. The mechanism underlying these responses is incompletely understood and whether the molecular responses that are seen occur due to the effects of IFN $\alpha$  on JAK2V617F mutant stem cells is debated.

#### Results

Using a murine model of Jak2V617F MPN, we investigated the effects of IFN $\alpha$  on Jak2V617F MPN-propagating stem cells in vivo. IFN $\alpha$  treatment induces hematological responses in the disease model and causes depletion of Jak2V617F MPN-propagating cells over time. IFN $\alpha$  treatment prevented Jak2V617F-MPN from developing in transplanted recipient mice, demonstrating functional depletion of disease-specific stem cells. Mechanistically, IFN $\alpha$  treatment preferentially induced cell-cycle activation of Jak2V617F mutant long-term hematopoietic stem cells and promoted a predetermined erythroid-lineage differentiation program.

#### Conclusion

These findings provide insights into the differential effects of IFN $\alpha$  on Jak2V617F mutant and normal hematopoiesis and suggest that IFN $\alpha$  achieves molecular remissions in MPN patients through its effects on MPN stem cells. Furthermore, these results support combinatorial therapeutic approaches in MPN, by concurrently depleting dormant JAK2V617F MPN-propagating stem cells with IFN $\alpha$  and targeting the proliferating downstream progeny with JAK2-inhibitors or cytotoxic chemotherapy.

**Keywords** Jak2 V617F, MPN, Stem cells

**Conflict of interest** No

Tuesday 30 October  
HSANZ Young Investigators Symposium  
O077

0830-1000  
Plenary 2 (Auditorium)  
0945-1000

## The Role of PET-CT Scans in Post Remission Surveillance of Patients with Diffuse Large B-cell Lymphoma

CY Cheah<sup>1</sup>, MS Hofman<sup>1,2</sup>, M Dickinson<sup>1,2</sup>, A Wirth<sup>1,2</sup>, D Westerman<sup>1</sup>, SJ Harrison<sup>1,2</sup>, K Burbury<sup>1</sup>, M Wolf<sup>1,2</sup>, EH Januszewicz<sup>1</sup>, HM Prince<sup>1,2,3</sup>, DA Carney<sup>1,2</sup>, DS Ritchie<sup>1,2</sup>, JF Seymour<sup>1,2</sup>

<sup>1</sup> Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia

<sup>2</sup> University of Melbourne, Parkville, Victoria, Australia

<sup>3</sup> Monash University, Clayton, Victoria, Australia

### Aim

To determine the role of PET-CT in the follow up of patients with diffuse large B-cell lymphoma (DLBCL) achieving complete metabolic response after primary therapy, identify patterns of relapse and define a risk-adapted strategy.

### Results

We included 115 patients with *de novo* DLBCL treated at our centre between 2002 and 2009 with a negative post-treatment PET-CT, and at least one surveillance PET-CT scan. With a median follow up of 53.6 months (range 8.2 – 133 months), 456 surveillance scans were performed (range 1 – 10 per patient). Thirteen patients (11%) relapsed with >80% of these occurring in the first 2 years following treatment. In 7/13 (54%) cases, the relapse was suspected based on symptoms and in 6/13 (46%) cases the relapse was sub-clinical. Although numbers were small, there was no difference in survival between the groups. PET-CT had high sensitivity (100%) and specificity (98%) with positive predictive value 56% in the cohort of patients with a low IPI (<3) compared with 80% if the IPI was ≥3. Across the entire cohort, the average number of patients in remission needed to scan to detect one sub-clinical relapse within the first 18 months was 42. However, for those with an IPI ≥3 the number needed to scan to detect one sub-clinical relapse was 22. Surveillance PET-CT had a very low yield after 18 months had elapsed from the conclusion of primary therapy (1 true positive among 170 scans). Second malignancies were detected by PET-CT in eight patients (6.9%).

### Conclusion

PET-CT has high sensitivity and specificity in the detection of relapsed DLBCL, with moderate to high positive predictive value depending on patient IPI risk profile. Use of surveillance PET-CT should be restricted to the first 18 months following completion of primary therapy and to patients with an IPI at diagnosis of ≥3.

**Keywords** Diffuse large B-cell lymphoma, surveillance imaging, PET-CT

**Conflict of interest** No conflicts of interest to declare

Tuesday 30 October  
ANZSBT Free Communications 1  
O078

0830-1000  
Room 219/220  
0830-0845

### **A New Method of Testing for Anti-IgA Antibodies in Adverse Transfusion Reactions**

Katie Havelberg, Gail Pahn, Bruce Dawkins, Mark Burton, Greg Jones  
*Australian Red Cross Blood Service*

#### **Aim**

The presence of anti-IgA in a transfused patient can result in an adverse transfusion reaction presenting with allergic or anaphylactic reactions. These reactions can range from uncomfortable to life-threatening. Historically, results from anti-IgA testing were not available for up to one month in our laboratory, due to a range of testing constraints. We have developed a fluorescent microbead-based test for anti-IgA1 and anti-IgA2 antibody detection. This method is fast, reliable, allows a rapid result turn around and is much less labour intensive than the previous in-house ELISA method.

#### **Method**

The method utilises a simple immunocomplex reaction. This reaction takes place on an antigen-coated polystyrene microbead. Flow cytometry detects anti-IgA that is bound to the coated microbeads, using a fluorescent detector antibody. Anti-IgA1 and anti-IgA2 can be detected separately but simultaneously in the same reaction tube. A number of samples were evaluated using the new method, and compared with the results from in house ELISA and commercial ELISA and PaGIA methods.

#### **Results**

The microbead method detected 100% of antibodies that were detected in either one or all of the in-house ELISA, commercial ELISA and PaGIA methods. Three positive samples had varying results using each of the comparison methods, with the beads being the only method able to detect the antibody in all three samples. This suggests that the bead method has a higher sensitivity than the other methods. Following successful validation, the anti-IgA microbead method has been utilised for routine testing within our laboratory for the past 12 months.

#### **Conclusion**

The results of the validation testing were satisfactory, with all parameters meeting the acceptance criteria. The use of the new assay has resulted in a reduction in both time and labour involved in testing for anti-IgA antibodies, and consequently a faster result turn-around time. Investigations can be completed within 48 hours of a transfusion reaction – providing a result that is more useful for clinicians in their decisions regarding further treatment, including the use of IgA deficient blood products if necessary.

**Keywords** Anti-IgA, Adverse Transfusion Reaction

**Conflict of interest** No conflict of interest to disclose

Tuesday 30 October  
ANZSBT Free Communications 1  
O079

0830-1000  
Room 219/220  
0845-0900

## Sero-prevalence of Antibodies to *Leptospira* Among Blood Donors in High-risk Areas of Queensland

Helen Faddy<sup>1</sup>, Vanessa Racloz<sup>2</sup>, Colleen Lau<sup>2</sup>, Robert Flower<sup>1</sup>, Philip Weinstein<sup>3</sup>  
<sup>1</sup>Research and Development, Australian Red Cross Blood Service; <sup>2</sup>School of Population Health, University of Queensland; Brisbane, Qld. <sup>3</sup>Barbara Hardy Institute, University of South Australia, Adelaide, SA

### Aim

To examine the sero-prevalence of antibodies to *Leptospira spp* in blood donors residing in high-risk areas of Queensland.

### Methods

A total of 498 plasma samples were collected from blood donors residing in high-risk areas of northern Queensland, based on notification rates of leptospirosis (including Ingham, Innisfail, Mareeba, Tully, Cairns, Townsville and Brisbane) during 2009 and 2011. All samples were tested for the presence of antibodies to 22 leptospiral serovars by microscopic agglutination. Samples with a titre of 1:400 or higher against any serovar were described as serologically suggestive of a recent infection, while samples with a titre greater than 1:50 were described indicative of previous infection.

### Results

Of the 498 plasma samples tested, none had antibody titres suggestive of a recent infection. However, seven donors (1.41% 95%CI: 0.37 – 2.44%) had titres suggestive of previous infection.

### Conclusion

Leptospirosis is an acute febrile illness, with concomitant bacteraemia, and transfusion transmission of *Leptospira* is possible. Infection is relatively uncommon in Australia, however, higher rates of infection are observed in northern Queensland. Management of the risk of transfusion-transmitted leptospirosis at the Australian Red Cross Blood Service involves total product restrictions for 3 months following a diagnosed infection, as well as product use restrictions for abattoir workers. Blood component quarantine or recall for donors reporting any illness within 7 days of donation and bacterial contamination screening are additional safety measures. In this study, we did not find evidence of recent infection in Queensland blood donors residing in high-risk regions. Collectively, our study provided novel data to underpin evidence-based risk assessment and policy development relating to *Leptospira* and the safety of the Australian blood supply, and supports the appropriateness of our current relevant donor selection policy.

**Keywords** Sero-prevalence, blood safety, leptospirosis

**Conflict of interest** No



**Tuesday 30 October**  
**ANZSBT Free Communications 1**  
**O080**

**0830-1000**  
**Room 219/220**  
**0900-0915**

**Alterations in Red Blood Cell (RBC) Band 3 and 4.1R Proteins Effect Viability During Storage**

VM Ng, MF Veale, G Healey, RL Sparrow  
*Research & Development, Australian Red Cross Blood Service, Victoria, Australia*

Maintaining RBC shape, deformability and elasticity involves complex interactions between protein 4.1R, transmembrane protein band 3 and the cytoskeletal network. Band 3 and 4.1R are crucial to the maintenance of RBC structure and function, including RBC membrane phospholipid asymmetry, membrane integrity and viability.

**Aim**

The purpose of this project was to monitor the RBC changes occurring in band 3 and 4.1R, and to correlate these with viability during RBC storage and aging.

**Methods**

Leukocyte-depleted RBCs ( $n \leq 6$ ) were processed according to standard Blood Service procedures. Samples were collected aseptically at day 1, 14, 28 and 42. Standard haematological parameters were tested. At each time point, density fractionated young and old RBCs and ghosts were prepared. RBC shape and size were measured by flow cytometry (FCM). Changes to band 3 were determined using a fluorescent dye, eosin-5-maleimide (EMA) and the mean fluorescence intensity (MFI) was measured using FCM. RBC viability over storage was measured using calcein a fluorescent vital dye. The changes to the 4.1R protein in RBC ghosts was determined by using SDS-polyacrylamide gel electrophoresis and Coomassie Blue and silver staining. Densitometric analysis was performed using an Image Quant analyser. Results were statistically analysed using ANOVA.

**Results**

RBC size increased over storage and differences to surface complexity were greatest between young and old RBCs ( $p < 0.05$ ). EMA MFI decreased significantly at day 14 in young and old RBCs, suggesting changes to band 3 distribution in the RBC membrane ( $p < 0.01$ ). Calcein MFI decreased in all RBCs over storage with significant differences between young and old RBCs ( $p < 0.001$ ) suggesting that RBC viability and membrane integrity decrease over storage. Changes to the 4.1R in old RBCs suggest that alterations occur to the cytoskeletal network of proteins.

**Conclusion**

These results suggest that RBC membrane alterations that occur in band 3 and 4.1R during storage and aging and may contribute to decreases in RBC viability.

**Keywords** band 3, protein 4.1R, red blood cells

**Conflict of interest** No

Tuesday 30 October  
ANZSBT Free Communications 1  
O081

0830-1000  
Room 219/220  
0915-0930

## Older Stored Red Blood Cells Promote Increased Adhesion of Fresh Allogeneic Leucocytes with Endothelial Cells

William Xu<sup>1</sup>, Olivier Huet<sup>2</sup>, Margaret F Veale<sup>1</sup>, Jaye PF Chin-Dusting<sup>2</sup>, Amrita Sran<sup>1</sup>, Gerry Healey<sup>1</sup> and Rosemary L Sparrow<sup>1</sup>

<sup>1</sup>Research & Development, Australian Red Cross Blood Service, Melbourne, Victoria, Australia; <sup>2</sup>Vascular Pharmacology, BakerIDI, Melbourne, Victoria, Australia

### Aim

Transfusion of older red cells (RBCs) has been linked to poorer clinical outcomes in which endothelial perturbation may be implicated. Using *in vitro* EC flow perfusion and *ex vivo* perfused vessel models designed to simulate *in vivo* blood transfusion, this study investigated the interaction of allogeneic leucocytes (representing the patient's cells) and endothelial cells (ECs) (representing the patient's blood vessel wall) that were pre-treated with stored RBCs or their supernatant.

### Methods

RBCs were prepared by standard Blood Service procedures. Samples were collected during 42 days of storage. Human ECs were cultured on perfusion slides (EC-perfusion assay). Fresh murine aortic vessels were mounted in a customised perfusion chamber. ECs/vessels were pre-perfused at 37°C with stored RBCs, RBC supernatant or medium alone. Fresh allogeneic whole blood (WB) was obtained from healthy volunteers and neutrophils (PMNs) were isolated for the EC-perfusion assays. PMNs were perfused across the ECs at 0.5 dyne/cm<sup>2</sup> and PMN adhesion recorded by a CCD camera (n=8 experiments). For the *ex vivo* vessel model (n=5), WB was fluorescently labelled with Vybrant-Dil dye and perfused through the vessel at 0.12mL/min. Leucocyte adhesion to the vessel wall was recorded by 10 sec-videos of two fields of view taken over 15 min by a CCD camera fitted to the fluorescence microscope. Unpaired t-tests and ANOVA determined significance.

### Results

ECs pre-perfused with supernatant or RBCs from RBC units stored for >35 days resulted in increased PMN adhesion compared to control (for supernatant pre-perfusion, 17±2 vs 6±1 PMNs/field; *p*<0.0002) (for RBCs, 23±3 vs 6±1 PMNs/field; *p*<0.002). For the vessel model, vessels pre-perfused with 42 days-stored RBCs resulted in increased leucocyte adhesion (13±2 vs 2 ±0.5 leucocytes/field; *p*<0.001).

### Conclusion

Exposure of ECs to stored RBCs or RBC supernatant promotes increased interaction of allogeneic leucocytes with ECs in our *in vitro* transfusion models. These findings may potentially lead to better understanding the effects of stored RBCs on EC interactions, which may be implicated in poorer clinical outcomes.

**Keywords** red blood cells, endothelial cells, leucocytes

**Conflict of interest** No

**Tuesday 30 October**  
**ANZSBT Free Communications 1**  
**O082**

**0830-1000**  
**Room 219/220**  
**0930-0945**

# **Weak D Type 1, 2 and 3 in the Western Australian Patient Population**

Dianne Grey<sup>1</sup>, Jill Finlayson<sup>1</sup>, Elizabeth Fong<sup>1</sup>, John Beilby<sup>2</sup>, Christopher Newbound<sup>2</sup>

*Departments of <sup>1</sup>Haematology & <sup>2</sup>Molecular Genetics, PathWest QEII, WA*

## **Aim**

To determine the proportion of weak D types 1, 2 and 3 using TaqMan PCR in serologically identified weak D samples in a Western Australian patient population.

## **Method**

60 patient samples sent for routine blood grouping reacting with anti-D at a grading <4 in Diaclon ABO/D gel cards (Biorad), were further evaluated using a panel of twelve monoclonal anti-D reagents (Alba Bioscience, Scotland) to differentiate weak D from partial D. Molecular analysis of the weak D samples to characterise weak D type 1, 2 and 3 was performed by TaqMan realtime PCR using custom TaqMan (Assay-by-Design<sup>SM</sup>, Applied Biosystems) MGB primer-probe mixes for the specific amplification of *RHD* and exclusion of *RHCE*. DNA sequencing of exons 1, 6 and 9 was performed using previously described primers<sup>1</sup>

## **Results**

The percentage of patients in the Western Australian population identified as weak D type 1, 2 or 3 by TaqMan PCR are shown in Table 1. The serological reactivity patterns with monoclonal anti-D were variable for all weak D types.

Table 1: % of patients identified as weak D types 1, 2 or 3 using TaqMan PCR.

Molecular Characterisation by TaqMan	% Patients (n=60)
Weak D Type 1	40 (24)
Weak D Type 2	50 (30)
Weak D Type 3	5 (3)
Unknown	5 (3)

## **Conclusion**

Weak D type 2 was the prevalent allele in the Western Australian population contrasting with many other geographical locations where type 1 is reported to predominate. Although 95% of patients serologically identified as weak D were confirmed as weak D type 1, 2 or 3 significant heterogeneity in anti-D reactivity was observed.

<sup>1</sup> Wagner F, Frohmajer A, & Flegel W. RHD positive haplotypes in D negative Europeans. BMC Genetics 2009 2:10.

**Keywords** weak D, monoclonal anti-D, TaqMan PCR **Conflict of interest** No

Tuesday 30 October  
ANZSBT Free Communications 1  
O083

0830-1000  
Room 219/220  
0945-1000

## Clinically Significant DEL-associated *RHD* Alleles Exist Within the Australian RhD Negative Blood Donor Panel

Stacy Scott<sup>1</sup>, Catherine Hyland<sup>1</sup>, Yew-Wah Liew<sup>2</sup>, Robert Flower<sup>1</sup>

<sup>1</sup>Research and Development and <sup>2</sup>Red Cell Reference Laboratory, Australian Red Cross Blood Service, Australia

### Aim

RBC donations with weakly expressed RhD antigen, or 'DEL' donations, are typed and managed as RhD negative. r'r and r"r donors have been genotyped, and 8 DEL-associated *RHD* alleles have been characterised. DEL-associated alleles which have been reported to immunise RhD negative patients, 1227G>A and *hom IVS5-38del4*, have been detected. As a result of these initial findings, the study was continued to define the frequency of these types in the RhD negative donor panel

### Method

gDNA from r'r and r"r donors (*n*=1026) was screened for *RHD* exon 4, 5, 10 by qPCR. Donors with a *RHD* signal were SNP analysed using a DNA microarray to fully characterise the *RHD* allele.

### Results:

Donor Group	N	RHD Alleles
1: Carry <i>RHD</i> alleles not expected to be immunogenic	53/1026	Non-functional <i>RHD</i> alleles
2: Carry potentially immunogenic DEL-associated alleles	21/1026	<i>RHD</i> -CE(4-9)-D; <i>IVS3+1G&gt;A</i> ; <i>IVS3+2T&gt;A</i> ; M295I; 94insT; 1177C
3: Carry DEL-associated alleles reported to be immunogenic	9/1026	1227G>A and <i>hom IVS5-38del4</i>

Using qPCR and SNP data, the r'r and r"r donors tested were divided into groups of different risk of RhD immunogenicity. Group 1 donors, 5.17% (95% CI: 3.81–6.52%), carry non-functional *RHD* alleles that do not encode for the RhD antigen and are not expected to be immunogenic. RBC donations from Group 2 donors, 2.05% (95% CI: 1.18–2.91%), are potentially immunogenic as donors carry a DEL-associated allele predicted to express a DEL phenotype. Group 2 alleles have not been reported to immunise RhD negative patients. Donors from Group 3, 0.88% (95% CI: 0.31–1.45%), do carry DEL-associated alleles that have been reported to immunise.

### Conclusion

Clinically significant DEL alleles 1227G>A and *hom IVS5-38del4* were detected in 0.88% of an Australian r'r and r"r blood donor sample. To reduce the risk of DEL immunisation, policy in relation to the issue of these DEL donors should be developed.

**Keywords:** RhD; DEL; immunise

**Conflict of interest:** No

**Tuesday 30 October**  
**ANZSBT Free Communications 2**  
**O084**

**0830-1000**  
**Room 218**  
**0830-0845**

**Lessons from the Albumin Quarantine 2012**

Bev Quested, Trish Roberts

*Australian Red Cross Blood Service, BloodSafe, Adelaide, SA, Australia*

**Aim**

Information was collated from BloodSafe Transfusion Nurses and key transfusion personnel from South Australian public and private sectors regarding the quarantine of Albumin. The experience and learnings from the Albumin quarantine process in South Australia 2012 have been summarised.

**Results**

The quarantine highlighted a number of issues

- Imprest (official and unofficial)
- Traceability
- Communication to and within the organisations
- Clear lines of responsibilities for acting upon communication
- Cases where it was used (or not)
- Roles of transfusion nurse, transfusion service provider and transfusion committee involvement in the process.
- Human factors can still corrupt sound processes

**Conclusion**

The profile of Albumin being a blood product and its traceability requirements were raised within some organisations. It also reinforced the hospitals role in managing blood and blood products safely and efficiently. Imprest levels of Albumin official (and other) have been reassessed and modified. As a result of this experience communication lines have been tested and reassessed along with responsibilities clarified, allowing for more succinct future communications if the need arises. The role of the transfusion nurses varied depending upon which hospitals they supported. Despite some hospitals handling the process extremely well human factors can still intervene.

**Keywords** Albumin, quarantine, transfusion nurses

**Conflict of interest** No conflict of interest to disclose.

Tuesday 30 October  
ANZSBT Free Communications 2  
O085

0830-1000  
Room 218  
0845-0900

# **Does the Duration of Blood Storage Impact on the Prognosis of Critically Ill Patients - A Multicentre Observational Study?**

C Aubron<sup>1</sup>, M Bailey<sup>1</sup>, Z McQuilten<sup>2,6</sup>, D Pilcher<sup>1</sup>, C Hegarty<sup>3</sup>, A Martinelli<sup>4</sup>, G Magrin<sup>5</sup>, P Diaz<sup>6</sup>, D Irving<sup>6</sup>, J Cooper<sup>1</sup>, R Bellomo<sup>1</sup>  
<sup>1</sup> *Australian and New-Zealand Intensive Care-Research Centre, DEPM, Monash University,*, *Transfusion Research Unit DEPM, Monash University,* <sup>3</sup> *Intensive Care Unit Austin Hospital,* <sup>4</sup> *Transfusion Service Austin Hospital (Melbourne),* <sup>5</sup> *Transfusion Service Alfred Hospital (Melbourne),* <sup>6</sup> *Australian Red Cross Blood Service*

## **Aim**

To assess the impact of Red blood cells (RBC) storage duration on the outcomes of patients hospitalised in intensive care unit (ICU).

## **Methods**

This retrospective multi-centre study was conducted over a 10-year period. All adults admitted to ICU in two tertiary hospitals who received at least one RBC were included. The impact of storage duration of the RBC (or age of blood) on ICU and hospital mortality and length of stay (LOS) was evaluated using the mean, maximum and minimum ages of blood divided into deciles. A multivariate analysis was performed adjusting for potential confounders. Subgroup analysis was performed on patients with no RBC transfusion prior to ICU and patients who received leukodepleted RBC exclusively.

## **Results**

Between 2001 and 2011, 8416 patients were transfused with a median of 4 (IQR=2-7) units per patient. The overall ICU and hospital mortality was 10% and 26%, respectively. The mean age of the first RBC unit received per patient was 18.1±8.5 days and was lower for the survivors compared to the non-survivors (17±0.2 days versus 18.3±0.1 days, p=0.0001). In multivariate analysis adjusting for factors associated with the risk of death on univariate analysis (Apache 3 score, admission category type, number of RBC units received, study centre and year), neither the mean, maximum or minimum age of RBC was associated with mortality. Similar results were obtained for the subgroup analyses. However, the age of the oldest RBC and the mean age were both independently associated with an increased ICU and hospital LOS, in the overall population and in the sub-groups analyses.

## **Conclusion**

In this large study, RBC storage duration was not associated with mortality but was independently associated with ICU and hospital LOS. These results support the need for a multicentre randomised trial to determine whether, compared to standard care, transfusion of the freshest available RBC leads to clinically relevant benefits.

**Keywords** age of blood, critically ill patients, outcome

**Conflict of interest** Nothing to disclose

ANZSBT Free Communications 2

O086

Room 218

0900-0915

# **Validation of Transfusion Laboratory Information System Data is Important in Data Linkage Studies: Results from a Validation Study**

Zoe McQuilten<sup>1,2,3</sup>, Nick Andrianopoulos<sup>1</sup>, Joanne Enticott<sup>1</sup>, Erica Wood<sup>1,2,3</sup>, Merrole Cole-Sinclair<sup>1,4</sup>, John McNeil<sup>1</sup>, Peter Cameron<sup>1</sup>, Julian Smith<sup>1,3</sup>, Christopher Reid<sup>1</sup>, Louise Phillips<sup>1</sup> on behalf of the Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) Steering Committee

<sup>1</sup>Monash University, <sup>2</sup>Australian Red Cross Blood Service, <sup>3</sup>Monash Medical Centre and <sup>4</sup>St Vincent's Hospital, all in Melbourne, Victoria

## **Aim**

Data from transfusion laboratory information systems (LIS) are increasingly being linked with clinical data to inform transfusion practice. Accurate data are essential for correct estimates of the risks and benefits of transfusion and appropriate interventions. However studies to validate data for this purpose are lacking. This study aimed to validate LIS data against the Australian and New Zealand Society of Cardiac and Thoracic Surgeons ANZSCTS Cardiac Surgery Database (CSD).

## **Methods and Results**

Data regarding transfusion episodes from surgery until discharge date in all patients who underwent cardiac surgery at 6 Victorian sites in 2008 were analyzed. During the study period, 2689 patients underwent cardiac surgery. LIS data were extracted on 2685 patients for 2709 procedures and matched to CSD. Kappa value (95% Confidence Interval) for agreement between LIS and CSD was 0.76 (0.74-0.79) for transfusion of one or more red blood cell (RBC) unit, 0.71 (0.67-0.74) for 5 or more RBC units and 0.83 (0.80-0.85), 0.86 (0.84-0.89), 0.83 (0.80-0.87) for one or more doses of platelets, fresh frozen plasma or cryoprecipitate, respectively. Total number of mismatches for any RBC unit between LIS and CSD was 321 (12%) and for 5 or more RBC units 235 (9%). In 55 (2%) procedures, the difference in RBC units transfused was outside limits of agreement on Bland-Altman plot. Compared with the patient medical record, 54 (98%) had missing RBC data in the LIS dataset. Problems identified included discrepant patient identifiers, the data query used and incorrect dates recorded in the LIS extract.

## **Conclusion**

There was very good to excellent agreement between LIS and CSD data on transfusion episodes, supporting use of LIS for data linkage purposes. In those cases with a large discrepancy between the two data sources, the most common cause of error was inaccurate data extraction from the LIS.

**Keywords** Transfusion, data, cardiac surgery

**Conflict of interest** No

**Tuesday 30 October**  
**ANZSBT Free Communications 2**  
**O087**

**0830-1000**  
**Room 218**  
**0915-0930**

**Has Transfusion Practice Improved in Australian Hospitals? Evidence from the Blood Matters Program Audits**

Linley Bielby, Lisa Stevenson, Jo Perillo, Bridget Glazebrook, Erica Wood  
*Blood Matters Program, Department of Health, Melbourne, Victoria, Australia*

**Aim**

The audit aimed to determine whether health services had a blood administration policy that is consistent with national guidelines and that everyday transfusion practice at the health service adheres to the policy.

**Method**

In 2011, 155 public and private hospitals across Victoria, Tasmania, Northern Territory and Australian Capital Territory were invited to participate in a comparative audit (2005&2007). The process included a desk top audit of existing blood transfusion policy alignment to guidelines related to specimen collection, labelling, consent, patient identification (ID), observations and adverse reaction management. A prospective bedside observational audit of 30 transfusion episodes including: location, patient consciousness, ID, monitoring, documentation and adverse event management. Data were entered electronically by participants.

**Results**

Eighty-five hospitals responded, 69 public (81%) and 15 private hospitals (17%), 1 no longer provided transfusions. All sites had a hospital-wide transfusion policy, improving from 66% and 84% respectively in 2005 & 2007. Eighty-two hospitals reported 1595 transfusion episodes. Transfusions mainly took place between 8am-8pm (90%) and most patients (95%) wore ID bands. Patients were not asked to confirm ID at 34 hospitals; as high as 70% at one, and 40% at four others. Generally documentation remained the same or improved. Adverse effects associated with transfusion were reported by 23/82 hospitals, 16% had no documentation of the event compared to 30% (2007), and 48% no documentation of reporting to the laboratory.

**Conclusion**

Transfusion practice has improved in hospitals contributing to the audits. The quality of hospital policies available to guide clinical practice has seen ongoing improvement from 2005 to 2011. There are still areas where practice can be improved, such as the reporting and management of adverse events, and more vigilant patient identification

**Keywords** Policy, practice, patient identification, transfusion safety

**Conflict of interest** No conflict of interest.



**Tuesday 30 October**  
**ANZSBT Free Communications 2**  
**O088**

**0830-1000**  
**Room 218**  
**0930-0945**

## **Procedural Adverse Events in Transfusion: What Do We Know?**

Marija Nedeljkovic<sup>1,2</sup>, Lisa Stevenson<sup>1,3</sup>, Bridget Glazebrook<sup>1,3</sup>, Linley Bielby<sup>1,3</sup>, Erica Wood<sup>1,2,3</sup> on behalf of the STIR expert group

<sup>1</sup> Australian Red Cross Blood Service, <sup>2</sup> The Royal Melbourne Hospital & <sup>3</sup> Blood Matters Program - Victorian Department of Health, Melbourne, Vic, Australia

### **Aim/Background**

The Victorian Serious Transfusion Incident Reporting (STIR) haemovigilance system that also includes reporting from Tasmania, Northern Territory and ACT, was established in 2007 after a successful pilot. Incident data related to transfusion are reviewed with the aim to identify potential areas for improvement and focus strategies for prevention. Procedural adverse events related to transfusion at the hospital end of the transfusion chain account for a large proportion of these incidents.

### **Method**

Reports from January 2007 to March 2012 were reviewed, utilising the categories of incorrect blood component transfused (IBCT), wrong blood in tube (WBIT) and near miss for procedural incidents.

### **Results**

A total of 992 incidents were reported to STIR over this time, of which 42% were procedural adverse events. The majority of these were WBITs and other types of near miss events where there is a potential for harm. There were 67 IBCTs (7%), including 5 ABO incompatible red cell transfusions, which contributed to serious harm for patients.

The majority of these errors occurred during the ordering and prescribing of blood products (21.5%), in the laboratory (22%) and at the bedside (21.5%), with patient/product identification as a key issue with all types of events. Factors contributing to errors included lack of awareness by junior doctors of patients' special requirements for blood products, poor communication between staff, failure to follow procedures regarding bedside pre-transfusion checks, and environmental conditions predisposing to risk, such as storage of blood in satellite fridges.

### **Conclusion**

A large number of these process-related incidents are preventable. Near miss events provide valuable learning opportunities as they occur more frequently than actual harm events, and are preceded by the same patterns of error as adverse events causing harm. Given the complexity of hospital-based transfusion practice, interventions to improve transfusion safety aimed at this part of the transfusion chain depend on a combined approach, including staff training, simplifying procedures relating to transfusion and monitoring of performance standards.

**Keywords** Transfusion, Errors, STIR

**Conflict of interest** No

**Tuesday 30 October**  
**ANZSBT Free Communications 2**  
**O089**

**0830-1000**  
**Room 218**  
**0945-1000**

## **Predicting the Probability of Red Cell Transfusion in Surgical Patients**

Romi Sinha, Susan Ireland, Kathryn Robinson  
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### **Objectives**

To predict the probability of red cell transfusion in patients undergoing cardiothoracic, colorectal and orthopaedic surgery based on pre-operative factors.

### **Methods**

A linked electronic database developed for SA public hospitals using clinical, epidemiological and transfusion data was used. Admissions for a range of major surgical procedures over between January 2009 and June 2010 were included. Pre-operative variables including age, sex, type of surgery and haemoglobin [Hb] (up to 8 weeks prior to date of surgery) were analysed using logistic regression to model the probability of red cell transfusion.

### **Results**

A total of 2821 surgical admissions including primary total arthroplasty of the hip [THR] (530), primary total arthroplasty of the knee [TKR] (643), right sided colorectal surgery (332), left sided colorectal surgery (305), coronary artery bypass grafting [CABG] (621) and on-bypass valve replacement surgery (390) were identified. The independent predictors of transfusion were older age ( $\geq 65$  years: odds ratio [OR] = 1.7; 95% confidence interval [CI] = 1.4-2.1,  $p = 0.001$ ), female sex (OR = 1.4; 95% CI = 1.1-1.8,  $p < 0.001$ ) as well as type of surgery and Hb level. Compared with TKA, the OR of transfusion was higher with left sided colorectal surgery (OR = 2.0; 95% CI = 1.4-2.9,  $p < 0.001$ ), THR (OR = 2.3; 95% CI = 1.7-3.2,  $p < 0.001$ ), valve replacement surgery (OR = 14.1; 95% CI = 9.9-20.1,  $p < 0.001$ ) and CABG (OR = 14.8; 95% CI = 10.6-20.6,  $p < 0.001$ ). The model showed that the odds ratio of transfusion was significantly higher if the pre-operative Hb was between 100-110 g/L (OR = 11.9 95% CI = 7.6 to 18.6,  $p < 0.001$ ) compared to a pre-operative Hb  $> 140$  g/L. Graphs showing the predicted probability of transfusion derived from the model (based on age, sex, type of surgery and pre-operative Hb) can be used in pre-operative assessment.

### **Conclusion**

Based on the findings from the model, a number of preoperative factors can be used to better assess the probability of transfusion in individual patients. The results, after further validation, could be used to help target and prioritise patient blood management (PBM) initiatives.

**Keywords:** red cell transfusion, major surgery, pre-operative haemoglobin

**Conflict of interest:** No

Tuesday 30 October

APSTH/ASTH Free Communications 3: Laboratory Methods

O090

0830-1000

Room 210

0830-0845

## The Determination and Characterisation of Anti-ADAMTS13 Autoantibodies

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<sup>3</sup> Centre for Thrombosis and Haemophilia. Murdoch University, Perth, Western Australia

### Aim

A deficiency of ADAMTS13, a multi-domain, anticoagulant protein, specifically responsible for the cleavage of von Willebrand Factor (vWF), can result in Thrombotic Thrombocytopenic Purpura (TTP). The acquired form results from inhibitory anti-ADAMTS13 autoantibodies directed against the protein's functional domains. Not all antibodies however, are inhibitory. Current methods designed to identify ADAMTS13 autoantibodies do not discriminate between pathological and benign forms, or identify the binding regions. This study aims to develop a method for screening patient plasma samples to determine autoantibody binding sites.

### Methods

The specific domains of the ADAMTS13 protein were selected as the antigens in an ELISA that was optimised for screening the patient plasma samples. The individual peptide regions were generated from the corresponding sequence of a synthetic cDNA construct of the full ADAMTS13 coding sequence, in a bacterial expression system. Two patient groups were analysed in the screening process; stroke patients with high titres of anti-ADAMTS13 autoantibodies with normal activity levels, that did not develop TTP, and a group of TTP patients with low level ADAMTS13 (<5%).

### Results

The non-TTP patient plasma samples were screened where distinct polyclonal antibody populations were detected against several of the peptides, mainly the CUB 2 domain, a region outside of the catalytic portion, involved in binding to the A3 domain of VWF whilst in its globular quiescent state. In contrast, the TTP patients demonstrated strong binding to a short sequence within the spacer domain (Tyr<sup>572</sup>-Asp<sup>579</sup>), directly involved in binding to the A2 domain of VWF, to initiate proteolysis.

### Conclusion

We have developed a simple to use ELISA that can differentiate the binding regions of anti-ADAMTS13 autoantibodies, and that may discriminate pathological from benign anti-ADAMTS13 autoantibodies.

**Keywords** ADAMTS13 TTP Autoantibodies

**Conflict of interest** No conflict of interest to disclose.

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APSTH/ASTH Free Communications 3: Laboratory Methods

O091

0830-1000

Room 210

0845-0900

# **Establishment of a Reliable Method for Cilostazol Monitoring in the Presence of PGE<sub>1</sub>**

Kaneo Satoh<sup>1</sup>, Isao Fukasawa<sup>2</sup>, Masato Ohta<sup>1</sup>, Yukio Ozaki<sup>1</sup>

<sup>1</sup> *Department of Clinical and Laboratory Medicine, Faculty of Medicine, University of Yamanashi, Japan.* <sup>2</sup> *Division of Neurosurgery, Kofu Jounan Hospital, Japan*

## **Aim**

Cilostazol has been shown to be effective for prevention and treatment of cerebral infarction. However, there appears to be no widely accepted method appropriate for monitoring cilostazol. We attempted to establish an assay system for cilostazol monitoring, using platelet aggregation induced by arachidonic acid (AA) in the presence of PGE<sub>1</sub> which upregulates intracellular cyclic AMP.

## **Methods**

Blood was drawn from stroke patients before and after cilostazol intake. AA-induced platelet aggregation after pretreatment with 0–30nM PGE<sub>1</sub> for 2 minutes was measured by light transmittance aggregometry.

## **Results**

AA-induced platelet aggregation was 73±2% in the absence of PGE<sub>1</sub>, and pretreatment with 30nM PGE<sub>1</sub> had virtually no inhibitory effect on platelet aggregation prior to cilostazol intake. In contrast, after cilostazol intake, 30nM PGE<sub>1</sub> significantly inhibited platelet aggregation to 13±5% (p<0.0001), while in the absence of PGE<sub>1</sub> platelet aggregation remained similar to that of prior-to-cilostazol value (71±4%). The plasma concentration of cilostazol ranged from 0.55 to 3.51 µM. In the presence of 30nM PGE<sub>1</sub>, all the patients with cilostazol concentrations exceeding 1µM had their platelet aggregation inhibited almost completely. ROC analysis suggests that AA-induced platelet aggregation in the presence of 30nM PGE<sub>1</sub> had the excellent sensitivity (91%) and specificity (88%) for monitoring cilostazol.

## **Conclusion**

AA-induced platelet aggregation in the presence of 30nM PGE<sub>1</sub> could give good estimate on plasma concentrations of cilostazol. It is suggested that this system is a good tool for monitoring cilostazol.

**Keywords** cilostazol, antiplatelet therapy, prostaglandin E<sub>1</sub>

**Conflict of interest** No conflict of interest to disclose.

Tuesday 30 October

APSTH/ASTH Free Communications 3: Laboratory Methods

O092

0830-1000

Room 210

0900-0915

## Longitudinal Investigation of the Effect of Centrifugal Continuous Flow Left Ventricular Assist Devices (cfLVADS) on Haemostatic Parameters

Ken Ly<sup>1</sup>, Kavitha Muthiah<sup>1</sup>, David Connor<sup>1</sup>, Jianlin Qiao<sup>2</sup>, Elizabeth Gardiner<sup>2</sup>, Robert Andrews<sup>2</sup>, Khumud Dhital<sup>1</sup>, Paul Jansz<sup>1</sup>, Christopher Hayward<sup>1</sup>, Joanne Joseph<sup>1</sup>

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<sup>2</sup> Australian Centre for Blood Diseases, Monash University, Melbourne, Australia

### Aim

Patients implanted with cfLVADS routinely receive aspirin and/or clopidogrel and anticoagulation. Therapy related bleeding is therefore common, however it may also reflect changes secondary to shear forces arising from the implanted device. This study aims to assess haemostatic parameters in patients with cfLVADS.

### Methods

8 patients (7M/1F), implanted with HeartWare cfLVAD were recruited. Baseline blood samples were collected pre-implant and at 1, 7, 30, 90 and 180 days post-implant. Platelet counts were measured by routine methods. Soluble P-selectin (sP-Selectin), soluble GPVI (sGPVI), von Willebrand Factor Antigen (vWF-Ag) and vWF Collagen Binding Activity (vWF-CBA) were measured using ELISA.

### Results

Compared to baseline, platelet counts were significantly lower at day 1, and higher at 1 month ( $193.13 \pm 27$  to  $151.63 \pm 16$  and  $361.63 \pm 56 \times 10^9/L$ , respectively,  $p=0.021$  and  $0.012$ ). sP-Selectin levels were elevated at day 0, 1 and 7 for 5 out of 7 patients tested but fell to within normal ranges for all but 1 patient at days 90 and 180. sGPVI levels were elevated at all timepoints (including baseline), and most significantly increased at day 30 compared to baseline ( $76.52 \pm 10.6$  to  $66.07 \pm 13 \text{ ng/mL}$ ,  $p=0.029$ ). vWF-Ag levels were significantly lower at day 90 in comparison to baseline ( $222 \pm 34\%$  to  $154.14 \pm 13\%$ ,  $p=0.027$ ). vWF-CBA was also significantly decreased at days 30 and 90 compared to baseline ( $205 \pm 42\%$  to  $71 \pm 12\%$  and  $65 \pm 8.6\%$  respectively,  $p=0.006$  and  $0.010$ ). vWF ratio (CBA:Ag) fell significantly from 1.03 at baseline to 0.42 at day 30 ( $p=0.008$ ). vWF multimers are pending.

### Conclusion

Our data shows a persistent elevation in sGPVI levels and decline in vWF-Ag, vWF-CBA and vWF ratio (CBA:Ag) over time in patients supported with HeartWare cfLVAD. This likely reflects effects of shear forces from the device itself. Platelet activation is highest in the immediate post-operative period and normalises with time. Patient recruitment is ongoing and additional haemostatic parameters (including microparticle analysis) are being studied.

**Keywords** Left Ventricular Assist Devices, Platelets, von Willebrand Factor

**Conflict of interest** No

Tuesday 30 October

APSTH/ASTH Free Communications 3: Laboratory Methods

O093

0830-1000

Room 210

0915-0930

# **The Same Mutations in Patients with Congenital Dysfibrinogenemia Leading to Different Clinical Manifestations**

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<sup>1</sup>State Key Laboratory of Medical Genomics; <sup>2</sup>Shanghai Institute of Hematology, Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine Shanghai, China; <sup>3</sup>Department of Laboratory Medicine, Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China.

## **Aim**

This study is to investigate the different clinical manifestations appeared in two unrelated dysfibrinogenemia families. Proband 1 has haemorrhagic manifestations, while proband 2 undergoes thrombosis performance.

## **Methods**

PCR amplification and direct sequencing technology were used to identify the mutations in these patients. The antigen level (Fg:Ag) and activity level (Fg:C) of fibrinogen in plasma of the patients were detected by ELLISA and Clauss assays, respectively. Fibrinogen clottability, fibrin polymerization and fibrinolysis measurement were applied to evaluate the functional fibrinogen in patients. A $\alpha$ , B $\beta$  and r chains of fibrinogen were detected by Western blot.

## **Results**

The ratio of Fg :C and Fg :Ag was lower than 0.5 in both patients, which can dignose them as dysfibrinogenemia. We identified the same compound heterozygous: FGA g.4198G>A (Arg289Gln), which is a novel mutation, and FGG g.7475C>T (Arg275Cys) in these two patients, as well as their family members. The fibrinogen clottability rate was 18.15% and 47.26%, respectively, in proband 1 and proband 2, compared to that in normal control. The fibrinogen polymerization curve of proband 1 was almost on the baseline, suggesting bleeding manifestation in patients. The takeoff time in the curve of proband 2 was earlier than that in normal control, indicating its thrombosis risk, although its peak value was relatively lower.

## **Conclusion**

Our results were consistent with clinical manifestations of the patients, which can further explain the different manifestations in these patients. We have excluded all the other polymorphisms related to thrombosis. The underlying mechanism why the same mutations can result in different clinical manifestations is undertaken.

**Keywords** dysfibrinogenemia, clinical manifestation, gene mutations

**Conflict of interest** No conflict of interest to disclose

Tuesday 30 October

APSTH/ASTH Free Communications 3: Laboratory Methods

O094

0830-1000

Room 210

0930-0945

## **Soluble EMMPRIN is Associated With Reduced Coagulation Potential in NSTEMI**

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### **Aim**

EMMPRIN (CD147) is an extracellular matrix metalloproteinase inducer that is upregulated on platelets and leukocytes under prothrombotic/proinflammatory conditions such as coronary artery disease (CAD). CD147 has been examined in CAD, but as yet soluble CD147 levels (sCD147) have not been investigated. The aims of this study were to determine whether sCD147 is elevated within the subgroups of CAD and the potential relationship between sCD147 and the Overall Haemostatic Potential (OHP).

### **Method**

Peripheral blood was collected in EDTA and CTAD collection tubes from a total of 63 subjects [48 CAD patients (22 with stable angina, 13 with unstable angina and 13 with non-ST elevated myocardial infarction (NSTEMI)) and 15 control subjects. Levels of sCD147 were assessed by ELISA, CTAD plasma samples from a subset of each group were used to assess the OHP. Risk factors for CAD, clinical presentation and medication use were recorded. Differences were assessed by ANOVA, post-hoc - Tukey's Multiple Comparison Test. Correlations were assessed by Pearson Correlation.

### **Results**

Analysis revealed significant differences in sCD147 levels between the groups ( $p=0.043$ ) and post-hoc analysis demonstrated a significant increase in the NSTEMI group compared to controls ( $p=0.03$ ). Further investigation of the NSTEMI group revealed significant negative correlations between sCD147 OHP parameters, overall coagulation potential (OCP) ( $r=-0.97$ ,  $p=0.0002$ ,  $n=7$ ), OD Max ( $r=-0.81$ ,  $p=0.027$ ,  $n=7$ ) and Max slope ( $r=-0.81$ ,  $p=0.027$ ,  $n=7$ ); the same relationship was observed for the control population subset.

### **Conclusion**

sCD147 is upregulated in CAD, particularly in NSTEMI patients. The strong negative correlation observed between sCD147 and OCP suggests a possible association between sCD147 and reduced coagulation potential in NSTEMI and controls.

**Keywords** Soluble CD147, Coronary Artery Disease, Overall Haemostatic Potential

**Conflict of interest** No

Tuesday 30 October

APSTH/ASTH Free Communications 3: Laboratory Methods

O095

0830-1000

Room 210

0945-1000

**Using an Age-adjusted Cut-off Level Improved the D-dimer Assay Performance for the Exclusion of Venous Thromboembolism (VTE) in Elderly Patients with a Non-high Pretest Probability (PTP)**

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<sup>1</sup>St-Roch University Hospital, Dept of Hematology, Nice, France and <sup>2</sup>Emergency Department, Cochin University Hospital, Paris, France

**Background**

D-dimer levels below a well defined cut-off level enable to safely rule out VTE in patients with non-high PTP. Ageing is associated with higher levels of D-dimer. To address the issue of their usefulness in elderly patients, we firstly managed data from 644 out-patients with non-high PTP included in a multicenter management study of a D-dimer assay (C.Legnani et al. 2010). We then validated our results in a cohort of 1,042 consecutive patients investigated using another assay.

**Results**

In the first part, D-dimer level, evaluated using the HemosIL D-dimer HS500 assay (Instrumentation Laboratory), was above the cut-off level (500 ng/ml) in all 88 patients with VTE and in 299 of those without (53.8%). Test negative predictive value (NPV) and sensitivity were 100%. Overall test specificity was 46.2%, but decreased in an age-dependent manner over 70 y. ROC-analysis of test results obtained in patients classified according to age allowed us to propose an age-adjustment of the cut-off level calculated by increasing its level by 100 ng/ml per 10 y-increments in patients aged over 59 y e.g. 600 ng/ml between 60 et 69 y, 700 ng/ml between 70 et 79 y. Using those age-adjusted cut-off levels yielded to significantly improve test specificity (56.1%), particularly in old patients. If an 84 y old patient (D-dimer=625 ng/ml) was missed, NPV remained high (99.6%). That strategy was then validated by evaluating data from 1,042 consecutive out-patients, investigated using the Vidas D-dimer assay (BioMérieux). When applied, that age-adjusted cut-off levels led to improve test specificity 62.2% vs. 55.1% using the fixed cut-off level. 3 additional patients, with D-dimer >500 ng/ml but below age-adjusted cut-off level, were missed but overall NPV remained high 99.0% vs. 99.4%.

**Conclusions**

Age-adjusted cut-off levels for D-dimer, calculated by increasing the traditional cut-off level (500 ng/ml) by 100 ng/ml per 10 y-increments in patients aged over 59 y, significantly increased the test specificity. Correlatively, NPV and sensitivity were slightly decreased, as some patients, with D-dimer above 500 ng/ml but below the age-adjusted cut-off level, could be misdiagnosed. However such a strategy was safe, as NPV remained above 99% in our studied populations.

**Keywords:** D-dimer, VTE diagnosis, elderly patients

**Conflict of interest** No



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0830-1000  
Room 211  
0830-0845

## Endothelial Progenitor Cell-based Therapy for Hemophilia A

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*Departments of Regulatory Medicine for Thrombosis<sup>1</sup> and Pediatrics<sup>2</sup>, Kashihara, Nara, Japan, Institute of Advanced Biomedical Engineering and Science<sup>3</sup>, Tokyo Women's Medical University, Tokyo, Japan*

### Aim

Ex vivo gene therapy for hemophilia A might be good for the therapeutic alternative because coagulation factor VIII (FVIII) is a secreted protein and the tight regulation of its expression is not necessary. Blood outgrowth endothelial cells (BOECs) are considered to be an attractive candidate to treat hemophilia A. In fact, we previously demonstrated that therapeutic levels of plasma FVIII were documented from hemophilia A mice over 6 months, in which lentivirally-engineered BOECs mixed with Matrigel were implanted subcutaneously. It is unclear, however, whether the gradual loss of plasma FVIII could reflect a breakdown of the scaffold material or cell death.

### Methods

In the present study, FVIII-transduced BOECs were cultured on temperature-responsive culture dishes and made BOECs sheets. Subcutaneous transplantation studies of BOECs sheets were performed in hemophilia A mice.

### Results

All hemophilia A mice that were treated with BOECs sheet without any prior treatment developed neutralizing anti-FVIII antibodies between 5 and 25 Bethesda units in titer. In contrast, FVIII at therapeutic levels between 20-50 mU/mL were confirmed to sustain for up to 30 weeks without developing anti-FVIII inhibitory antibodies, when hemophilia A mice were treated with peri-transplantation with cyclophosphamide. Histological examination in the part of the mice revealed that the transplanted BOECs sheets were structured as flat clusters without any cells infiltration.

### Conclusion

Tissue engineering approach using genetically modified BOECs sheet is viable for persistent tissue survival and providing therapeutic values. This novel ex vivo gene transfer strategy can provide the safe and efficacious delivery of FVIII in hemophilia A.

**Keywords:** Hemophilia A, Endothelial Progenitors, Cell Therapy

**Conflict of interest:** This research was supported by Bayer Hemophilia Awards (H.M).

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O097

0830-1000  
Room 211  
0845-0900

## A Possible Mechanism for Inv22-related *F8* Large Deletions in Severe Hemophilia A Patients with High Responding Factor VIII Inhibitors

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### Aim

Intron 22 inversion (Inv22) of the coagulation factor VIII (FVIII) gene (*F8*) is a frequent cause of severe hemophilia A (HA). A variety of *F8* mutations causing HA have been reported, however, only a few of these large deletions have been fully characterized at the nucleotide level. In this study, we investigated to identify and further characterize gene abnormalities in three severe HA patients, and to elucidate the etiopathogenic mechanism of their complex X chromosomal rearrangements.

### Methods/Results

In the previous study, the patients have shown to carry atypical Inv22 combined with the large deletion of *F8*. Inverse-shifting PCR for Inv22 genotyping revealed that these patients carried an Inv22 pattern in the centromere side, but a normal pattern in the telomere side. We further analyzed their X chromosomes by PCR mapping and inverse PCR, and found that they had different centromeric breakpoints in the Inv22 X chromosomes, adjacent to the palindromic regions containing *int22h-2* or *-3*, respectively. The connections appeared to be shifted toward the telomere of the normal *F8* wild-type Xq28, resulting in a new telomere with an additional intact *int22h* copy.

### Conclusion

These gene rearrangements might result from double-strand breaks in the most distal regions of the long arms of the Inv22 X chromosomes, followed by DNA restorations using the normal *F8* wild-type Xq28 by nonhomologous end joining or break-induced replication; thus leading to large *F8* deletions in severe HA patients. Elucidation of such complex gene rearrangements will help to understand the mechanism behind gross X chromosomal abnormalities causing HA.

**Keywords** *F8*, intron 22 inversion, gene rearrangement

**Conflict of interest** No conflict of interest to disclose.

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**O098**

0830-1000  
Room 211  
0900-0915

**Mapping the Amino-acid Site of Interaction Between VWF A1 Domain and A3 Domain**

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**Aim**

VWF is a multimeric glycoprotein composed of multiple functional domains and it interacts with the platelet membrane receptor GPIIb-IX-V complex through its A1 domain and with collagen fibres through its A3 domain. Several researches indicate that the A1-A2-A3 triple domain has a more complicated secondary structure rather than the three single domains. Recently, we demonstrated that there was interaction between A1 and A3 domain. The interaction generally will be interrupted by conformational change of A1 and A3 each. To map the interaction binding site in detail, we introduced 11 amino-acid mutations of A1 domain and 13 mutations of A3 domain. The mutants were expressed as recombinant VWF A1, VWF A3 as well as full-length VWF. The role of mutation site in A1-A3 interaction was evaluated in a binding assay using snap/clip-tag technology.

**Results**

The Asp(506), Tyr(508), Lys(549) of A1 domain and Arg(1026), Pro(1027) of A3 domain significantly reduced binding affinity of VWF A1-A3 interaction. Arg(632), Lys(643), Leu(964) and Gln(966) has weak impact on A1-A3 binding affinity. In addition, the reduction of the affinity enhanced the binding of platelet GPIIb to VWF A1 domain.

**Conclusion**

It was predicted using bioinformatics methods that some amino-acid sits on VWF A1 domain or A3 domain were related with interaction of these two domains. Our results confirmed that some sites of them can indeed interfere with the affinity of VWF A1-A3 interaction. Interestingly, several sites were in line with VWD mutation site. These results help us to reveal the function of VWF A domain and the pathogenesis of VWD.

**Keywords** von Willebrand factor(VWF), mutation, VWD

**Conflict of interest** No

Tuesday 30 October  
ASTH Emerging & Young Investigators Awards  
O099

0830-1000  
Room 211  
0915-0930

## Decreased TIM-3 and Its Correlation With Th1 in Patients With Immune Thrombocytopenia

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### Aim

T-cell immunoglobulin- and mucin-domain-containing molecule 3 (TIM-3) is a novel transmembrane protein that is involved in the regulation of T-helper 1 (Th1)-cell-mediated immunity, and has been shown to influence autoimmune diseases; however, its function in immune thrombocytopenia (ITP) has not been well-defined. This study was undertaken to investigate the expressions of TIM-3 in CD4<sup>+</sup> T cells in patients with ITP.

### Method

Plasma IL-18, IFN- $\gamma$  and IL-4 levels, as well as platelets counts were measured in patients with active ITP (n=21), ITP in remission (n=20) and in healthy subjects (n=31) by enzyme linked immunosorbent assay (ELISA). Using real-time quantitative polymerase chain reaction (RT-PCR), the mRNA expression of IL-18, IFN- $\gamma$ , IL-4, T-box (T-bet) and TIM-3 were studied in all blood subjects. CD4<sup>+</sup> Tim-3<sup>+</sup> cells population in the blood were evaluated by flow cytometry and expressed as a percentage of the total number of CD4<sup>+</sup> cells.

### Results

TIM-3 expression was decreased on CD4<sup>+</sup> T cells in the blood of ITP patient. TIM-3 positive cells population in circulating CD4<sup>+</sup> cells in newly diagnosed patients were significantly decreased compared to controls, while T-bet, IL-18 and IFN- $\gamma$  levels were significantly elevated in patients. TIM-3 mRNA expression was significantly lower in the blood of ITP patients compared to controls. During remission stages, the levels of these cytokines were comparable to those of healthy controls.

### Conclusion

TIM-3 is closely associated with ITP disease and may play an important role in the pathogenesis of ITP. TIM-3 may exert its suppressive effect on ITP disease activity by modulation of regulatory Th1 cells and reinforcement of TIM-3 may be a reasonable therapeutic strategy for ITP.

**Keywords** Immune thrombocytopenia; T-cell immunoglobulin- and mucin-domain-containing molecule 3; Th1; IL-18; T-bet

**Conflict of interest** No conflict of interest to disclose

Tuesday 30 October  
ASTH Emerging & Young Investigators Awards  
O100

0830-1000  
Room 211  
0930-0945

## HIF1 Signalling Mediates Cancer-Associated Thrombosis

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<sup>1</sup>University of Cambridge, <sup>2</sup>King's College London, UK, and <sup>3</sup>Salk Institute, USA

### Aim

Hypoxia-inducible factor (HIF) 1-mediated responses are heavily involved in the progression of venous thrombosis and cancer, which often occur simultaneously. Our aim was to determine whether HIF1 is responsible for the positive association between these conditions by investigating the effect of up- and down-regulating HIF1 levels on cancer-associated coagulation and thrombosis.

### Methods

Systemic or endothelial and myeloid HIF1 levels were altered (via administration of HIF1 agonist L-mimosine or via cre-Tie2 driven HIF1 deletion respectively) in established mouse models of thrombosis and breast cancer (polyoma middle T mutation driven by mouse mammary tumour virus). Protein arrays were used to quantify the expression of 25 factors that regulate coagulation, thrombosis, and cancer progression. Coagulation assays (plasma clotting time) and image analysis were used to quantify coagulability and thrombosis respectively.

### Results

Thrombus formation or upregulation of HIF1 increased the circulating expression of 10 factors that regulate thrombus remodelling including the tumourigenic vascular endothelial growth factor (VEGF,  $2 \pm 1$  vs  $1 \pm 0.2$  pg/ml in controls,  $n=6$ /group,  $P<0.05$ ), hepatocyte growth factor (HGF,  $10 \pm 5$  vs  $7 \pm 2\%$  in controls,  $n=8$ /group,  $P<0.05$ ), and stromal cell-derived factor 1 (SDF1,  $14 \pm 6$  vs  $2 \pm 1\%$  in controls,  $n=8$ /group,  $P<0.05$ ). Upregulation of HIF1 also increased the expression of thrombotic tissue factor (TF,  $10 \pm 4$  vs  $1 \pm 0.4\%$  in controls,  $n=8$ /group,  $P<0.05$ ), tissue inhibitor of matrix metalloproteinase 1 (TIMP1,  $57 \pm 9$  vs  $25 \pm 9\%$  in controls,  $n=8$ /group,  $P<0.05$ ), and 7 other factors that regulate coagulation in thrombosed mice. Conversely, HIF1 deletion in mammary tumour cells ablated their hypoxia-induced increase in coagulability ( $23 \pm 1$  vs  $31 \pm 1$  secs in controls,  $n=5$ /group,  $P<0.005$ ). Fibrin deposition in mammary tumours was also reduced following endothelial and myeloid HIF1 deletion ( $5 \pm 1$  vs  $13 \pm 3\%$  in controls,  $n=7$ /group,  $P<0.05$ ) and this was associated with reduced tumour size ( $n=14$ ,  $R=0.7$ ,  $P<0.01$ ).

### Conclusions

HIF1-signalling regulates the progression of venous thrombosis and breast cancer, and could also be responsible for the positive association between these conditions. Cell- and tissue-specific HIF1 is therefore an attractive putative target for treatments that aim to reduce cancer-associated thrombosis.

**Keywords:** Hypoxia, thrombus, cancer. **Conflict of interest:** No conflict of interest.

Tuesday 30 October  
ASTH Emerging & Young Investigators Awards  
O101

0830-1000  
Room 211  
0945-1000

### **A Novel Association Between a PROC Variant and Ischemic Stroke in a Chinese Han Population**

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#### **Aim**

Protein C (PC) is a well-characterized anticoagulant enzyme. However, the association between PC and ischemic stroke (IS) remains controversial. The aim of the present study was to investigate whether any genetic variant in the human protein C gene (PROC) was associated with susceptibility to IS in the Chinese Han population.

#### **Methods**

All exons and the 5'- and 3'-untranslated regions of PROC were initially sequenced to identify informative variants. Potential abnormal variants were analysed in a population of 788 IS patients and 1200 healthy controls and this analysis was stratified by stroke aetiology.

#### **Results**

A 3-nucleotide duplication/deletion variant (c.574\_576del, rs199469469) was identified and found to be significantly associated with IS (OR=2.386; 95% CI: 1.320-4.313,  $p=0.004$ ). Stratification by stroke aetiology after adjustment for IS risk factors showed that this association persisted in the lacunar and cardioembolic subtypes ( $p<0.001$  and  $p=0.017$ , respectively) but not in the atherothrombotic and undetermined subtypes ( $p=0.126$  and  $p=0.998$ , respectively).

#### **Conclusions**

Our results suggested that the novel PROC c.574\_576del variant is a possible genetic determinant of an increased risk of IS and diminished anticoagulant activity of PC.

Note: Nucleotide sequence data for PROC c.574\_576del are available in GenBank under the accession number rs199469469.

**Keywords** ischemic stroke, Protein C, PROC

**Conflict of interest** No

Tuesday 30 October

Nurses Free Communications 3: Apheresis - More Than Meets the Eye

O102

0830-1000

Room 212

0830-0845

**The Use of High Dose Etopophosphate for Stem Cell Mobilisation of a Patient with Mycosis Fungoides and Sezary Syndrome - A Single Case Study Experience**

Alicia Cianflone

*Nepean Hospital, Penrith, NSW, Australia*

**Background**

The case study is a 52 year old male with Mycosis Fungoides and Sezary Syndrome who required an Autologous Bone Marrow Transplant for further management of his disease. The patient was on Interferon for maintenance treatment at the time. According to two published studies from the Hammersmith Hospital and the Charing Cross Hospitals High Dose Etoposide is a useful mobilisation regimen, especially in the use of managing Mycosis Fungoides.

**Method**

Interferon treatment was ceased two weeks prior to treatment for stem cell mobilisation in an attempt to optimise the ability of stem cells mobilisation. Prehydration was commenced with Sodium Bicarbonate until urine became alkalinised. The patient was also commenced on methylprednisone every 8 hours. Once alkalinised, the patient was given High Dose Etopophosphate intravenously as a 10 hour infusion with further Hydration running concurrently over 18 hours. High Dose Etopophosphate was given without incident. On day 3 the patient was commenced 10 micrograms/kg/day of G-CSF (divided up into BD doses) for 10 days. A follow up assessment took place on Day 7.

**Result**

On Day 7 patient had two ulcers on the tongue which were healing. Patient described feeling short of breath on exertion. Examination took place and chest x-ray was attended, which did not show any abnormalities. WCC was 0.3, Hb 120, platelets 62 and neutrophil count 0.0. Patient also noted a marked reduction to the presence of redness and plaques on his skin. On Day 10 CD34 was checked. Patient described severe intermittent bone pain which was not relieved with panadol. Patient's peripheral CD 34 was 63/micro litre and had a WCC 2.5. A single stem cell collection was attended with a CD34 yield of 3.6 per kg which was sufficient for Autologous Bone Marrow Transplant. Bone pain subsided within 24 hours of completing the G-CSF.

**Conclusion**

High Dose Etopophosphate was an affective agent for mobilising stem cells for our case study patient with mycosis fungoides. However as it is only a single case, further comparison is needed to its efficacy.

**Keywords** High Dose Etoposide, Mycosis Fungoides, Stem Cell Mobilisation

**Conflict of interest** No

**Tuesday 30 October**

**Nurses Free Communications 3: Apheresis - More Than Meets the Eye**

**O103**

**0830-1000**

**Room 212**

**0845-0900**

**The Role of Red Cell Exchange in the Treatment of Erythropoietic Protoporphyria**

Kari Mudie, Glen Kennedy

*Royal Brisbane & Women's Hospital, Brisbane, Queensland, Australia*

**Aim**

To investigate the role of red cell exchange, as supportive therapy, in the management of erythropoietic protoporphyria (EPP).

**Methods**

A review of recent literature regarding the various supportive and potentially curative treatment strategies in the management of EPP was performed. The recent case of a patient with a severe form of this disorder, treated at this metropolitan hospital, was reviewed and response to treatment examined.

**Results**

Whilst there is little information in the published literature, therapeutic plasma exchange and red cell exchange are both supportive treatment options that may be employed to reduce circulating pools of protoporphyrin. It has been suggested that red cell exchange may prove a more effective and efficient supportive therapy however, as it both removes circulating pools of protoporphyrin whilst also contributing to reduction of protoporphyrin production.

This case study demonstrates that red cell exchange is an effective supportive treatment strategy in a 25 year old female with severe EPP who was also treated with hepatic and allogeneic haemopoietic stem cell transplants.

**Conclusion**

EPP is a rare inherited disorder of the haem biosynthetic pathway, caused by a partial failure of a critical enzyme, ferrochelatase. The scant available literature suggests that sequential liver / haemopoietic stem cell transplant may be a curative strategy in this group of patients. This case study further highlights the important role of red cell exchange in the maintenance therapy of EPP.

**Keywords** Erythrocytapheresis, protoporphyria, transplantation

**Conflict of interest** No conflict of interest to disclose



**Tuesday 30 October**

**Nurses Free Communications 3: Apheresis - More Than Meets the Eye**

**O104**

**0830-1000**

**Room 212**

**0900-0915**

**Predicting the Day of Mobilisation – RBWH Retrospective Review**

Samantha Easton, Kari Mudie, Glen Kennedy, Simon Durrant  
*Royal Brisbane and Women's Hospital (RBWH), Brisbane, Qld, Australia*

**Aim**

To retrospectively review current chemotherapy protocols used in mobilising autologous haematopoietic progenitor cells (HPC), to improve the predicted day of mobilisation.

**Method**

A retrospective review of all autologous HPC collections performed within this apheresis unit from January 2008 to June 2012. Data was collected on the mobilisation chemotherapy protocol, the diagnosis, the day HPC collection was commenced and the peripheral CD34+ count.

**Results**

A comparison between the different chemotherapy protocols highlights the diversity between the efficacy and predictability of sufficient mobilisation of CD34+ cells for autologous HPC collection. This review does not contain data on prior chemotherapy administered or other contributing factors that may influence mobilisation (i.e. co-morbidities), however it will identify where further study may be focussed for this bone marrow transplant centre.

**Conclusion**

A number of chemotherapy protocols are used at the RBWH for mobilisation of autologous HPC. These include but are not limited to high dose cyclophosphamide, CHOP, ESHAP, DHAP, (D)ICE, BFM90 and Hyper CVAD (both A and B cycles) all of which contain either alkylating agents, antimetabolites, antitumour antibiotics, mitotic inhibitors, and platinum compounds or a combination of the same. Granulocyte-Colony Stimulating Factor (G-CSF) (10mcg/kg/day) should commence 24 hours after chemotherapy irrespective of the protocol used and continued until HPC collection is completed. The timing schedules of CD34+ testing should be revised regularly, ensuring an effective and predictable service.

**Keywords** Mobilisation, Haematopoietic Progenitor Cells

**Conflict of interest** No conflict of interest to disclose

Tuesday 30 October

Nurses Free Communications 3: Apheresis - More Than Meets the Eye

O105

0830-1000

Room 212

0915-0930

### Validation of the Spectra OPTIA® Apheresis System for HPC Collection

J Parrington<sup>1</sup>, M Darby<sup>1</sup>, K Stokes<sup>1</sup>, D Wall<sup>1,2</sup>, A Mouminoglu<sup>1</sup>, HM Prince<sup>1,2</sup>, P Gambell<sup>1</sup>, SJ Harrison<sup>1,2</sup>

<sup>1</sup>Centre for Blood Cell Therapies, Peter MacCallum Cancer Centre & <sup>2</sup>Sir Peter MacCallum Dept of Oncology, University of Melbourne, Parkville, Australia

#### Introduction

Autologous haematopoietic progenitor cells (HPC-A) are routinely collected for re-infusion following high dose therapy (HDT) to expedite haematopoietic recovery. As part of an evaluation process to identify a replacement for the aging Cobe® Spectra (CS) system, we performed an internal validation of the Spectra Optia® (OP) system, measuring HPC-A collection efficiency (CE) and engraftment kinetics (EK), as compared to CS.

#### Methods

Inclusion criteria: patients were scheduled to undergo HDT as part of their first or second line of therapy; prospective plan to collect sufficient cells for >2 infusions; pre CD34 counts >10 cell/μL.

CE calculated for the first 20 OP collections. CE's were compared between products collected on both systems for each patient. CE was calculated as per 2009 NPAAC guidelines. EK was analysed for the first 10 reinfusions using OP product alone and compared against long term CS average collected for routine QC. Haemopoietic recovery defined as; neutrophil count >5x10<sup>9</sup>/L post nadir, unsupported platelet count >20x10<sup>9</sup>/L.

#### Results

Demographics; 15 patients (MM=14, BCL=1), 8 male, 7 Female, median age 60 (30-70). 10 of 15 collected on OP day 1. OP returned a higher median collection efficiency, OP 66% (38-143) v CS 55% (34-117). There was a shift to longer EK for patients receiving OP product compared with CS (Time to Neutrophil Recovery = 13days[11-15] Vs 11 days[8-17]). Subgroup analyses indicate that inclusion of trial (Litvacc study) patients impacted on time to neutrophil recovery (Litvacc Optia median = 13 days (12-15) vs Optia excluding Litvacc 12 days (11-14). Within the Litvacc population, neutrophil recovery was slower in the Optia group compared with Litvacc product collected on the COBE Spectra over the 2009-2011 period (Median 13 days vs 12 days p< 0.05).

#### Conclusions

The OP achieved a higher median CE compared with CS. From a small sample size, validation findings indicate delays to Haemopoietic recovery are likely due to patient population variables rather than machine type. Validation to be extended to increase sample size and determine trend significance.

**Key Words.** Apheresis, Validation, Spectra Optia®

**Conflict of interest** This research was supported by TherumoBCT. The company had no roll in analysing the data or preparing the abstract.

**Tuesday 30 October**  
**HSANZ Lab Haematology: Symposium 1**

**0830-1000**  
**Room 213**

### **Clinically Relevant Molecular Markers in Acute Leukaemia**

Andrew Wei

*Department of Clinical Haematology, The Alfred Hospital, Melbourne, Australia*

Our understanding of the molecular biology of AML has changed radically in the last few years. The completion of the Human Genome Project on April 14, 2003 followed by the sequencing of the first AML genome in 2008 will permit researchers to catalogue the vast majority of recurrent AML mutations within the next few years. The rapidly increasing number of molecular lesions linked to AML prognosis has made the situation highly complex, presenting dilemmas for haematologists as to what markers should be made available and the most efficient way to do this in a hospital environment. This review will focus on the role of molecular markers most relevant to clinical practice and the challenges faced in introducing some of the more recently discovered markers to the clinic.

**Keywords** AML, molecular, prognosis

**Conflict of interest** No

Tuesday 30 October  
HSANZ Lab Haematology: Symposium 1

0830-1000  
Room 213

## Molecular Haematology – A Parallel Future?

Anthony H Bell  
*Peter MacCallum Cancer Centre, Melbourne, Australia*

The sequencing of the human genome and the development of new molecular techniques has resulted in an explosion in the understanding of the molecular basis of disease. For the past 30 years, the gold standard Sanger method for DNA sequencing has dominated this space. However, the demand to deliver faster, inexpensive and accurate genome information lead to the 2005 commercial launch of the first “next generation sequencing” (NGS) platform. This revolutionary & timely technological advance has initiated a new era of high-throughput genomic analysis.

World-wide efforts to catalogue mutations in multiple cancer types (including haematological neoplasms) has led to new discoveries that are rapidly being translated into clinical practice. In particular, molecular markers are being applied to disease diagnosis / classification; prognostic stratification & informing therapeutic targets; and individualised monitoring of minimal residual disease.

Next generation sequencing has now matured to the point where it is being seriously considered for introduction by molecular laboratories into routine diagnostic use and will undoubtedly impact the future practice and care of patients with haematological malignancies.

**Keywords** NGS, Molecular, Haematology

**Conflict of interest** No

Tuesday 30 October  
HSANZ Lab Haematology: Symposium 1

0830-1000  
Room 213

### Acute Leukaemia MRD: What's the Fuss About?

David A Westerman

*Department of Pathology, Division of Cancer Medicine, Peter MacCallum Cancer Centre, East Melbourne & University of Melbourne, Parkville, Victoria Australia*

The paradigms and understanding of abnormal, in diagnostic pathology, are based on a strong foundation and knowledge of "normality". This concept is pivotal for everyday practice in multiparameter flow cytometry (MFC). Delineation of normal antigen maturation and expression patterns of the targeted cell populations enable the detection of aberrancy. There is a large body of literature supporting the routine diagnostic utility of immunophenotyping in haematologic malignancies, however in the MRD setting paediatric ALL is leading the way, having demonstrated in prospective clinical trials clearly improved endpoints in the absence of minimal residual disease (MRD) detection at the appropriate time points.

In adult AML, there is a great potential for using MFC MRD rather than molecular MRD given quick result turnaround, broader availability of flow technology, and around 90% antigenic aberrancy detection at diagnosis in AML, hence MFC has wide applicability. Despite the promise and a number of publications over a 10 year period showing prognostic significance post induction, post consolidation and preallogeneic transplantation with MRD there are a number of standardisation issues that remain unresolved. These diminish the power of MFC MRD as a routine modality across different centres. These issues are preanalytical and analytical, some include different antibody panels and fluorochrome combinations used, different gating techniques for MRD determination, different MRD cutoff's used in studies, and different levels of normal antigen expression in immature precursors between centres. Thus a powerful tool AML MRD flow can be, but standardization remains a key for progress in this field to more positively impact on patient care.

**Keywords** MRD, multiparameter flow cytometry

**Conflict of interest** No

Tuesday 30 October

HSANZ Free Communications 9: Aggressive Lymphomas

O106

1030-1130

Plenary 2 (Auditorium)

1030-1045

## Risk Factors and Response to Therapy in Heart and Lung Transplant Recipients with Post-transplant Lymphoproliferative Disease

Orly Lavee<sup>1</sup>, Gayathri Kumarasinghe<sup>2</sup>, Rebecca Pearson<sup>3</sup>, Christopher Hayward<sup>2</sup>, Peter Macdonald<sup>2</sup>, Anne Keogh<sup>2</sup>, Monique Malouf<sup>3</sup>, Marshall Plit<sup>3</sup>, Alan Glanville<sup>3</sup>, Eugene Kotlyar<sup>2</sup>, Adrian Havryk<sup>3</sup>, Anthony Dodds<sup>1</sup>, Sam Milliken<sup>1</sup>, Joanne Joseph<sup>1</sup>, Keith Fay<sup>1</sup>, Andrew Parker<sup>4</sup>, John Moore<sup>1</sup>

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4. Department of Histopathology St Vincent's Hospital, Sydney, Australia.

Post-transplant lymphoproliferative disease (PTLD) is a heterogenous, well-described complication in patients who have received solid organ transplants and has a high mortality rate. Knowledge of the disease is expanding though to date only small studies exist with limited analysis specific for heart-lung transplant patients.

### Aim

To compile a registry of heart and lung transplant recipients with PTLD to identify unique clinical features in order to better understand risk and prognostic factors and further develop treatment regimens and prophylaxis.

### Methods

We conducted a retrospective analysis of data collected at a single centre heart and lung transplantation institution from 1984 to 2011. Patient demographics, diagnostic information, treatment, response, complications and outcomes were studied and compared by multivariate analysis.

### Results

Sixty nine patients with PTLD were identified (4.32% of all patients who have undergone heart and lung transplantation in the unit). Of these, 45 were heart recipients (5.41% of all heart transplants), 18 were lung recipients (2.63% of all lung transplants) and 6 were heart-lung recipients (7.50% of all heart-lung transplants). The mean age at the time of transplant was  $42.3 \pm 15.0$  years. Early analysis of PTLD classification suggests that Diffuse Large B Cell Type is seen most commonly (52%). The mean transplant to PTLD time was  $5.0 \pm 5.1$  years and the 3 year overall survival rate for the group was 33%. Data collection on EBV status, HLA matching, treatment regimen and related outcomes is ongoing.

### Conclusions

This large analysis of heart and lung transplant patients with PTLD shows promise in giving a more detailed insight into risk factors and responses to therapy than previous studies have been able to demonstrate.

**Keywords:** PTLD, Transplantation, Malignancy.

**No conflict of interests to disclose.**

Tuesday 30 October

HSANZ Free Communications 9: Aggressive Lymphomas

O107

1030-1130

Plenary 2 (Auditorium)

1045-1100

# **Safety and Efficacy of R-CEEP in Elderly Patients (>70) with Diffuse Large B-cell Lymphoma (DLBCL): A Retrospective Single Centre Experience**

Wei-Hsun Hsu<sup>1</sup>, Stephen Larsen<sup>1</sup>, Danny Hsu<sup>2</sup>, Ibrahim Tohidi-Esfahani<sup>1</sup>, John Gibson<sup>1</sup>, Harry Iland<sup>1</sup>, Joy Ho<sup>1</sup>, Scott Dunkley<sup>1</sup>, Christina Brown<sup>1</sup>, Claire Davies<sup>1</sup>, Douglas Joshua<sup>1</sup>

<sup>1</sup> *Institution of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia*

<sup>2</sup> *Department of Haematology Liverpool Hospital, Sydney, NSW, Australia*

## **Aim**

Elderly patients with DLBCL who have significant co-morbidities have limited treatment options such that many are not offered anthracycline-containing chemotherapy regimens due to concerns regarding toxicity. We describe our single center experience with R-CEEP, a lower intensity regimen, for elderly patients with newly diagnosed or relapsed DLBCL whom are deemed inappropriate for R-CHOP based chemotherapy.

## **Method**

All elderly DLBCL patients >70 years old treated with R-CEEP at RPAH from 2000 to 2012 were retrospectively reviewed. R-CEEP (cyclophosphamide 300mg/m<sup>2</sup> D1, epirubicin 50mg/m<sup>2</sup> D1, etoposide 100mg/m<sup>2</sup> D1, and prednisolone 50mg D1-D5, rituximab 375mg/m<sup>2</sup> D1) was administered every 2 weeks. Baseline characteristics, number of cycles, treatment response and toxicity were assessed.

## **Result**

A total of 29 patients were identified with a median age 79 years old (range 71-93 years). Five patients did not receive rituximab. The median number of cycles of R-CEEP was 6 (range 2 – 9). Three patients required dose reduction, while 5 patients required delays in treatment due to haematological toxicity. An overall response rate of 89.3% was observed with complete response (CR) of 64.3% and partial response (PR) of 25%. Three out of the 7 patients with relapsed disease, who were previously exposed to R-CVP, demonstrated CR. Thirteen patients are still in CR at a median of 2 years with one patient still in CR at 8.5 years. Twelve patients had febrile neutropenia and grade 3-4 haematological toxicity was observed in 72% of patients. There were no treatment-related deaths.

## **Conclusion**

This retrospective single center experience shows that R-CEEP is effective and well tolerated in elderly patients deemed unsuitable for R-CHOP and provides a viable treatment option.

**Keywords** diffuse large B cell lymphoma, elderly, chemotherapy

**Conflict of interest** No

Tuesday 30 October

HSANZ Free Communications 9: Aggressive Lymphomas

O108

1030-1130

Plenary 2 (Auditorium)

1100-1115

## Outcome of Intensive chemotherapy With Hyper-CVAD in Patients With Untreated High-Risk Diffuse Large B cell Lymphoma

Anupkumar George<sup>1</sup>, Nicholas Weber<sup>2</sup>, Michael Dickinson<sup>1</sup>, David Ritchie<sup>1</sup>, Miles Prince<sup>1</sup>, Max Wolf<sup>1</sup>, Henry Januszewicz<sup>1</sup>, Simon Harrison<sup>1</sup>, Kate Burbury<sup>1</sup>, Dennis Carney<sup>1</sup>, Peter Mollee<sup>2</sup>, Paula Marlton<sup>2</sup>, John F Seymour<sup>1</sup>

<sup>1</sup> Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.

<sup>2</sup> Princess Alexandra Hospital, Brisbane, Queensland, Australia.

### Aim

To analyse the outcome of patients treated with the Hyper-CVAD (HCVAD) regimen for high-risk diffuse large B-cell Lymphoma (DLBCL).

### Background

R-CHOP remains the standard of care in treating DLBCL but the efficacy in patients with IPI  $\geq 3$  remains inadequate; 3 yr PFS 55-60% (Pfreundschuh JCO 2010).

### Method

We retrospectively analysed 28 patients with DLBCL treated with HCVAD at our centre from 01/99-12/11 on an "intention-to-treat" basis. (An updated analysis with an additional 32 patients from PAH will be presented)

### Results

Of the 28 patients, 18 were male. Median age was 43.5yrs (range 16–61). Diagnostic biopsy showed DLBCL (*de novo*) (71%), transformed follicular NHL (11%) and primary mediastinal B cell lymphoma (18%). Median LDH was 699U/L (1.52xULN). 20 pts had IPI  $\geq 3$ . 16 (58%) pts had high-risk features for CNS disease (van Besien Blood 1998). Four received one cycle of R-CHOP prior to HCVAD. Median number of treatment (A+B cycles) received was six. Therapy was de-escalated to R-CHOP in one due to toxicity. 50% did not receive rituximab. 85% achieved CR at the end of treatment. There were no deaths due to toxicity. Median follow-up was 53.7 months. The actuarial PFS and OS rates at 5yrs were 54% and 75% respectively. To date, there are no statistically significant differences related to rituximab use. Amongst those who failed therapy (11/28), three had primary refractory disease; 8 relapsed (6 within 1yr) and 54% did not receive Rituximab. 72% of these patients remain alive and in remission after successful salvage at the time of analysis. There were no CNS relapses.

### Conclusion

Intensive treatment with Hyper-CVAD is safe, effective and achieves encouraging long-term survival rates in selected patients with high-risk diffuse large B cell lymphoma. The PFS and OS rates obtained are comparable to other intensive regimens used in similar cohort of patients. In addition, HCVAD provides excellent protection against CNS relapses in these high-risk patients.

**Keywords:** DLBCL, hyperCVAD, high-risk **Conflict of interest:** No



Tuesday 30 October

HSANZ Free Communications 9: Aggressive Lymphomas

O109

1030-1130

Plenary 2 (Auditorium)

1115-1130

## Mantle Cell Lymphoma with CNS involvement: A Report from the European Mantle Cell Lymphoma Network

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### Aim

To comprehensive assess the incidence, clinical features, treatment and outcomes of patients with CNS involvement of MCL across a large, multicentre database.

### Results

The crude incidence of CNS involvement was 57/1396 patients (4.1%). Of patients with CNS disease, 70% were male, with median age 61 years (range 38 to 83 years). At diagnosis, blastoid histology was found in 28% of patients, Ki67 was 30% or higher in 18/27 cases with data (67%). 91% of patients were stage IV, 53% had B symptoms at presentation, and the median white cell counts was 10.9 x 10<sup>9</sup>/L. High MIPI score present in 61%. LDH was increased in 38/51 (75%), beta 2 microglobulin raised in 17/22 (77%) and ECOG performance status was 2 or worse in 16/43 (37%) patients with data. Extranodal involvement at 2 or more sites was present in 35 (62%) cases. Excluding patients with CNS disease at diagnosis, the median time to CNS relapse was 15.2 months from diagnosis, and median survival from time of CNS diagnosis 3.7 months (range 0.9 to 63.2 months). White cell count <10.9 x 10<sup>9</sup>/L at time of original diagnosis consolidation with stem cell transplant, salvage with high dose anti-metabolite therapy and achievement of CNS complete remission following salvage therapy predicted longer overall survival.

### Conclusion

CNS involvement in Mantle Cell Lymphoma is associated with highly proliferative disease, is more common at relapse and carries a poor prognosis. Once diagnosed, treatment with high dose antimetabolite therapy followed by stem cell transplant appears to be the only strategy resulting in long term survival.

**Keywords** Mantle Cell Lymphoma, clinical, CNS involvement

**Conflict of interest** None

Tuesday 30 October

HSANZ Free Communications 10: Globin Gene Biology

O110

1030-1130

Room 216

1030-1045

## Generation of Fluorescent Reporter Gene Constructs for $\alpha$ - and $\beta$ -Globin Gene Transcription, Splicing and Translation Studies

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### Aim

During the routine investigation of patients suspected of having haemoglobin disorders a number of novel mutations have been identified. An in vitro system has been developed to assess the pathophysiologic relevance of these mutations.

### Methods

Using the two-step Sense Strand Release-Polymerase Chain Reaction (SSR-PCR) protocol, the open reading frames (ORF) of two reporter genes, EGFP and mCherry were inserted into the C-termini of the  $\alpha$ - and  $\beta$ -globin genes in a reporter construct. The alpha and beta globin reporter gene constructs were transiently transfected into human bladder carcinoma (5637) cells, allowing real time expression analysis of both globin genes.

### Results

The transfection of both reporter genes constructs showed cells emitting green and or red fluorescence indicating accurate splicing of each gene and in-frame insertion of both fluorophores. To prove the functionality of the system, we generated  $\beta$ -Globin<sup>Cd39/Cd55/mCherry</sup>-pGEM-T, a construct carrying a well-known  $\beta$ -globin premature termination mutation [HBB:c.118C>T] and showed that cells transfected with this construct did emit any red fluorescence.

### Conclusion

Our results have demonstrated the usefulness of these expression/reporter constructs as molecular tools that enable us to study the pathophysiological relevance of various mutations that affect the transcription, splicing and translation of  $\alpha$ - and  $\beta$ -globin genes in real time without the need for protein visualization by immunodetection.

**Keywords:**  $\alpha$ - and  $\beta$ -globin, Fluorescent Reporter gene, Haemoglobin disorder

**Conflict of interest:** "No conflict of interest to disclose".

Tuesday 30 October

HSANZ Free Communications 10: Globin Gene Biology

O111

1030-1130

Room 216

1045-1100

# **Modification of the Common Alpha deletional Multiplex PCR to Include the $-\alpha^{3.7III}$ and $-\alpha^{3.5}$ Deletions**

KM Weekes, EJ Hoskin, JM Brasch, AP Twomey, M Gavrilu, R Li, A Adrahtas, P Matthews, JN Wells, A Alam, S Pasricha, DK Bowden  
*Clinical Genetics Laboratory, Monash Medical Centre, Clayton Vic, Australia.*

## **Aim**

To redesign the single-tube multiplex polymerase chain reaction (PCR) commonly used as a screening method to detect large deletions causing alpha thalassaemia to include the  $-\alpha^{3.7III}$  allele found in Melanesian and Polynesian populations and the  $-\alpha^{3.5}$  deletion found in the Indian population.

## **Method**

We replaced two primers in the multiplex PCR of Tan et al., Blood 2001 leaving all other conditions as previously described. Primer  $\alpha 2$ -R was replaced with  $\alpha C6$  – 5' CTC CAG CCG GTT CCA GCT ATT GC 3' and primer  $\alpha 2/3.7F$  was replaced with  $\alpha C8$  – 5'GAG CCT GGC CAA ACC ATC AC 3'. The substitution of these two primers allows for continued discrimination between the normal ( $\alpha 1$  gene) and the three  $-\alpha^{3.7}$  amplicons and enabled detection of the  $-\alpha^{3.5}$  deletion. Expected amplicon sizes are 2023 bp for the normal  $\alpha 1$  gene, 2093/2096/2097 bp respectively for the three  $-\alpha^{3.7}$  deletions and 978 bp for the  $-\alpha^{3.5}$  deletion. A separate PCR now needs to be performed to determine zygosity for the  $-\alpha^{4.2}$  deletion.

## **Results**

We have confirmed our multiplex PCR by testing 100 archival samples of known genotypes including all three  $-\alpha^{3.7}$  alleles and several  $-\alpha^{3.5}$  alleles and is now currently in routine use as the first line screening method for alpha thalassaemia deletions in our laboratory.

## **Conclusion**

These simple modifications to the screening alpha multiplex PCR have resolved a significant number of cases with microcytic, hypochromic anaemia in our laboratory and this method was found to be a reliable and inexpensive way of detecting common alpha deletions in a large population.

**Keywords** Alpha thalassaemia, multiplex PCR,

**Conflict of interest** No

Tuesday 30 October  
HSANZ Free Communications 10: Globin Gene Biology  
O112

1030-1130  
Room 216  
1100-1115

### **Triplicated Alpha Globin Genes in Heterozygous Beta Thalassaemia: Genotypes and Clinical Phenotypes**

Sudhakshini Raghunath, Asif Alam, Kerry Weekes, Mioara Gavrilă, Donald K Bowden, Sant-Rayn Pasricha  
*Thalassaemia Service, Monash Medical Centre, Southern Health, Melbourne, Victoria, Australia*

#### **Aim**

In beta thalassaemia, imbalanced globin chain synthesis results in an excess of alpha chains, causing red cell destruction and ineffective erythropoiesis. Coinheritance of beta thalassaemia trait and excess alpha genes may produce a clinically significant thalassaemia syndrome. We sought to describe the clinical picture of this under-recognised condition.

#### **Methods**

All genetic testing for thalassaemia in Victoria is performed in the Clinical Genetics Laboratory at Southern Health. Testing for a Triplicated Alpha Gene has been available since 2000. We reviewed the results of all patients identified with a triplicated alpha gene whom had co-inherited beta thalassaemia trait.

#### **Results**

We identified 26 subjects with both beta Thalassaemia trait and triplicated alpha gene, 9 of whom also had clinical data available. There was wide clinical variation. Haemoglobin values varied between 58g/L and 136g/L with a median of 88g/L. A haemoglobin of <120g/L was found in 92% of patients (Hb<100g/L in 73%, Hb <90g/L in 46%, Hb <80g/L in 27% and Hb <70g/L in 1 patient). MCV was between 57.7-78fL with a median of 64fL, MCH was between 18.4-26pg with a median of 20pg. For the 9 patients for whom clinical data was available, records showed 5 with splenomegaly (3 of whom required splenectomy), 3 patients on regular transfusion regimes and 4 requiring occasional transfusions. Three cases had "dominant" inheritance where beta mutations and triple alpha were inherited from one parent.

#### **Conclusion**

Coinheritance of a triplicated alpha globin gene should be considered in patients with unusually severe thalassaemia trait. In some patients this can cause transfusion-dependant anaemia. This has major implications for prenatal screening of thalassaemia as a triplicated alpha gene alone is phenotypically silent and testing is not currently routinely performed. Furthermore, a child can inherit beta thalassaemia and triplicated alpha from one parent, and thus this syndrome can have 'dominant' inheritance.

**Keywords** Beta Thalassaemia Trait, Triplicated Alpha Globin Gene

**Conflict of interest** No

Tuesday 30 October

HSANZ Free Communications 10: Globin Gene Biology

O113

1030-1130

Room 216

1115-1130

## Inactivation of Transcription Factor Binding Sites Within the Alpha-Globin Core Promoter Significantly Reduces HBA2 Transcription Level

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<sup>1</sup>Department of Haematology, PathWest Laboratory Medicine, QEII Medical Centre, Nedlands, Western Australia, <sup>2</sup>School of Pathology and Laboratory Medicine, University of Western Australia, Nedlands, Western Australia and <sup>3</sup>Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

### Aim

In a recent publication we proved the existence of two differentially expressed long and short transcripts for both HBA2 and HBA1 in healthy individuals. We provide experimental evidence that the marked reduction in transcription levels of the longer transcripts HBA2L and HBA1L is a result of the incorporation of the TATA Binding Protein site (ATAAA) into the first exon of the HBA2L and HBA1L isoforms which effectively inactivates the TATA binding protein site.

### Methods

Using our previously designed HBA2-WT (wild type) expression constructs and mutagenesis protocol we generated the following constructs HBA2:c.-44\_-40delGGCCG, HBA2:c.-65\_-60delATAAA, and HBA2:c.-108\_-104delCCAAT. Human 5637 cells were transfected with either the wild type or the mutated constructs followed by HBA2 gene transcription analysis using Real-Time PCR.

### Results

The analysis showed a 96.5% reduction in HBA2 transcripts level in cells transfected with HBA2:c.-65\_-60delATAAA, compared to the HBA-WT group. When compared with the HBA-WT the HBA2 transcript levels were reduced by 86.2% and 97.2% in HBA2:c.-44\_-40delGGCCG and HBA2:c.-108\_-104delCCAAT groups respectively.

### Conclusion

With this work we have provided experimental evidence that the inactivation of transcription factors binding sites within alpha-globin core promoter region significantly reduces the expression levels of HBA2 transcripts.

**Keywords:** Alpha-Globin promoter, Transcription Factor recognition site

**Conflict of interest:** No

Tuesday 30 October

HSANZ Free Communications 11: Haematopoietic Stem Cell Biology

O115

1030-1130

Room 217

1030-1045

## The Bone-Bone Marrow Organoid as a Unique Model to Decrypt the Genesis of the Haematopoietic Endosteal Niche

Mirieli Ho<sup>1,2</sup>, Hatem Salem<sup>1</sup>, Stephen Livesey<sup>2</sup>, Kathy Traianedes<sup>2</sup>

<sup>1</sup>*Department of Clinical Haematology, Monash University, Melbourne, Victoria, Australia,* <sup>2</sup>*Department of Clinical Neurosciences, St Vincent's Hospital, Melbourne, Victoria, Australia*

Haematopoiesis is in part dynamically regulated by cells residing at the endosteal niche. Elucidating the contributions of each player in mature bone is challenging due to the intricate crosstalk between bone and bone marrow (B-BM) cells, matrix, and factors.

### Aim

The aim of this study is to characterise the development of this marrow in relation to bone formation.

### Method

We have established a novel model to study the spatial and temporal development of *de novo* B-BM using rat demineralised bone matrix (rDBM) in an ectopic site. Time course experiments were performed by implanting rDBM into the hind-limbs of nude mice up to 35 days.

### Result

Histological analysis of the explanted nodules at specified time-points revealed sequential neovascularisation and recruitment of cells over 35 days. Repopulation of the DBM by mesenchymal and haematopoietic lineage cells was demonstrated by specific gene expression profiles and immunohistochemical evaluation. In addition, colony forming cell assays suggested that functional haematopoietic progenitors were present by day 11 and were equivalent to those present in the long bones of the same animal. The student's t-test was employed to determine significance.

### Conclusion

Collectively, our results verified the haematopoietic potential and established the major sequence of events in the genesis of ectopic B-BM. This model will facilitate the study of communication between B-BM cells, and potentially assist in identifying new putative regulators of haematopoiesis.

**Keywords** bone, bone marrow, haematopoiesis

**Conflict of interest** No

Tuesday 30 October

HSANZ Free Communications 11: Haematopoietic Stem Cell Biology

O116

1030-1130

Room 217

1045-1100

## Pro-Apoptotic Bcl-2 Proteins Are Important in Numerical But Not Functional Maintenance of Haematopoietic Stem Cells (HSCs)

Kylie D Mason<sup>1,2,3</sup>, Dominic Fong<sup>4</sup>, Angela Georgiou<sup>1</sup>, David C.S. Huang<sup>1</sup>, Andrew Roberts<sup>1,3</sup>

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### Aim

Novel anti-cancer agents, the BH3 mimetics, act by directly targeting the pro-survival Bcl-2 proteins. They are well tolerated and have demonstrated efficacy in haematologic malignancy. Our aim was to determine the role of the Bcl-2 family in HSCs and examine the potential effect of Bcl-2 prosurvival inhibition on HSC survival and function.

### Method

Bone Marrow(BM) harvested from mice heterozygous for Bcl-2 family proteins were analysed using a flow cytometric panel for ckit, Sca-1, CD48, CD150, lineage markers and propidium iodide, to identify primitive populations of LSKs and SLAMs. BM was incubated with ABT-737 (inhibitor of Bcl-2, Bcl-x, Bcl-w) for 24hr, and viability assessed by exclusion of propidium iodide. Functional assays including colony assays and competitive serial *in vivo* transplant assays were also performed. Mice were serially transplanted every 8 weeks.

### Results

ABT-737 exerts dose dependent apoptosis of LSKs and SLAMs. This is enhanced by the absence of cytokines. *In vitro* imaging demonstrates rapid apoptosis of LSKs, with the majority of cell death occurring by 6 hours. Heterozygosity for pro-survival proteins Bcl-2, Bcl-x, Mcl-1, is insufficient alone to reduce LSK or SLAM populations when compared to WT controls. Colony formation was not significantly impaired following incubation with ABT-737. Both competitive transplant and serial transplant assays have shown no functional deficit in the ability of HSC's treated with ABT-737 *in vivo* to engraft post transplant.

### Conclusion

Pro-survival Bcl-2 proteins are important for maintenance of HSC numbers but do not appear to play a critical role in HSC function. This is particularly important with regards to the treatment of haematologic malignancy with the BH3 mimetics.

**Keywords** HSC, apoptosis, Bcl-2 **Conflict of interest** No

Tuesday 30 October

HSANZ Free Communications 11: Haematopoietic Stem Cell Biology

O117

1030-1130

Room 217

1100-1115

## Establishing a Humanized Mouse Model of Multiple Myeloma

Rebecca Delconte<sup>1</sup>, Andy Hsu<sup>1,2</sup>, Joshua Noske<sup>1</sup>, Alex Davenport<sup>1</sup>, David Ritchie<sup>1,2</sup>, Paul Neeson<sup>1,2</sup>

<sup>1</sup> Haematology and Immunology Translational Research laboratory, Peter MacCallum Cancer Center, East Melbourne, Victoria, Australia.

<sup>2</sup> The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia

### Aim

To create a novel humanized mouse model of multiple myeloma (MM) by reconstituting immunodeficient mice with autologous MM patient G-CSF mobilised stem cells and primary tumour cells.

### Methods

Cord bloods (CB) were collected from the Royal Children's Hospital. MM patient mobilised stem cells were processed at Peter Mac. Rag2<sup>-/-</sup>γc<sup>-/-</sup> mice were housed at the animal facility at Peter Mac. Stem cell engraftment was monitored by FACS. Student T test /ANOVA were used for statistical analysis where appropriate.

### Results

We have shown that intrahepatic injection of MM patient derived CD34<sup>+</sup> stem cells into newborn Rag2<sup>-/-</sup>γc<sup>-/-</sup> mice results in effective engraftment (up to 30%) of human multilineage (B and T) CD45<sup>+</sup> cells. Interestingly, we report evidence of thymic development and selection as some mice harbour 100% human CD1a<sup>+</sup> T cells in the thymus. However, this level of engraftment is suboptimal and human CD45<sup>+</sup> cells decrease over time. In comparison, CB-derived stem cells result in efficient (up to 90%) and long term engraftment. This difference is likely attributed to the low frequency of pluripotent CD34<sup>+</sup>CD38<sup>-</sup> stem cells found in MM patient mobilized stem cells. It is essential for our model to show multilineage, long term engraftment, thus we addressed this by using StemRegenin-1 (SR-1) supplemented with cytokines to induce a 20-fold increase in the number of MM patient mobilized stem cells and an expansion in the pluripotent population over 21 days.

### Conclusion

By optimizing stem cell engraftment for the development of a humanized mouse model of MM, this will provide a powerful tool to study the biology of MM and novel therapeutics in a more physiologically relevant model.

**Keywords** Multiple Myeloma, Humanized, Stem Cells

**Conflict of interest** No



Tuesday 30 October

1030-1130

HSANZ Free Communications 12: Stem Cell Transplantation - Late Complications Room 218  
O118

1030-1045

# **Outcomes in Australasian Patients Receiving Second Allogeneic Haematopoietic Transplants for Haematopoietic Malignancy using Fludarabine-Melphalan Conditioning**

Ian Bilmon<sup>1</sup>, Ian Nivison-Smith<sup>2</sup>, Anthony Dodds<sup>1</sup>, Mark Hertzberg<sup>3</sup>, David Ritchie<sup>4</sup>, Matthew Greenwood<sup>5</sup>, John Moore<sup>1</sup>

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## **Aim**

To examine the outcomes in recipients of a second allogeneic haematopoietic transplant (HCT) using Flu-Mel reduced intensity conditioning (RIC) reported to the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR).

## **Method**

The 'Flu-Mel' study retrospectively examined the outcomes of all patients receiving Flu-Mel RIC allografts between 1998 and 2008 reported to the ABMTRR. Those patients receiving a Flu-Mel allograft as their second transplant were identified and examined separately as part of a planned sub-analysis. Rates of relapse and Graft-versus-Host Disease (GvHD) are presented as cumulative incidences, with death as a competing risk.

## **Results**

There were 15 cases identified across 6 Australian transplant centres, with a median age of 47 years (range 22 – 65). The diagnoses were AML (n=10), CML (n=4), and NHL (1 case). The indication for second transplant was relapse after initial transplant in 13 cases, with one case each of refractory ITP and therapy-related AML. At transplantation 6 patients were not in remission. The donor was HLA-identical sibling in 9 cases, and 6 patients were transplanted with a different donor from that used with their first transplant. After 3 years the overall and disease-free survival rates were 53% and 29% respectively. Cumulative incidence of grade II-IV acute GVHD was 43%, transplant-related mortality (TRM) at 1 year 13%, and relapse at 1 year 33%. There were 4 patients alive and relapse-free at their last follow-up, with a median survival of 4.17 years. Factors relating to the first and second transplants associated with improved overall and disease-free survival will be presented.

## **Conclusion**

Long term disease-free survival is possible with RIC Flu-Mel conditioned second allografts in select patients.

**Keywords** Allogeneic HCT, RIC, Second transplant

**Conflict of interest** No conflict of interest to disclose.

Tuesday 30 October

1030-1130

HSANZ Free Communications 12: Stem Cell Transplantation - Late Complications Room 218  
O119

1045-1100

## Second Malignancies in Recipients of Autologous HSCT: Findings from the Cancer after Stem Cell Transplantation (CAST) Study

Renate Thielbeer<sup>1</sup>, Claire Vajdic<sup>2</sup>, Tracey O'Brien<sup>3</sup>, Anthony Dodds<sup>4</sup>, Ian Bilmon<sup>4</sup>, Leonie Wilcox<sup>5</sup>, Lesley Ashton<sup>1</sup>

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<sup>4</sup>Haematology and Bone Marrow Transplantation Unit, St Vincent's Hospital, Darlinghurst, NSW, Australia. <sup>5</sup>Australasian Bone Marrow Transplant Recipient Registry, Darlinghurst, NSW, Australia

### Aim

To examine rates of second malignancies in adult recipients of autologous hematopoietic stem cell transplantation (HSCT) in Australia.

### Method

The CAST study includes over 13,000 HSCT recipients reported to the Australasian Bone Marrow Transplant Recipient Registry since 1992. Linkage with the Australian Cancer Database was performed to identify primary invasive cancers diagnosed in this cohort. Cumulative incidence curves and standardised incidence ratios (SIRs) were generated for 7765 autologous HSCT recipients aged 15 or older.

### Results

By the end of 2007, 298 second malignancies had developed in 287 autologous HSCT recipients. The overall cumulative incidence was 7.3% for all second malignancies, 5.6% for solid malignancies and 1.8% for haematological malignancies. 1.4 times more second malignancies occurred in this cohort than expected in the Australian general population (SIR=1.4, 95%CI=1.2-1.6). Observed malignancy rates were found to approach expected rates as age at HSCT increased and as the transplant era became more recent. The ratio of observed to expected malignancies was greatest beyond 10 years from HSCT (SIR=2.1, 95%CI=1.4-3.1). Specific cancers that occurred at greater than expected rates were melanoma (SIR=2.5, 95%CI=1.9-3.3), non-Hodgkin lymphoma (SIR=3.3, 95%CI=1.6-7.0) and acute myeloid leukaemia or myelodysplasia (SIR=20.6, 95%CI=15.6-27.1).

### Conclusion

These results demonstrate the importance of surveillance and early detection of post-transplant malignancies, particularly melanoma, non-Hodgkin lymphoma and acute myeloid leukaemia or myelodysplasia.

**Keywords** autologous HSCT, second malignancies, late effects

**Conflict of interest** No

Tuesday 30 October

1030-1130

HSANZ Free Communications 12: Stem Cell Transplantation - Late Complications Room 218  
O120

1100-1115

## Incidence of Secondary Malignancy in Recipients of Allogeneic Stem Cell Transplantation – 20 Year Experience in a Single Institution

S Avery<sup>1,2</sup>, T Walker<sup>1,2,3</sup>, S Patil<sup>2</sup>, A Wei<sup>2</sup>, A Schwarer<sup>2</sup>, Y Ling<sup>2</sup>, J Muirhead<sup>2</sup>, A Spencer<sup>2,3</sup>

<sup>1</sup>Late Effects Clinic, <sup>2</sup>Dept of Malignant Haematology and Stem Cell Transplantation, Alfred Hospital, Melbourne; <sup>3</sup>Dept of Clinical Haematology, Monash University

### Introduction

Steady improvements in the outcome of allogeneic stem cell transplantation (SCT) have resulted in increasing numbers of long-term survivors. While durable remissions are achievable, long-term survivors remain at risk of late effects including second cancers related to prior therapies including SCT.

### Method

The incidence of second malignancy excluding non-melanomatous skin malignancy in 502 consecutive patients undergoing allogeneic SCT at a single institution over a 20 year period between January 1992 & March 2012 was examined. The probability of developing secondary cancer was estimated by cumulative incidence (CI).

### Results

As of March 2012, 212 of 502 patients were alive with a median follow up of 311 days (range 4–5842). Median age at transplantation was 41 years (range 15–66). 17 patients developed a total of 18 new solid malignancies. Nine of these were diagnosed within 5 years post-transplant and 7 thereafter. Involved sites included prostate (n=5), cervix (n=5), and one each of oesophagus, tongue, melanoma, PTLN, thyroid, liver/pancreas and lymphoma. One patient had 2 carcinomas involving the tongue and oesophagus, respectively. CI of secondary malignancy continued to rise over time post-transplant and was 2.6% and 7.3% after 5 and 10 years respectively. CI of secondary solid neoplasia for those patients surviving more than 5 years post-transplant (n=103) were 9.4% and 12.3% at 10 and 15 years, respectively. Of the 17 patients with new solid neoplasms, 5 have died, 3 as a consequence of their second cancer and 2 of unrelated causes. Time to death following second neoplasm diagnosis was 61 days (oesophagus), 64 days (pancreas), and 7.3 years (prostate).

### Conclusion

Our analysis of 502 allogeneic SCT recipients reveals a high incidence of secondary malignancy without evidence of a plateau with time. While the curative potential of allogeneic transplantation outweighs the risk of late complications, all transplant recipients remain at risk of new neoplasms and associated mortality. These data highlight the importance of late effects clinic attendance for long-term follow up and screening to detect second malignancies at the earliest possible stage.

**Keywords** Second malignancy, Transplantation, Survivorship

**Conflict of interest** No

Tuesday 30 October

1030-1130

HSANZ Free Communications 12: Stem Cell Transplantation - Late Complications Room 218  
O121

1115-1130

## **Late Relapse After Allogeneic Stem Cell Transplantation for Chronic Myeloid Leukaemia. Clinical Characteristics and Outcomes**

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Vic, Australia*

### **Introduction and Aim**

Prior to the widespread availability of the tyrosine kinase inhibitor imatinib mesylate (IM) for the treatment of chronic myeloid leukaemia (CML), this disease was the commonest indication for allogeneic stem cell transplantation (SCT) and the majority of patients (pts) treated with SCT were apparently cured. The observation that some relapses occurred late after SCT prompted a review of these pts to identify their clinical characteristics and outcomes. Patients were identified from our institutional BMT database and relevant data extracted and tabulated.

### **Results**

157 pts were transplanted for CML from 1996 to 2002. Of these, 97 pts survived at least 2 years and 19 of these relapsed with CML more than 2 years post transplant. Median time to relapse was 68 months (range 26-139 months). The majority of pts relapsed with molecular or cytogenetic markers only (n=17), with 2 having overt haematologic disease. All pts received treatment post relapse. Donor lymphocyte infusion (DLI) (n=7), IM (n=9) or both DLI and IM (n=1) were administered. 17 pts achieved a complete molecular remission (CMR) and are alive in ongoing CMR, while 2 pts died from disease progression. Of 10 pts treated with IM, 8 have ceased the drug and remain in CMR more than 2 years post cessation.

### **Conclusion**

Effective salvage of patients relapsing late after SCT for CML is feasible in almost all patients. The subsequent withdrawal of IM therapy appears possible for almost all of them without loss of CMR. This suggests that a graft versus leukaemia effect may persist or be restored in many of these patients. Diligent monitoring of disease state with regular molecular testing is indicated indefinitely for patients who have had SCT for CML.

**Keywords** CML, Transplant, Relapse

**Conflict of interest** No conflict of interest to disclose

Tuesday 30 October  
ANZSBT Symposium 7: Patient Perceptions

1030-1130  
Rooms 219/220

## **Recipients' Perceptions of Red Cell Transfusions and Anti-D Administration**

Christopher Corkery, Richard Charlewood, Suzie Rishworth, Angela Wright, Fiona King, Liz Thrift, Rachel Donegan, Jacquie Raynes  
*New Zealand Blood Service, New Zealand*

### **Aim**

The primary aim was to ascertain whether patients were satisfied with the information received before the administration of the blood component/product and what concerns they had about blood transfusions.

### **Method**

Using a combined modified version of a questionnaire by Gray & Murphy (1993) and Horne, Hankins & Jenkins (2001) patients at eight main hospitals in New Zealand were interviewed by Transfusion Nurse Specialists.

### **Results**

356 red cell recipients and 191 Anti-D recipients were interviewed. All recipients were aware of having received either red cells or Anti-D. All recipients of Anti-D felt part of the decision making process whereas only 87% of those receiving red cells felt part of the process. Anti-D recipients were less concerned about receiving a blood product. The primary concern voiced by both groups was of contracting a viral infection. Anti-D recipients voiced no concerns post transfusion whereas 7% of red cell recipients still had concerns. Those patients who received written information were statistically less concerned about receiving blood transfusions compared to those who received verbal information only.

### **Conclusion**

This survey provides a useful picture of the perceptions of various groups of patients receiving two different blood products. The results are relevant to the practice of transfusion medicine in New Zealand and provide a baseline of patient's perceptions toward blood transfusions. Results indicate that both Anti-D and red cell recipients were less likely to have concerns if given written information about their transfusion. However a significant minority of red cell recipients felt they weren't involved in the decision making process.

**Keywords** Consent, perception, satisfaction

**Conflict of interest** No

**Tuesday 30 October**  
**ANZSBT Symposium 7: Patient Perceptions**

**1030-1130**  
**Rooms 219/220**

### **The Knowledgeable Patient**

Sophie Hill

Abstract not available at time of going to print

Tuesday 30 October  
ASTH/APSTH Symposium 6: Challenges in VTE

1030-1130  
Room 210

## **Surgical Versus Medical Treatment for Proximal Deep Vein Thrombosis**

Simon McRae  
*Royal Adelaide Hospital, SA Pathology*

Post-thrombotic syndrome remains a common complication of deep vein thrombosis, and accounts for much of the cost associated with this condition. Recent research has focused on the effect of more aggressive up-front treatment of deep vein thrombosis on reducing the long-term incidence of PTS. This talk will focus on recent studies examining the impact of up-front thrombolysis or surgical intervention in patients with acute DVT, and will discuss whether the potential benefits justify routine application of these methods to unselected patients with proximal DVT.

**Keywords** Post-thrombotic syndrome, thrombolysis, deep vein thrombosis

**Conflict of interest** Nil to declare

**Tuesday 30 October**  
**ASTH/APSTH Symposium 6: Challenges in VTE**

**1030-1130**  
**Room 210**

### **Distal Vein DVT – To Treat or Not To Treat?**

Eileen Merriman  
*North Shore Hospital, New Zealand*

Approximately 50% of symptomatic episodes of deep venous thrombosis will be limited to the calf veins (distal DVT). The natural history of these is unknown, and there are concerns regarding the specificity of ultrasound for distal DVT, suggesting that a proportion of patients treated for the condition will not actually have thrombus present. The optimal management is therefore controversial. The epidemiology and management of distal DVT will be reviewed, along with suggestions for management.

**Keywords**

**Conflict of interest** No



Tuesday 30 October  
ASTH/APSTH Symposium 7: Bleeding disorders in Asia-Pacific

1030-1130  
Room 211

## Factor Replacement in Haemophilia – Unresolved Issues and Options in the Asia-Pacific Area

Alok Srivastava  
*Christian Medical College, Vellore, India*

While the unquestioned superiority of prophylactic clotting factor concentrate (CFC) has been well established over episodic “on-demand” replacement therapy in the management of haemophilia, questions remain with regard to the optimal regimen. The unresolved issues include age at which to initiate therapy, dose at which to start, frequency of administration, escalation and de-escalation protocols based on clinical response. The type of CFC to be used (recombinant or plasma derived) and definitions of different types of prophylaxis (primary and secondary) are also issues to be addressed. While these are considerations in countries where there is relatively unrestricted access to CFC, the major issue in the majority of countries in the Asia Pacific region are related to the decision on when to initiate prophylaxis as opposed to episodic treatment as a policy and who to start with. The vast majority of these countries, even those with 1-2 iu/capita of CFC, continue to practice episodic replacement of CFC for treatment of bleeds on the understanding that prophylaxis should only be initiated when of approximately 3 iu/capita of CFC becomes available. This is the paradigm which needs to be questioned for several reasons. First, there is sufficient data to show that episodic treatment of haemophilia after bleeding does not improve long term musculoskeletal (MSK) outcome over a wide range of dosage (100 – 2000 iu/kg/year). Second, there is increasing evidence that prophylaxis started early even at lower doses can significantly reduce the frequency of bleeding, the best surrogate marker of reduced long term damage to joints and muscle. What are needed therefore are large well designed multicenter studies that address these questions which lead to new challenges. For those who have access to the high end of CFC replacement, why should they subject themselves to efforts at finding optimal doses and for those just beginning to get access to modest quantities of CFC, can they question conventional paradigms and start prophylaxis at lower doses. Scientific and socioeconomic reasons around the world require that this be done along with careful documentation of MSK outcome. While there are now several validated clinimetric instruments to measure such outcome, the new challenge is to convince physicians, patients, health authorities and research funding agencies that such studies are critical to moving CFC replacement practices in haemophilia from being based on traditional wisdom to that on good evidence.

**Keywords** haemophilia, clotting factor concentrate, replacement therapy

**Conflict of interest** None

Tuesday 30 October  
ASTH/APSTH Symposium 7: Bleeding disorders in Asia-Pacific

1030-1130  
Room 211

## vWD and TTP in China

Changgeng Ruan

*Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China*

### Aim

Von Willebrand disease, as a most common inherited bleeding disorder, is characterised by either qualitative or quantitative deficiency of von Willebrand factor. However, thrombotic thrombocytopenic purpura (TTP) is caused by multiple von Willebrand factor rich microthrombi due to a lack of a plasma metalloprotease named ADAMTS13. There were both hemostasis disorders involving vWF. To investigate the current diagnosis and treatment of von Willebrand disease (vWD) and TTP in China, we evaluate the data from main Chinese working groups and review the results in modern Chinese medical literature.

### Results

We collected the clinical data of vWD patients mainly from Jiangsu Institute of Hematology and Chinese National Hemophilia Data Management Center. Among them 57.8% were type 1, 12.8% were type 3 and 29.4% were type 2 (2A, 2B, 2M and 2N took up 13.7%, 9.8%, 2.0% and 3.9% respectively). Significant reverse relationship was found between vWF:Ag levels and the bleeding score of vWD patients. The test panel of FVIII:C, vWF:Ag, vWF:CB, vWF:Rco and vWF multimer analysis was applied in increasing numbers of patients, while genetic test was only performed for research purpose. Replacement therapy, DDAVP, and antifibrinolytic drugs were the main treatment choices for vWD patients in China. As to the female vWD patients, oral contraceptives were used to manage menorrhagia except for basic treatments. Meanwhile, regarding to the clinical data of TTP patients from Jiangsu Institute of Hematology, 7 congenital and 51 acquired TTP patients were registered. A combination of FRET-vWF and residual collagen-binding assay was utilised to measure the plasma activity of ADAMTS13 and its inhibitor. Fresh plasma transfusion or plasma replacement with immune suppressive agents was performed to treat congenital or acquired TTP respectively.

### Conclusion

Most cases of vWD and TTP patients were still not diagnosed while one-quarter of vWD patients were not precisely typed. The vWD discriminatory laboratory tests such as vWF multimer analysis, vWF:CB, vWF:FVIII binding assay and ADAMTS13 activity assay were not available in many hospitals. The care of vWD and TTP patients in China is at an early stage. Multiple medical centres should collaborate to pay more attention to the diagnosis of vWD and TTP patients.

**Keywords:** von Willebrand disease, thrombotic thrombocytopenic purpura , diagnosis, treatment, China

**Conflict of interest:** No

Tuesday 30 October

1030-1130

Nurses Free Communications 4: End of Treatment Issues. The Interface Between Survivorship and Palliative Care

Room 212

O122

1030-1045

### Development of a Survivorship Project – ‘Positive Change for Life’

H McLauchlan, D Klarica, T Walker, S Avery

*Late Effects Clinic, Dept of Malignant Haematology and Stem Cell Transplantation, Alfred Hospital, Melbourne; <sup>3</sup>Dept of Clinical Haematology, Monash University*

#### Introduction

Lifestyle modification is an increasingly important component of cancer survivorship to ameliorate the effects of treatment, minimise risk of associated co-morbidities & promote long-term wellness. Although curative for many people with blood cancer, stem cell transplant (SCT) survivors often carry a burden of associated morbidity. Within an established Late Effects Clinic, assessments of modifiable risk factors has revealed a large proportion of SCT survivors are obese (60%), have hypertension (51%), elevated triglycerides (55%) & continue to smoke (14%). Survivors are also at risk of osteoporosis with 59% having low bone mineral density & increased risk of fractures. Lifestyle modification focusing on nutrition & physical activity has the potential to impact substantially on these cardiovascular & bone health risk factors.

#### Aim

To provide a range of physical activities, support & health education opportunities to enable SCT survivors, at any fitness level, to take an active role in their ongoing physical and emotional healing to benefit health, wellbeing & quality of life.

#### Method

Long-term autologous or allogeneic SCT survivors ( $\geq 2$  years in ongoing complete remission) attending the Alfred Late Effects Clinic will be offered the opportunity to participate in the ‘Positive Change for Life’ project. Each participant will enter a 12 month program integrating the key components of dietary advice, tailored individual and group physical activity, motivational strategies & GP support.

#### Results

Recruitment & data collection is commencing. Changes from baseline over time for dimensions of interest relating to anthropometric measurements, laboratory parameters and questionnaire items relating to quality of life, barriers to physical activity, leisure time exercise habits, dietary intake and fatigue will be assessed.

#### Conclusion

The completion of cancer treatment can represent an opportunity to make changes to improve health & wellbeing. A critical need for SCT survivors to address lifestyle has been identified. Regular exercise, good nutrition & maintaining a healthy weight are all strategies that may improve health, wellbeing and quality of life for long-term survivors of curative SCT.

**Keywords** Survivorship, Lifestyle, Quality of Life

**Conflict of interest** No

Tuesday 30 October

1030-1130

Nurses Free Communications 4: End of Treatment Issues. The Interface Between Survivorship and Palliative Care

Room 212

O123

1045-1100

# **Facilitating the Possibility of Travel Whilst Maintaining the Long term Care and Safety Needs of Eculizumab-treated Paroxysmal Nocturnal Haemoglobinuria Patients**

Michael Brown<sup>1</sup>, Alexandra Wood<sup>2</sup>, Gemma Brooksbank<sup>2</sup>, Richard Kelly<sup>2</sup>, Anita Hill<sup>2</sup>, Elizabeth Marshall<sup>3</sup>, Jeff Szer<sup>1</sup>, Peter Hillmen<sup>2</sup>, Louise Arnold<sup>2</sup>

<sup>1</sup>Royal Melbourne Hospital, Melbourne, Australia; <sup>2</sup>St James University Hospital, Leeds, United Kingdom; <sup>3</sup>OneSource Australia and New Zealand, Sydney, Australia

## **Aim**

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare, acquired bone marrow disorder. Historically the management of PNH was largely supportive, relatively ineffective and resulted in frequent visits to hospital, admissions, and an inability to function normally including loss of employment or other daily activities. PNH is a chronic condition and in most patients persists for the remainder of the patient's life. Eculizumab has been reported to improve all symptoms due to haemolysis in PNH as well as preventing the common complications, such as thrombosis and renal failure. However the nature of the fortnightly intravenous administration presents some difficulties. Through international collaboration we present a number of case studies where we have successfully been able to balance the patients' needs whilst continuing to maintain their treatment and safety as they travel to, and through Australia.

**Case study 1:** a 24 year old UK male on a 12 month working holiday in Australia.

**Case study 2:** a 38 year old Victorian male taking a 6 month camping holiday throughout remote North/ Western Australia.

**Case Study 3:** a 32 year old female UK based Australian citizen on 2 month holiday complicated by family emergency necessitating an extended stay.

## **Conclusion**

Recent studies have proven that the eculizumab treated patients now have similar life expectancy to that of an age matched population. Its use is effective in enabling life events such as returning to work, safer pregnancy and general wellbeing. As such there are distinct trends emerging in lifestyle expectations and requirements in this patient population. Location of care and the need to travel (for both holiday and employment) are presenting themselves as the most notable of these trends. This presents a unique challenge in caring for these patients, and will only become more prevalent in the future clinical management of patients with PNH. As nurses caring for these patients we have a duty of care to, where available, help balance this requirement with ongoing safety whilst also ensuring long term patient compliance.

**Keywords** Paroxysmal Nocturnal Haemoglobinuria, Eculizumab, Travel

**Conflict of interest:** This research was supported by Alexion Pharmaceuticals. The company had no role in analyzing the data or preparing the abstract

**Tuesday 30 October**

**1030-1130**

**Nurses Free Communications 4: End of Treatment Issues. The Interface Between Survivorship and Palliative Care**

**Room 212**

**O124**

**1100-1115**

**A Coordinated Care Program for the Palliation of Patients with Acute Leukaemia**

Bianca Hopkins<sup>1</sup>, Andrew Wei<sup>2</sup>

<sup>1</sup> *The Alfred, Melbourne, Vic, Australia.* <sup>2</sup> *Department of Clinical Haematology, The Alfred, Monash University, Melbourne, Vic, Australia.*

**Aim**

The aim of this project was to develop a systematic approach of the transition to palliative care for patients with terminal blood cancers, such as acute leukaemia.

**Method**

A working group was established to 1) determine palliative factors specific to haematology patients 2) agree upon appropriate levels of care 3) determine processes for referral and documentation 4) formalise an integrated palliative care plan 5) develop a patient information booklet and 6) evaluate outcomes. The project plan was approved by Alfred Health Research and Ethics Committee in December 2010. Recruitment of patients commenced March 2011 and 21 patients have been recruited thus far. Pre and post patient and staff evaluation questionnaires have been developed.

**Result**

A 'Haematology Supportive Care Plan' form was developed and implemented. Education sessions for health professionals were delivered on a regular basis. Completed care plans are stored electronically under patient medical records and a copy is given to the patient. Post evaluation is planned after an 18 month period from when patients were initially recruited for the project.

**Conclusion**

The transition from acute to palliative care of haematology patients is challenging. This study has implemented a coordinated care approach that focuses on optimising communication between staff and patients, early involvement of the palliative care team, management of the transition by a nurse coordinator, documentation of decisions made and provision of written information to patients. The study will evaluate patient acceptance of the transition to palliative care, staff understanding of the transition processes and resource utilisation outcomes.

**Keywords** Haematology, Palliative Care, Supportive Care

**Conflict of interest** No

**Tuesday 30 October**

**1030-1130**

**Nurses Free Communications 4: End of Treatment Issues. The Interface Between Survivorship and Palliative Care**

**Room 212**

**O125**

**1115-1130**

## **Haematology and Palliative Care – It Does Work!**

Lisa Speedy, Alison Rowe

*Wellington Blood and Cancer Centre, Wellington Hospital, New Zealand*

### **Introduction**

Evidence suggests that there are significant obstacles in the integration of palliative care in haematology. Haematological patients may therefore receive less best-practice palliative care than other cancer patients. There are various reasons for this including:

1. Over adherence to the biomedical model by clinical haematology teams.
2. The specific needs of the haematology patient population, particularly the very rapid transition from active to palliative treatment.
3. Differences of professional perspective in managing bone marrow failure in patients with incurable haematological conditions.

### **Aim**

To investigate the referral patterns from the Wellington Hospital Haematology Service to the Palliative Care Services and to determine the level of integration of the two services.

### **Results**

Data from three consecutive years was analysed. The results indicated that the two services were increasingly collaborative with referral numbers from Haematology to Palliative Care increasing annually over the period studied. Most patients were initially referred for symptom control. The data showed that the input from palliative care could be intermittent but there was also a significant group of patients that had a long term relationship with palliative care services.

### **Conclusion**

Wellington Regional Hospital has developed an exciting collaborative approach between palliative care services and clinical haematology which has significantly enhanced care for patients, families and health professionals alike. The relationship between the services has developed to such an extent that new initiatives are being explored with haematopoietic stem cell transplant patients.

**Keywords** Haematology, Palliative Care, collaboration

**Conflict of interest** No

**Tuesday 30 October**  
**HSANZ Lab Haematology: Symposium 2**

**1030-1130**  
**Room 213**

**QAP in the Era of 8-10 Colour Flow Haematology**

Mary Sartor

Abstract not available at time of going to print

Tuesday 30 October  
HSANZ Lab Haematology: Symposium 2

1030-1130  
Room 213

## Molecular Genetics of Myeloproliferative Neoplasms

Meaghan Wall<sup>1</sup> da J Campbell<sup>1,2</sup>

<sup>1</sup> Victorian Cancer Cytogenetics Service, St Vincent's Hospital Melbourne, Fitzroy, Victoria, Australia <sup>2</sup> Department of Medicine (St Vincent's Hospital Melbourne), Melbourne University, Fitzroy, Victoria, Australia

Philadelphia negative myeloproliferative neoplasms (MPN) comprise three classical disorders and several rare variants. Direct or indirect activation of the JAK2 signal transduction pathway is common to the three classical BCR-ABL1 negative disorders: polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF). Deregulation of tyrosine kinase signalling pathways is important in chronic eosinophilic leukaemia and systemic mastocytosis which are often associated with abnormalities in PDGFRA or PDGFRB and KIT signalling respectively.

In BCR-ABL1 negative classical MPN, identification of a mutation in the *JAK2* gene indicates the presence of clonal haemopoiesis. Other recurrently mutated genes are *TET2*, *CBL*, *IDH1*, *IDH2*, *ASXL1*, *EZH2* and *DNMT3A*. Abnormality rates for conventional cytogenetics in PV and ET in clinical trial cohorts have been estimated at 20-30% and 10% respectively. However, review of all cases of PV and ET referred to our institution over a 10 year period revealed lower abnormality rates (13% in PV and 6% in ET). Common recurrent abnormalities are gain of 1q, trisomy 8, trisomy 9 and del(20q). Despite the low diagnostic yield in ET, cytogenetics may be used to exclude the presence of a Philadelphia chromosome. In myelofibrosis, the rate of cytogenetic abnormalities is higher (20-50%) and the karyotype has been validated as an independent prognostic variable. Conventional cytogenetics, FISH and molecular tests can be employed to identify patients with variant MPN and abnormalities of PDGFRA, PDGFRB or FGFR1 that may be amenable to therapy with small molecule inhibitors. Thus, combined application of cytogenetics, FISH and molecular techniques provides diagnostic, prognostic and predictive information for the management of patients with MPN.

**Keywords** Myeloproliferative neoplasm, JAK2, cytogenetics

**Conflict of interest** No conflict of interest to disclose.



Tuesday 30 October  
ANZSBT: Ruth Sanger Oration

1130-1230  
Plenary 2 (Auditorium)

## The Marvellous Land of Oz - 2030

Ken Davis

IMVS Pathology, Transfusion Medicine, Adelaide, SA, Australia

*In the Wonderful Wizard of Oz, Dorothy, Scarecrow, Tin Woodman, and Cowardly Lion followed the yellow brick road [YBR] on their first adventure. In the Munchkin Forest it was broken by two deep crevices, and nearer the Emerald City a river cuts through it. In the Marvellous Land of Oz, Tip and Jack Pumpkin head followed another branch of this road as they rode the Sawhorse from the Gillikin Country to the Emerald City.*

*The road is famous, paved with smooth yellow bricks, which connects various sections of the Land of Oz to the Emerald City. It is broad, but not straight. It wanders over hill and dale. It's smooth except in a few places where bricks have crumbled or been removed, leaving holes. Following any stretch of the yellow brick road is a hazardous journey.*

The YBR could well describe the many roads and directions that transfusion medicine has taken and is an analogy that may still be relevant as we attempt to look forward to 2030 and beyond. For many years we have awaited a range of new technologies to provide suitable alternatives or substitutes in lieu of some of our current treatment options. Success in bringing R&D to a therapeutic alternative has occurred with the recombinant clotting proteins FVIII, FIX and FVIIa. In the area of red cell alternatives and specific recombinant antibodies, we have seen promising developments slowed, halted or abandoned.

Are we being overly optimistic in anticipating that the fields of protein chemistry, genetic manipulation, cryobiology and large scale manufacture of artificial red cells or platelets in bioreactors will provide a new array of safe, cost-effective therapeutic alternatives?

Whether the rate of exponential development will continue at the pace of the last 20 years is yet to be seen.

**Tuesday 30 October**  
**HSANZ/ASTH Combined Symposium**

**1330-1500**  
**Plenary 2 (Auditorium)**

**Monitoring and management of L-Asp associated thrombosis**

Nicola Goekbuget

Abstract not available at time of going to print

Tuesday 30 October  
HSANZ/ASTH Combined Symposium

1330-1500  
Plenary 2 (Auditorium)

## **Assessment and Management of Splanchnic Vein Thrombosis**

Walter Ageno  
*University of Insubria, Varese, Italy*

Splanchnic vein thrombosis (SVT) is defined by the formation of a thrombus in one or more abdominal veins draining from different organs, including small and large bowel, liver, spleen and pancreas, which may be associated with a rather heterogeneous spectrum of underlying disorders, either local or systemic, and which results in variable clinical presentations. SVT risk factors can be classified as abdominal (local) or systemic; temporary or permanent; congenital or acquired. Myeloproliferative neoplasms, abdominal cancer, intra-abdominal inflammatory conditions, surgery, cirrhosis, and portal hypertension are the most common identified acquired risk factors. Among the inherited thrombophilia, Factor V Leiden mutation has shown stronger association with BCS than with PVT, while the converse has been reported for prothrombin G20210A mutation. JAK2 mutation is, together with flow cytometry for CD55 and CD59, the main new biological marker of subclinical risk factors. The optimal treatment of SVT remains an open issue, since there are no randomized controlled trials and current recommendations are based only on cohort studies and expert opinion. The management of acute PVT and MVT requires prompt anticoagulation with the aim to prevent intestinal infarction and long-term complications of chronic portal hypertension. Patients with known esophageal varices have an important risk of bleeding complications.

**Keywords** Splanchnic vein thrombosis, risk factors, anticoagulant treatment

**Conflict of interest** No

**Tuesday 30 October**  
**HSANZ/ASTH Combined Symposium**

**1330-1500**  
**Plenary 2 (Auditorium)**

## **Modern Management of Budd-Chiari Syndrome**

Adam Testro  
*Liver Transplant Unit, Victoria, Austin Health*

This presentation will focus on the modern management of Budd-Chiari syndrome. The experience of the Austin Liver Unit over the past decade will be presented, with a particular focus on the typical patient presentation, initial diagnostic modalities, including haematological investigations, and the range of therapeutic options, with an emphasis on radiological interventions such as transjugular intrahepatic portosystemic shunts (TIPS). The longer term outcome of these local patients will be discussed in the context of the larger published case series and consensus recommendations.

**Keywords** liver, thrombosis, Budd-Chiari

**Conflict of interest** No

Tuesday 30 October  
ANZSBT Symposium 8: Platelet Storage

1330-1500  
Rooms 219/220

## Evaluation and Characterization of Cryopreserved Platelets

Larry J Dumont<sup>1</sup>, Jose Cancelas<sup>2</sup>, Ann Galan<sup>3</sup>, Gines Escolar<sup>3</sup>, Kathy Grindle<sup>1</sup>, Melissa Barber<sup>1</sup>, Michelle Dumas<sup>1</sup>, Sharry Baker<sup>1</sup>, Sue-Ann Connary<sup>1</sup>, Kathleen Grindle<sup>1</sup>, Rene Geissler<sup>1</sup>

<sup>1</sup>The Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

<sup>2</sup>Hoxworth Blood Center, University of Cincinnati, Cincinnati, OH, USA

<sup>3</sup>Serv Hemoterapia-Hemostasia, Hospital Clinic, Barcelona, Spain

### Aim

The availability of 22°C stored platelets is severely limited in some settings. Our objectives were to develop a cGMP manufacturing process for 6% DMSO frozen platelets (CPP), and to characterize the *in vivo* and *in vitro* performance of CPP including storage stability at -80°C.

### Results

We performed a randomized, Phase 1 study analysing platelet viability and *in vitro* function in consenting healthy subjects. CPP were prepared from apheresis platelets (AP) suspended in 6% DMSO, concentrated and placed at  $\leq -65^{\circ}\text{C}$  for 7 to 13 days, thawed at  $37^{\circ}\text{C}$  and resuspended into 25mL 0.9% NaCl. Autologous recovery and survival of CPP were determined and compared to fresh autologous platelets as a control. CPP 24-hour recovery ( $41.6 \pm 9.1\%$ ) was lower than AP ( $74.2 \pm 18.5\%$ ,  $p < 0.0001$ ) and did not meet the current FDA criterion. CPP had diminished survival compared to fresh platelets ( $6.8 \pm 2.1$  vs.  $8.2 \pm 1.3$  days, respectively,  $p = 0.018$ ), but did meet and exceed the FDA criterion for survival. *In vitro* tests of CPP stored up to 6 months revealed CPP retained  $77 \pm 6\%$  of AP platelet yield, showed increased platelet associated P-selectin ( $72 \pm 10\%$ ), reduced responses to agonists, and platelet microparticle content of  $2.8 \times 10^6$  per unit. Platelet coverages for platelet depleted whole blood in the Impact-R and Baumgartner perfusion chamber were restored by CPP in a dose dependent manner. Compared to 5-day old plasma stored platelets, CPP were more efficient at enhancing fibrin formation on vascular surfaces approaching that of whole blood. Clot viscoelastic properties were evaluated in the TEG and ROTM devices, and thrombin generation by F1+2 and response in the calibrated automate thrombogram.

### Conclusion

While 24-hour recovery does not meet FDA criteria for liquid stored platelets, the CPP survival of circulating platelets was surprisingly high and exceeded the FDA criteria. CPP retains a measure of adhesive functions and clot viscoelastic properties. These data support proceeding with additional studies to evaluate the clinical effectiveness of CPP.

**Keywords** cryopreservation, platelet, recovery

**Conflict of interest** No

Tuesday 30 October  
ANZSBT Symposium 8: Platelet Storage

1330-1430  
Rooms 219/220

## Platelet Storage: What Are We Doing in Australia?

Denese C Marks

*Research and Development, The Australian Red Cross Blood Service*

Platelets are the primary cellular mediators of haemostasis. In Australia over 130,000 platelet donations are supplied to hospitals every year for life-saving transfusions, most frequently for oncology patients or surgery and trauma. The shelf-life of fresh platelets is currently limited to 5 days due to the potential for bacterial contamination and development of the platelet storage lesion, which impair the quality of a platelet concentrate upon storage beyond 5 days. This presents a major challenge for managing a platelet inventory, and extension of the platelet shelf-life would be highly advantageous.

One way of prolonging the shelf-life of platelets is cryopreservation, allowing platelets to be stored frozen for up to 2 years. Cryopreserved platelets are not currently an approved blood component in Australia. There is, however, an unmet demand for such a product in remote and regional areas where platelets are either discarded due to infrequent demand or not immediately available in emergency situations. Similarly, cryopreserved platelets could be utilised by the Australian Defence Force (ADF) on deployments to austere environments where fresh blood products cannot be readily supplied.

The Netherlands Military Blood Bank has been using cryopreserved platelets since 2001 for peacekeeping and peace enforcing missions abroad, and they have been shown to be safe, effective and efficient in supporting combat casualty care. The ADF plans to adopt a similar capability for use in their deployments. The Australian Red Cross Blood Service has been working closely with the ADF to develop processes for producing and supplying deep frozen blood products, including cryopreserved platelets, based on published methods and those used by the Netherlands Military.

This presentation will highlight the methods we have been evaluating for freezing and thawing platelets, with emphasis on resuspension solutions and their effect on platelet quality. Some changes that occur to intracellular signalling will also be presented.

**Keywords** Platelet storage lesion, cryopreservation

**Conflict of interest** No

Tuesday 30 October  
ANZSBT Symposium 8: Platelet Storage

1330-1500  
Rooms 219/220

## Frozen Products in Remote Environments

Anthony Holley

*Royal Brisbane and Women's Hospital, Royal Australian Navy, University of Queensland, Brisbane, Qld, Australia*

Haemorrhage is a potentially reversible cause of trauma deaths. The concept of haemostatic resuscitation, characterised by transfusion of blood products in an immediate and sustained fashion is well established. Early transfusion with red blood cells, platelets and plasma in a 1:1:1 ratio appears beneficial. This paradigm demands products be readily available in austere environments. Investigation into synthetic products has failed to provide a viable alternative. Refrigerated liquid products are limited by short shelf lives, while fresh, warm whole blood has significant draw backs. Deep freezing is able to substantially extend the shelf life of blood products required for resuscitation, facilitating practical resupply.

Viable Storage Times		
	Standard Liquid Blood	Deep Frozen Blood
<i>Red Blood Cells</i>	42 days at 4°C	10 years at -80°C
<i>Platelets</i>	5 days at 22°C	2 years at -80°C
<i>Fresh Frozen plasma</i>	1 years at -30°C	7+ years at -80°C

Deep frozen blood product production requires cryopreservation with glycerol in the case of erythrocytes and dimethyl sulphoxide for the preservation of platelets. These agents protect the biological elements from the destructive potential of deep freezing. Prior to transfusion the products must be washed/prepared including removal of cryopreservatives. The development of a deep frozen blood product supply could substantially enhance the ability to provide high quality critical care for injured service personnel on deployment or civilians in remote centres. This presentation will describe the processes, training, Dutch/Australian experience and potential pitfalls of adopting "deep freezing" technology in the military environment.

**Keywords** Frozen blood products, military, remote

**Conflict of interest** No

Tuesday 30 October  
ASTH/APSTH Symposium 8: Fibrinolysis

1330-1500  
Room 211

## Plasminogen Activation and the Blood Brain Barrier: Implications for Ischaemic Stroke and Traumatic Brain Injury

Robert Medcalf

*Australian Centre for Blood Diseases, Monash University, Victoria, Australia*

### Aim

The plasminogen activating (PA) system is known for its role in fibrinolysis but also plays an important function in the brain. Tissue-type plasminogen activator (t-PA) is the more prominent plasminogen activator in this compartment and has been linked with memory formation and anxiety, the processing of neurotrophic factors, and in the response to drugs of addiction. Other roles for t-PA/plasmin became apparent under conditions of neuropathology: deficiency in t-PA can reduce the neurotoxic effects of glutamate, while in models of ischaemic stroke and traumatic brain injury (TBI), the absence of t-PA results in smaller infarct volumes and improved recovery times, respectively. t-PA has also been shown to increase permeability of the blood brain barrier (BBB) in humans and this may underlie the ability of t-PA to promote haemorrhage in patients with ischaemic stroke.

### Results

Using an *in vitro* BBB model, we demonstrate that the t-PA-mediated increase in BBB permeability is plasmin-dependent, requires engagement with LDL receptors (LDLRs) and activates the Rho-kinase signalling pathway in astrocytes. Selective inhibition of LDLR binding or Rho-kinase signalling reduced t-PA-induced BBB opening. We also subjected t-PA<sup>-/-</sup> mice and transgenic mice overexpressing t-PA in neurons to a computer controlled cortical impact model of TBI and evaluated changes in albumin extravasation in the brain at 3h post-TBI. t-PA<sup>-/-</sup> mice had less extravasation whereas t-PA overexpressing mice had increased extravasation and displayed more severe neurological impairment. Interestingly, t-PA was also shown to form complexes with its natural inhibitor, PAI-1 that resulted in an increase in levels of matrix metalloproteinase (MMP)-3. Inhibition of MMP-3 or blockade in the ability of t-PA:PAI-1 complexes to bind to LDLRs reduced extravasation post-TBI. t-PA:PAI-1 complexes and MMP-3 levels were shown to be elevated in the cerebrospinal fluid of severe TBI patients.

**Conclusion.** t-PA plays a natural role in the brain to modulate the BBB. However, under neuropathological conditions this can lead to significant extravasation and impairment. Inhibition of this process may offer translational opportunities in ischaemic stroke and in TBI.

**Keywords** tissue type plasminogen activator, blood brain barrier, stroke

**Conflict of interest** No



Tuesday 30 October

ASTH/APSTH Symposium 8: Fibrinolysis

### Cell-associated Positive Feedback Mechanism of Fibrinolysis

1330-1500

Room 211

Tetsumei Urano, Yuko Suzuki

*Department of Medical Physiology, Hamamatsu University School of Medicine,  
Higashi-ku, Hamamatsu, JAPAN*

#### Aim

Recently, we demonstrated that tissue plasminogen activator (tPA) stays on the surface of vascular endothelial cells (VECs) after secretion in a heavy chain dependent manner, employing green fluorescent protein (GFP)-tagged tPA and total internal fluorescence (TIRF) microscopy. In the present study we analysed how membrane-retained secreted tPA triggers cell-associated fibrinolysis on VECs and contributes to maintaining high fibrinolytic potential on VECs.

#### Results

1. Our confocal laser scanning (CLS) microscopy study revealed that Alexa Fluor 568-labeled plasminogen (plg-568) accumulated on cell surface at tPA-GFP retained spots as well as intercellular/matrix adhesive area. Either addition of epsilon-aminocaproic acid (EACA), a lysine analogue, or pre-incubation with carboxypeptidase B to remove carboxyl-terminal Lys residues attenuated accumulation of plg-568. Alexa Fluor 568-labeled mini-plasminogen, lacking four of five kringle domains of native plg, rarely accumulated, suggesting that the binding of plg was lysine binding site (LBS)-dependent.
2. Either employment of catalytically inactive mutant of tPA-GFP, or supplementation of inhibitors such as PAI-1, aprotinin or  $\alpha$ 2-antiplasmin, also attenuated accumulation of plg-568, indicating that generation of plasmin on cell surface seems to be essential.
3. Retained tPA appeared to initiate zonal clot lysis of a fibrin network that had been formed on VECs, which was preceded by the binding of plasminogen to the lysis front. Catalytically inactive mutant of tPA-GFP did not develop the lysis.

#### Conclusion

Generation of plasmin by secreted and retained active tPA on cell surface effectively facilitated further accumulation of plasminogen by exposing C-terminal lysine after cleavage of either surface or pericellular proteins. The retained tPA also effectively evoked fibrinolysis of the clot formed on GFP-tPA expressing VECs. This positive feedback loop in plasmin generation on cell surface as well as on fibrin by membrane-retained secreted tPA, seems essential in maintaining high fibrinolytic potential on VECs. Attenuation or overexpression of this feedback loop may result in a variety of pathological events by modifying either anti-thrombotic potential of VECs or permeability of vascular wall.

**Keywords** vascular endothelial cell (VEC), tissue plasminogen activator (tPA), fibrinolysis

**Conflict of interest** No

Tuesday 30 October  
ASTH/APSTH Symposium 8: Fibrinolysis

1330-1500  
Room 211

## Hyperfibrinolysis in Trauma

Herbert Schöchl  
AUVA Trauma Centre Salzburg, Austria

Trauma-induced coagulopathy (TIC) has been considered for a long time as being due to depletion of coagulation factors secondary to blood loss, dilution and consumption. Dysfunction of the remaining coagulation factors due to hypothermia and acidosis is assumed to additionally contribute to TIC.

Another phenomenon, which has been recognized recently as an important pathomechanism of coagulopathy is termed hyperfibrinolysis (HF). In a group of patients suffering from pronounced haemorrhagic shock and tissue trauma, *Brohi and co-workers* described an "endogenous" coagulopathy, independent of confounding factors such as hypothermia, acidosis or haemodilution. In the course of hypotensive low flow states, significant amounts of tissue plasminogen activator (t-PA) are released from endothelial cells and thrombomodulin is expressed on their surface. Thrombomodulin binds thrombin and subsequently activates the protein C pathway. Protein C together with its cofactor protein S slows down the accelerators of the coagulation process by inactivating FVIIIa and FVa. Furthermore, high amounts of protein C consume plasminogen activator inhibitor 1 (PAI 1), the major antagonist of t-PA. As a consequence, overwhelming amounts of t-PA are available, creating a profibrinolytic state. Hyperfibrinolysis results in a destruction of the fibrin network thus decreasing clot stability. In addition, degradation of fibrinogen leads to defibrination. However, it is still unknown to which degree HF contributes to coagulopathy.

In a large cohort of trauma patients *Cotton et al.* reported recently a 2% incidence of HF according to visco-elastic test. HF was associated with higher transfusion requirements and increased mortality. Results from a huge randomized, placebo-controlled trial suggest that the early treatment of trauma patients with tranexamic acid resulted in a significant reduction of trauma-associated mortality. Therefore TXA should be an integral treatment step in TIC.

Hyperfibrinolysis is an important contributor of coagulopathy in major trauma and strongly associated with poor outcome. Visco-elastic test provide rapid information on premature clot destruction. Antifibrinolytic therapy should be considered in all trauma patients with an injury severity score >15.

**Keywords:** Hyperfibrinolysis, trauma,

**Conflict of interest:** Herbert Schöchl received speakers' fees from CSL Behring and TEM international

Tuesday 30 October  
Nurses Symposium 5: Focus on Apheresis

1330-1500  
Room 212

## Gelofusine as a Sedimenting Agent for Granulocyte Apheresis

Aaron Hill, Claire Dowsing, Jeff Szer

*Department of Clinical Haematology & Bone Marrow Transplant Service, The Royal Melbourne Hospital, Melbourne Vic, Australia*

### Background and Aim

Erythrocyte sedimenting agents are essential for the efficient collection by apheresis of granulocytes for therapeutic use. The most commonly used and best studied sedimentation agent in this procedure is hydroxyethylstarch (HES) which is not available in Australia. Dextran 70 has been used as a substitute and was found to be a superior sedimenter than higher molecular weight products such as Pentastarch. Dextran 70 will soon no longer be accessible due to cessation of manufacturing. Our experience with Dextran 70 (MW 70,000 and superior as a sedimenting agent compared to HES, MW 480,000) suggested that factors other than molecular weight were important in sedimentation and that the powerful hypertonic action and the electrokinetic potential of the polysaccharide solution in Dextran 70 were identified as these factors. The gelatin product Gelofusine, a third generation fluid gelatin from B-Braun with a favourable safety profile when compared to Dextran 70 was identified as a potential substitute for efficacy in granulocyte apheresis and was evaluated. It is known to be associated with donor reactions at a rate similar to that of HES and is also compatible with sodium citrate. *In vitro* it has been shown to cause a significant increase in the sedimentation rate of erythrocytes.

### Results

Ten granulocyte collections using Cobe, Amicus and ComTec cell separators provided a mean granulocyte collection of  $155 \times 10^9/L$  (range 82-345) per infusion. Recipient mean neutrophil count increment was  $5.9 \times 10^9/L$  (range 0.2-10.7). There were no donor adverse reactions.

### Conclusion

With a lower anaphylaxis potential, lower rate of coagulation disturbances and a higher ceiling dose, Gelofusine should be considered for use as a step towards maximising donor safety. Collection of granulocytes using Gelofusine as the sedimenting agent also reliably provides a clinically relevant dose of granulocytes for infusion on multiple cell collection platforms.

**Keywords** Granulocyte, Gelofusine, Sedimentation

**Conflict of interest** No conflict of interest to disclose.

Tuesday 30 October  
Nurses Symposium 5: Focus on Apheresis

1330-1500  
Room 212

## Apheresis and Autologous BMT – A Regional Experience

Jenny Hempton

*Andrew Love Cancer Centre, Barwon Health, Geelong Hospital, Victoria, Australia.*

The Andrew Love Cancer Centre (ALCC) is a comprehensive cancer ambulatory treatment facility dedicated to the treatment of patients with haematological and solid cancers in a multidisciplinary environment. In conjunction with our 24 bed inpatient unit we provide radiotherapy and chemotherapy treatment modalities and access to national and international clinical trials.

Although therapeutic apheresis services commenced in ALCC in 1996, regional patients requiring harvesting of haemopoietic progenitor cells (HPC's) for subsequent haemopoietic stem cell transplantation (HSCT) were referred to Melbourne metropolitan centres. It was not until 2003 that a local autologous collection and transplantation programme was developed. Processing and storage of our HPC product continues to be provided off site under a service agreement between Barwon Health and Melbourne Health Shared Pathology Service.

Barwon Health services the Barwon South West region, which has a large geographical catchment area of over thirty three thousand square kilometres and a population of approximately three hundred and eighty thousand people. This provides many unique challenges for our patients and their primary health care providers as they navigate their way through their treatment and long term follow up. As well as coordinating and providing local treatment for a vast range of patients, our unit is also faced with the challenges of staffing a small service, and the complexities of NATA accreditation with the off site processing and storage of HPC's collected and subsequently transplanted at our facility.

How do we do it, and how do we improve from here into the future?

**Keywords** Regional Autologous Apheresis

**Conflict of interest** No

Tuesday 30 October  
Nurses Symposium 5: Focus on Apheresis

1330-1500  
Room 212

## Putting the Numbers into Practice: Clinical Indicators in Autologous Stem Cell Collection and Bone Marrow Transplantation

Rachel Prall, Gill Sheldon-Collins, Louise Nicholson  
*Statewide Bone Marrow Transplant Service, Royal Hobart Hospital, Tasmania*

### Aim

Rate-based indicators flag specific clinical issues, facilitating a multidisciplinary evaluation of transplant related activities. The Australian Council on Healthcare Standards (<http://www.achs.org.au/ClinicalIndicators/>) promotes the use of indicators 'to assess, compare and determine the potential to improve care'.

### Method

To provide objective measurement of significant aspects of autologous bone marrow transplantation, the Statewide Bone Marrow Transplant (BMT) Service has developed for analysis four sets of clinical indicators (CIs): patient selection and assessment, collection of haemopoietic progenitor cells (HPC), processing of cellular products and issue, reinfusion and early relapse following autologous bone marrow transplantation. The Service also reports and reviews a whole-of-service key performance indicator (KPI), the '30-Day Mortality Rate following Autologous BMT'.

### Results

Indicator monitoring allows specific issues to be graphically depicted as a rate within a percentile chart, rather than as figures in a spread-sheet or data tables. Reviewing service performance trends every three months has identified areas of non-conformance or poor outcomes as well as activities constantly achieving designated targets. The main areas identified as requiring improvement have been collection efficiency and the eligibility criteria for selection for autologous HPC collection.

### Conclusion

Indicator reporting is a useful quality improvement tool providing a visual display of performance outcomes in relation to designated targets. Evidence of poor outcomes or declining performance - in conjunction with audits, validations, incident reporting and patient surveys – facilitates analysis and focuses quality improvement initiatives.

**Keywords** Autologous BMT Indicators

**Conflict of interest** No

Tuesday 30 October  
HSANZ Symposium 7: What's New in B Cell Lymphoma

1530-1630  
Plenary 2 (Auditorium)

## An Overview of Current Therapy for Follicular Lymphoma

Robert Marcus  
*Kings College Hospital London UK*

Limited stage FL (LS) has traditionally been treated with local radiotherapy at varying doses, although there is now some consensus that 24Gy is sufficient to prevent local relapse. There is a continuous pattern of recurrence and until recently no evidence that the addition of chemotherapy immunotherapy or a combination of has any these impact on either progression free or overall survival. This may now change in the light of the US Lymphocare study (Friedberg JCO 2012 ) that shows , albeit in a retrospective and non randomised trial, that PFS is improved by the either substitution or addition of immunotherapy with the anti-CD20 monoclonal antibody Rituximab (R ) Should these data be confirmed in an RCT then there would be strong justification for a major change in practice for LS FL. Advanced Stage Asymptomatic (ASA ) FL, has traditionally been not been actively treated on the basis that since the disease is incurable and yet responsive to therapy at the time of symptomatic progression with little evidence that transformation risk or duration of response are altered by such a delay. This approach too may be called into question by 2 studies published in abstract form (Ardeshtna ASH 2010, Kahl ASH 2011) that observed significant prolongation of time to introduction of second line therapy by, once again, in the use of single agent R with ASA disease. We have no data that OS will be prolonged by this approach or whether the duration of second and subsequent responses will be compromised by this early use of single agent immunotherapy. In Advanced Stage Symptomatic (ASS)FL there are data that demonstrate that maintenance R prolongs PFS when added to induction with immuno-chemotherapy (Salles Lancet Oncology 2010) no OS benefit has yet been demonstrated. The question of which cytotoxic should partner R may be superseded if non chemotherapy based regimens such as R-Lenolidamide demonstrate superiority to cytostatics. We also look forward to the additional effect of novel antibodies, anti bcl2 agents and btk inhibitors in both increasing the duration of remission and reducing toxicity in the therapy of follicular Lymphoma.

### Conclusion

The addition of R has had a major impact in the outlook for patients with ASS FL , its lack of toxicity may also lead to a change therapy in LS and ASA disease with the incorporation of other non-cytotoxic agents early in the time course of the disease to maximise response duration whilst minimising toxicity

**Keywords** Rituximab , Follicular Lymphoma ,Cytotoxic-free therapy

**Conflict of interest** Honoraria and Travel support from Roche

**Tuesday 30 October**  
**HSANZ Symposium 7: What's New in B Cell Lymphoma**

**1530-1630**  
**Plenary 2 (Auditorium)**

**Treatment**

John Seymour

Abstract not available at time of going to print

Tuesday 30 October

ANZSBT Symposium 9: Transfusion Reactions in the Vulnerable Patient

1530-1630

Rooms 219/220

## Transfusion in the Vulnerable Trauma Patient

Herbert Schöchl

*AUVA Trauma Centre Salzburg, Austria*

Uncontrolled bleeding is the second most common cause of death following severe trauma just exceeded by major brain injury. Traditionally blood loss, consumption of coagulation factors and dilution of the remaining coagulation proteins has been assumed as the major components of trauma induced coagulopathy (TIC).

Moreover, hypothermia and acidosis are well known contributors of coagulopathy and frequently observed upon emergency room admission. Additionally primary hyperfibrinolysis has been identified recently as an additional important driver of TIC. Uncompressible diffuse microvascular bleeding results in increased transfusion requirements and is strongly associated with poor outcome. To avoid exsanguination the concept of "damage controlled resuscitation" has been implemented in military and civilian trauma centres. This concept addresses blood pressure control to minimise blood loss by "popping the clot", minimal prehospital volume therapy to avoid dilutional coagulopathy and aggressive rewarming. Furthermore early and consequent haemostatic therapy has been proven effective in avoiding and treating trauma related coagulopathy.

One of the core problems is to identify those patients early upon admission who are at risk for massive transfusion (MT). It has been shown that unnecessary exposure to FFP or platelet concentrates is associated with important side effects such as acute lung injury, transfusion related immune modulation and pathogen transmission. There is sound evidence that patients receiving less than 10 red blood cells within the first 24h do not benefit from early high ratio plasma or platelet transfusion. Therefore an early stratification regarding the potential risk of receiving MT is highly warranted. MT predictive scores based on both anatomical findings and rapidly available laboratory data, such as haemoglobin and the base deficit were developed in order to assess the risk of the individual patient for MT. Our group showed recently that thromboelastometric measurements are potentially useful to identify massive bleeders within 10 minutes upon ER admission. Patients with clot amplitude of 0 – 3 mm in the fibrin polymerization test (FIBTEM) showed an 85% risk for MT. The receiver characteristics operation curve was 0.83 which is similar to other published MT predictive scores. These retrospective data clearly have to be confirmed and validated in prospective studies.

**Keywords** Coagulopathy, trauma, risk stratification

**Conflict of interest** HS received speakers fees from CSL Behring and TEM international



Tuesday 30 October

ANZSBT Symposium 9: Transfusion Reactions in the Vulnerable Patient

1530-1630

Rooms 219/220

**Neonatal Exchange Transfusion – Challenges in the Modern Era**

Lisa Fox

*Royal Women's Hospital, Melbourne, Australia***Aim**

To describe morbidity and mortality associated with exchange transfusion in a 21<sup>st</sup> century tertiary neonatal unit.

**Results**

Sixty four exchange transfusions (ETs) were performed in 51 infants over a 10 year period, an average of 6.4 ETs per year. This compares with over 100 a year performed in this hospital in the 1960's. Thirty-six (71%) infants were Rhesus isoimmunised and 6 (12%) had ABO incompatibility. The highest SBR prior to ET was 782  $\mu\text{mol/l}$ . Of the 39 infants not on respiratory support prior to ET, 6 (15%) required mechanical ventilation afterwards; these infants were significantly more acidotic during the ET compared with those who were never ventilated (mean pH 7.309 and 7.153 respectively, mean difference -0.156, 95%CI -0.196 to -0.116,  $p < 0.001$ ). The three infants with the most severe hyperbilirubinaemia are known to have significant neurodevelopmental sequelae. Four (8%) infants died before a month of age.

**Conclusion**

Exchange transfusion is becoming a rare procedure in neonatal practice. Short term morbidity related to ET is common. Despite advances in neonatal care, an infant who undergoes ET remains at significant risk of dying in the neonatal period.

**Keywords** Neonate, exchange transfusion, complications**Conflict of interest** No

Tuesday 30 October  
ASTH/APSTH Symposium 9: Women's Health and Coagulation

1530-1630  
Room 210

## Management of Bleeding Disorders in Pregnancy

Jameela Sathar  
*Ampang Hospital, Malaysia*

### Aim

To discuss the management of patients with bleeding disorders during pregnancy, labour and delivery.

### Results

Pregnancy and childbirth pose a haemostatic challenge to patients with bleeding disorders. The optimal management of pregnancy in women with bleeding disorders requires a multidisciplinary approach and individualised treatment plan taking into account the obstetric risk factors in addition to their bleeding risk.

The risk of bleeding increases with the severity of the disorder. Severe disorders such as Glanzmann's thrombasthenia, Bernard Soulier and von Willebrand Disease type 3 can present with serious life-threatening haemorrhage following delivery. Specific disorders such as deficiency of fibrinogen and factor XIII are associated with antenatal complications including antepartum haemorrhage from miscarriage and placental abruption.

In general, factor levels rise during pregnancy but this is unpredictable hence levels should be measured at presentation and near term. Factor replacement, if required is given close to the time of delivery.

Regional anaesthesia should only be considered in close consultation with the anaesthetist and haematology team and is not contraindicated if the haemostatic defect can reliably be corrected by factor replacement.

The recommended method of delivery is spontaneous vaginal delivery. Vacuum extraction, the use of forceps and prolonged labour should be avoided. Ultimately the mode of delivery should be determined on obstetric grounds.

To reduce the risk of postpartum haemorrhage and surgical bleeding, factor levels should be maintained in the normal range for up to 7 days. Tranexamic acid can be used to control secondary postpartum haemorrhage.

### Conclusion

Patients with bleeding disorders should be managed preferably in a haemophilia centre or in close collaboration with a haematologist, with a written plan of management for the mother, and also a plan for investigation and management of the neonate, if appropriate.

**Keywords** Carriers of haemophilia, pregnancy, rare bleeding disorders, von Willebrand disease

**Conflict of interest** No

**Tuesday 30 October**  
**ASTH/APSTH Symposium 9: Women's Health and Coagulation**

**1530-1630**  
**Room 210**

### **Update on Diagnosis Venous Thromboembolism (VTE) in Pregnancy**

Sanjeev D Chunilal

*Department of Haematology Monash University, Monash Medical Centre, Australian  
Centre for Blood Disorders (ACBD)*

The diagnosis of VTE in pregnancy is challenging, as until recently there have been few prospective studies in this area. For the diagnosis of deep vein thrombosis (DVT) studies show that a pregnancy specific pre-test probability model, the LeFt rule, can safely identify woman at low risk of DVT (DVT). D-dimer levels are known to increase during a healthy pregnancy. A recent study has confirmed that if D-dimer assay is to be used for the assessment of pregnant women with suspected VTE then a pregnancy specific cut point is required to maintain D-dimer's specificity and clinical utility. However as neither D-dimer or the PTP have been validated all pregnant women should undergo a modified compression ultrasound with routine assessment of the iliac veins is required to exclude DVT. Furthermore, for those in whom clinical concern still persists (i.e. a high clinical PTP) and initial testing is negative, either serial testing or another imaging modality to assess the iliac vessels may be required. For women with suspected pulmonary embolism (PE) there is still uncertainty around whether ventilation perfusion (V/Q) scanning or computer assisted tomographic angiography (CTPA) is the most appropriate test. When both tests are available, VQ scanning is likely the safer test for the mother and exposes the foetus to acceptable levels of radiation, but has similar accuracy to CTPA and for these reasons is preferred. For any woman with a clinical suspicion of VTE it is not unreasonable for a single dose of low molecular weight heparin to be given pending the results of objective testing but a definitive answer from objective testing should be sought to guide further treatment.

**Keywords** VTE, pregnancy

**Conflict of interest** No

Tuesday 30 October  
ASTH/APSTH Symposium 10: Antiplatelet Therapy

1530-1630  
Room 211

## Antiplatelet Agents: A Cardiology Perspective

Philip Aylward  
*Flinders Medical Centre, Bedford Park, SA, Australia*

### Aim

Antiplatelet therapy is one of the mainstays of treatment of acute coronary syndromes (ACS). Aspirin was shown to be beneficial in patients with unstable angina and myocardial infarction in the 1980s. The CURE Study demonstrated the benefits of Clopidogrel in addition to Aspirin in ACS. In this study it was demonstrated that the improvements in ischemic outcomes was accompanied by an increase in bleeding but overall the benefits in terms of deaths, myocardial infarction and stroke outweighed the worsening in bleeding. The importance of coronary bypass bleeding was recognised. The development of glycoprotein IIb IIIa receptor inhibitors which blocked the final common pathway of platelet activation followed. These were shown to be particularly useful in patients undergoing percutaneous procedures where the thrombotic stimulus is large but came at a cost of significant bleeding.

### Results

New P2Y<sub>12</sub> platelet inhibitors Prasugrel and Ticagrelor have recently been studied in the TRITON-TIMI 38 and PLATO Trials and each has been shown to be superior to Clopidogrel when in combination with aspirin in ACS. Ticagrelor has been shown to reduce mortality. Both agents however increase bleeding which has been recognised as a serious complication for patients with ACS. Vorapaxar, a platelet thrombin receptor antagonist has been studied in the recent TRACER and TRA 2P Trial and the results disappointing.

### Conclusion

In summary platelet inhibitors have a major role in the management of cardiovascular disease but the balance between ischemic prevention and bleeding has become an increasing issue.

**Keywords** Platelet inhibitors, ACS, Percutaneous procedures

### Conflict of interest

Research support by Elli Lilly, Merck and Astra Zeneca  
Advisory Board and honoraria Elli Lilly, Astra Zeneca and Sanofi Aventis

Tuesday 30 October  
ASTH/APSTH Symposium 10: Antiplatelet Therapy

1530-1630  
Room 211

## Antiplatelet Therapies for the Treatment of Cardiovascular Diseases

Matthew D Linden

*Centre for Microscopy, Characterisation and Analysis, The University of Western Australia*

Because of the central role that platelets play in arterial thrombosis, antiplatelet therapy remains the cornerstone of treatment for cardiovascular disease and the prevention of atherothrombotic events. Established approaches to antiplatelet therapy target key pathways of platelet activation - including thromboxane synthesis inhibition by aspirin, ADP receptor signalling inhibition by thienopyridines, and fibrinogen receptor blockade by GPIIb-IIIa antagonists. In combination or alone, these approaches are very effective. However, there remains considerable variability in responsiveness and the risk of breakthrough atherothrombosis remains. New and emerging approaches to antiplatelet therapy attempt to circumvent this through more rapid, less variable and more complete inhibition of the existing targets, or development of agents with different molecular targets. This enhanced inhibition of platelets must be measured against the potential for greater bleeding.

Emerging ADP receptor antagonists prasugrel and ticagrelor have recently been approved for high risk patients. There is evidence that they may provide additional benefit in diabetes, where aspirin and clopidogrel are less effective. Cangrelor, which has a faster on-off effect is proposed as a bridging agent prior to surgery, while the development of elinogrel has recently been abandoned. Future developments in this field may include new agents which target multiple but synergistic molecules in the ADP signalling pathway.

Several agents, including atopaxar and vorapaxar, are in development or proposed for their inhibition of the platelet thrombin receptor PAR-1. These have yielded mixed results with reduced cardiovascular events alongside increased bleeding. Many other novel agents targeting different elements of platelet activation / aggregation have been considered, including antagonists of GPVI, integrin  $\alpha 2\beta 1$ , and 5HT<sub>2A</sub> serotonin receptors. Advances in understanding of the clinical predictiveness of antiplatelet monitoring and genetic testing opens the possibility for guided or personalised approaches, where agent selection and dosing strategy may be made on the basis of laboratory tests.

The ultimate objective remains a single agent to provide the most effective prevention of platelet-mediated thrombosis and ischaemia whilst balancing the consequent increase in bleeding risk.

**Keywords** Antiplatelet therapy, drug development, monitoring

**Conflict of interest** Speaker Honoraria: Novo Nordisk, Siemens

Tuesday 30 October  
Nurses Programme: Concurrent Workshops

1530-1620  
Room 212

### **Difficult Conversations – Managing them well – Strategies and Approaches**

Ralph Hampson  
*Ralph Hampson Consulting, Melbourne, Vic, Australia*

This workshop will be interactive and will provide participants with opportunities to discuss and practice the art of having 'difficult conversations' with people and their families who have cancer.

The workshop is based on the work Dr Hampson has undertaken over 30 years in the delivery of services, program evaluation, research and clinical practice. This has included being one of the original founders of CANTEEN, clinical work in hospitals and mental health services, policy development, evaluation and research in the cancer fields.

Participants will leave the workshop with practical approaches and techniques for having difficult conversations. It will involve small group exercises.

**Keywords:** difficult conversations, clinical skills, counselling

**Conflict of interest** No

**Tuesday 30 October**  
**Nurses Programme: Concurrent Workshops**

**1530-1620**  
**Room 212**

## **How to Integrate Principles of Palliative Care into Everyday Practice**

Marian Allison  
*Royal Melbourne Hospital, Melbourne, Vic, Australia*

Several vignettes will be used to illustrate and discuss the integration of palliative care principles into everyday practice. The practice of identifying and addressing physical symptoms, psychosocial and spiritual needs. Enhanced care co-ordination to facilitate an interdisciplinary approach to the patient and their family. The importance of communication and understanding patient and family values, preferences, goals and needs to ensure appropriate goal directed treatment options are be offered..

**Keywords:** Palliative principles integrated.

**Conflict of interest** No

**Tuesday 30 October**  
**Nurses Programme: Concurrent Workshops**

**1530-1620**  
**Room 212**

**How To Engage and Involve Patients/Consumer Participation and Communication**

Sophie Hill

Abstract not available at time of going to print



**Tuesday 30 October**  
**Nurses Programme: Concurrent Workshops**

**1530-1620**  
**Room 212**

### **How to Develop Survivorship Care Plan.....**

Priscilla Gates

This interactive workshop is facilitated by nurses associated with the Australian Cancer Survivorship Centre (A Richard Pratt Legacy) and will provide participants with an update regarding the most recent evidence for survivorship models and care plans using international, national and local sources. Discussions will involve identifying the enablers and challenges of implementing survivorship care plans and what education resources may be required. Following a case presentation, participants will have the opportunity to develop a survivorship care plan (SCP) relevant to their clinical setting and patient population. The ACSC project team hope to support cancer nurses specifically in the area of SCP and education regarding cancer survivorship issues.

Tuesday 30 October  
Nurses Programme: Concurrent Workshops

1530-1620  
Room 212

### **“Living Ethics” – How to Integrate Ethics Into Clinical Practice**

Susan Sherson

*Clinical Ethics Committee, The Royal Melbourne Hospital, Victoria.*

Nurses are making ethical decisions every day. How we allocate individual staff in a busy ward ? on a busy shift ? How we divide our time between tasks, between patients? Do we make time to listen to young Abigail who is desperately scared at the prospect of her upcoming chemotherapy or to sit a while with Mr Hassan who is dying and far from home ?

These may seem like practical questions, questions of time so familiar in everyday practice, however they are, at their core, ‘ethical’ questions. Questions of what we ‘ought to do’ in the particular situation.

How we do, or could, respond ethically within our individual or collective nursing practice is what this session aims to explore and the lived experience of clinical nurses is both its focus and its conclusion.

**Keywords** ethics, clinical practice, decisions

**Conflict of interest** No

**Tuesday 30 October**  
**Nurses Programme: Concurrent Workshops**

**1530-1620**  
**Room 212**

## **How to Critically Appraise Literature (and Get Your Head Around Basic Statistics)**

Anna Ugalde

*Department of Cancer Experiences Research, Peter MacCallum Cancer Centre*

Reviewing and appraising the literature is important in interpreting what research has been done and what should be done next. Reviewing a new topic in the literature can be overwhelming not only because of the number of articles but also the varying quality and messages in different manuscripts. This session will explore how to assess the quality of peer reviewed publications, through describing what to look for in high quality manuscripts. The introduction, methods, results and discussion sections will each be separately explored with examples from recently published manuscripts. A brief overview of how to interpret the results and analysis will be included. Participants will be provided with strategies and techniques to assist them in reviewing their own research topics critically.

**Keywords** Literature, reviewing, publishing

**Conflict of interest** No

Tuesday 30 October  
HSANZ Masterclass

1630-1730  
Room 216

## Myeloma

Philip McCarthy  
*Roswell Park Cancer Institute, Buffalo, NY, USA*

### Aim

Discussion of cases and strategies for multiple myeloma (MM) patient management

MM, a plasma cell cancer of the bone marrow (BM) can manifest as a low grade condition (smoldering MM) with low tumor burden that may not require treatment. The classic standards for therapy are: hyperCalcemia, Renal failure, Anemia and Bone disease (CRAB). Appropriate staging including: monoclonal immunoglobulin protein detection in urine and serum along with LDH, albumin and  $\beta$ -2 microglobulin ( $\beta$ -2M), BM metaphase karyotyping and fluorescence *in situ* hybridization, radiographic imaging by skeletal survey +/- PET and MRI. Molecular tests such as BM gene expression profiling (GEP) helps with further risk stratification. Other factors such as age, performance status, organ involvement, disease risk and consideration for intensive therapy such as autologous hematopoietic stem cell transplant (HSCT) are considerations for treatment strategies. In the USA, MM induction regimens include older agents including melphalan (M) and glucocorticoids (prednisone (P) or dexamethasone (D)) and cyclophosphamide (Cy). Incorporation of novel agents into induction regimens has improved response rates and duration of response. These novel agents include the proteasome inhibitor (PI) bortezomib (B, V or P) and the immunomodulatory drugs (IMiDs) thalidomide (T) and lenalidomide (R or L) have led to better responses for transplant-eligible patients before HSCT or before maintenance therapy. Newer doublets such as VD or Rd (low dose D) may be replaced by triplet regimens such as RVD, PAD, CyBorD and VTD for HSCT-eligible patients. For the non-HSCT MM patients, MPT, MPR, Rd and VD are appropriate regimens. The EMN 02 trial is examining the role of VMP versus ASCT, single versus tandem ASCT and VRD consolidation versus no consolidation. The IFM DFCI 2009 trial is examining single ASCT versus continued RVD. The BMT CTN 0702 trial is examining single, versus tandem ASCT versus single with consolidation RVD. All trials are followed by maintenance L.

There are several approaches for induction therapy for MM patients. Inducing a complete or near complete response remains a primary goal of induction with the long term goal of maintaining response.

**Keywords** Combination therapy for multiple myeloma

**Conflict of interest** Advisory board for Celgene, Speaker Janssen Pharm.

**Tuesday 30 October**  
**ANZSBT Masterclass: Meet the Expert**

**1630-1730**  
**Room 208**

**Meet the Expert**

Cees van der Poel

Abstract not available at time of going to print

Tuesday 30 October  
ANZSBT Masterclass: RBC storage advances and metabolomics

1630-1730  
Room 209

## RBC Storage Advances and Metabolomics

Larry J Dumont<sup>1</sup>, James C Zimring<sup>2</sup>, John D Roback<sup>3</sup>

<sup>1</sup>*The Geisel School of Medicine at Dartmouth, Lebanon, NH, USA*

<sup>2</sup>*Puget Sound Blood Center, Seattle, WA, USA*

<sup>3</sup>*Emory University Hospital, Atlanta, GA, USA*

### Aim

Understanding the cause and effect relationship between red blood cell (RBC) collections, processing and storage (including age); the RBC phenotype; and clinical outcomes is central to many R&D initiatives and investigations. Our aim is to examine the changes in the RBC metabolomics profile and identify candidate biochemicals and/or processes that may correlate to clinic outcomes and may be amendable to either screening and selection or to interventions in RBC for transfusion.

### Results

Broad metabolomic fingerprints covering over 270 metabolites from various RBC will be presented for discussion. These conditions include aerobic & anaerobic storage, storage times up to 6 weeks, various donors, gamma irradiated, and RBC treated with rejuvenation methods. The major RBC pathways of glycolysis, pentose phosphate, purine salvage, and glutathione homeostasis will be considered. Significant variations are observed between various RBC treatment methods and between individual donors. In a preliminary pilot study, levels of a few metabolites correlated with the autologous RBC recovery observed in four selected study subjects.

### Conclusion

These observations suggest that candidate metabolites and/or pathways may be identified for hypothesis driven studies evaluating the effects on clinical outcomes. Understanding the larger metabolic profile picture within the RBC may inform the mechanistic understanding of the RBC storage lesion.

**Keywords** RBC, metabolomics, storage

**Conflict of interest** LJD has received research support and/or consulting fees from New Health Sciences, Fenwal, and Citra Labs.

**Tuesday 30 October**  
**ASTH/APSTH Masterclass:**

**1630-1730**  
**Room 207**

## **Haemophilia Management**

Alok Srivastava

Abstract not available at time of going to print

Tuesday 30 October  
ASTH/APSTH Masterclass: Treatment of acquired aplastic anaemia

1630-1730  
Room 218

## Treatment of Acquired Aplastic Anemia

Jong Wook Lee

*Division of Hematology, Department of Internal Medicine, Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea*

Aplastic anaemia (AA) is generally an acquired disease that has been associated with hepatitis, chemicals, drugs and, rarely, pregnancy; however, in most patients, the disease is idiopathic. AA involves heterogeneous pathophysiological mechanisms including a lack of, or defect in, haematopoietic stem cells, an immunologically mediated destruction of haematopoiesis, or an abnormal marrow stromal microenvironment. In general, the epidemiology of AA in Asian regions is distinct from that in Europe; there is a higher overall incidence, it is prevalent at younger ages and idiopathic cases are more common.

### **Stem cell transplant (SCT)**

Candidates for SCT should have severe or very severe AA and be younger than 50 years of age; the availability of HLA-matched related donor is also essential. Over the years, survival rates following HLA-matched sibling SCT have improved because of better pretransplant immunosuppressive conditioning regimens and supportive care, with 90% of SCT recipients now being curative. However, chronic graft-versus-host disease is a long-term complication affecting 15% to 25% of transplant recipients and adversely affecting survival. For the large proportion of AA patients who lack a suitable sibling donor, HLA-matched unrelated donor SCT is an alternative treatment option. Characteristics of unrelated SCT candidates have usually failed other therapy (eg, IST), and have a long disease duration, a multiple transfusion history and iron overload. With development of high resolution DNA typing and optimal conditioning regimen, the long-term survival following unrelated SCT substantially improved to 80-90%.

### **Immunosuppressive therapy (IST)**

In older patients (>50 years) with severe AA, those who do not have an HLA-matched sibling donor, or patients with non-severe AA, IST may be a suitable treatment. IST regimens usually involve antithymocyte globulin (ATG) and cyclosporine (CsA). Combination IST regimens are now the standard of care as clinical trials have demonstrated that they associated with a more rapid response, higher response rates, and improved failure-free survival rates. However, IST is not curative; one third of patients may relapse and a significant proportion (10%) are at risk of developing secondary clonal diseases. Patients with shorter telomere have the increased risk of relapse and clonal evolution.

### **Supportive care**

All patients with severe AA require supportive care, and in some AA patients, supportive care may be the only therapeutic option, eg, patients who fail IST, cannot tolerate IST, or not candidate of SCT. Supportive care involves transfusions of red blood cells (when Hb <7.0 g/dL) and platelets (when thrombocyte count <10,000/mm<sup>3</sup>) and careful infection control.



**Tuesday 30 October**  
**ASTH/APSTH Masterclass: Atypical VTE**

**1530-1630**  
**Room 217**

## **Atypical VTE**

Walter Ageno  
*University of Insubria, Varese, Italy*

Clinical cases of patients with unusual site thrombosis will be presented and discussed. In particular, the session will focus on the management of patients with retinal vein occlusion, cerebral vein thrombosis, and splanchnic vein thrombosis. For each case, we will focus on the assessment of risk factors and on therapeutic decisions to be taken.

**Notes:**

**Wednesday 31 October**  
**HSANZ Symposium 8: Scanning the Myeloid Horizon**

**0830-1000**  
**Rooms 212/213**

**New Treatment Directions in Myelodysplasia**

Speaker to be advised

Abstract not available at time of going to print

Wednesday 31 October  
HSANZ Symposium 8: Scanning the Myeloid Horizon

0830-1000  
Rooms 212/213

## New Treatment Directions in AML

Charles Craddock

*Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, UK*

Despite advances in myelosuppressive chemotherapy and allogeneic transplantation the majority of adults with Acute Myeloid Leukemia (AML) still die of resistant or relapsed disease. This, coupled with the unacceptable toxicity of both treatment modalities in older patients confirms the importance of the identification of novel chemotherapeutic agents and more effective transplant strategies. It is increasingly apparent that the development of novel therapies will be predicated on advances in our understanding of both disease biology and the innate and adoptive immune system. Acquired abnormalities in chromatin structure are commonly documented in patients with myeloid malignancies and it is postulated that the consequent dysregulation of gene expression plays an important role in the pathogenesis of these malignancies. DNA methyltransferase inhibitors (DMTI) and histone deacetylase inhibitors (HDI) have the capacity to reverse acquired abnormalities in chromatin structure and recent studies have demonstrate significant activity of these agents when administered alone or in combination in patients with AML and represent an important new class of therapeutic agents particularly in older patients unable to tolerate intensive chemotherapy. However, their clinical utility is limited by the short median response duration (~9 months) and disease relapse is almost universally observed. Critically AML is a hierarchical disease and a small population of disease propagating cells stem-progenitor cells (LSC) can be identified in the great majority of patients at diagnosis. More recently our group has demonstrated co-existence of two distinct populations with a lymphoid-primed multi-potential progenitor-like (LMPP-like) (Lin-CD34+CD38-CD90-CD45RA+) and a granulocyte-macrophage progenitor (GMP-like) (CD34+CD38+CD123+/loBAH-1-CD45RA+). On the basis of limited *in vitro* data it has been postulated that the LSC population in patients with AML is relatively chemo-resistant compared with mature hematopoietic leading to the untested hypothesis that drug resistant LSC form a cellular reservoir which contributes to disease relapse. There are however only very limited studies of the impact of conventional chemotherapy on carefully defined LSC numbers and none have been performed in patients treated with epigenetic therapies. Our group has studied the impact of both myelosuppressive, epigenetic and allogeneic transplantation on LSC numbers identifying this population as a potentially important biomarker of response and relapse in AML. The potency of an immunologically mediated graft-versus-leukaemic (GVL) effect represents one of the most important, and hitherto under-exploited, curative strategies in older patients with AML. Strategies aimed at dissociating GVL and GVHD will be discussed.

**Keywords** Epigenetic therapies; leukaemic stem cell; graft-versus-leukaemia

**Conflict of interest** No

Wednesday 31 October  
HSANZ Symposium 8: Scanning the Myeloid Horizon

0830-1000  
Rooms 212/213

## New Treatment Directions in ET

Claire Harrison  
*Guy's and St Thomas' Hospitals, London, UK*

In 1934, Epstein & Goedel used the term “hemorrhagic thrombocythaemia” to describe a disorder characterized by permanent elevation of a platelet count to more than three times normal, hyperplasia of megakaryocytes and the tendency for venous thrombosis and spontaneous hemorrhage. Over the last 75 years, and particularly in the past 6 years major progress has been made in our understanding of ET and its pathogenesis with the identification of the highly prevalent *JAK2 V617F* and other mutations. Current management of this condition is based upon historical data and with treatments that haven't changed significantly for nearly two decades. New treatments which address fundamental biology of the disease are required. Here we discuss current data with novel treatments such as pegylated interferon alpha-2a; JAK inhibitors and other treatment modalities such as HDAC inhibitors.

**Keywords** ET, JAK inhibitors, interferon

**Conflict of interest** Speaker fees from Novartis, Shire, Cellgene, Sanofi Aventis; consultancy work for YM Bioscience, S\*Bio, Sanofi Avensis and research funding from Shire and Novartis

**Wednesday 31 October**

**ANZSBT Symposium 10: Managing the Patient Outside the Hospital**

**0830-1000**

**Rooms 219/220**

**Managing Patients in the Remote Setting**

Stephen Langford

Abstract not available at time of going to print

Wednesday 31 October  
ANZSBT Symposium 10: Managing the Patient Outside the Hospital

0830-1000  
Rooms 219/220

## Logistics of the Out-of-hospital Transfusion

Giles Kelsey  
*The Royal Melbourne Hospital, Melbourne, Vic*

Out of hospital transfusions pose challenges to the blood component provider and the treating team. Patient factors, laboratory processes, logistics, records and safety must be considered prior to embarking on an out of hospital transfusion program.

Out of hospital transfusions may occur at home, in residential care facilities, in clinics or at the site of emergencies. Whilst a similar approach is required to assess risk benefit and feasibility, each environment will have specific requirements and potential complications.

In conjunction with the transfusion service at the Royal Melbourne Hospital, Air Ambulance Victoria has developed an out of hospital transfusion capability designed to minimise the risk from life threatening haemorrhage prior to patient's arrival at hospital. Assessment of the requirements and logistics of providing this service are examined and preliminary data presented.

**Keywords** Transfusion, Logistics, Emergency

**Conflict of interest** No

**Wednesday 31 October**  
**ANZSBT Symposium 10: Managing the Patient Outside the Hospital**

**0830-1000**  
**Rooms 219/220**

**Transfusion in the Air**

Speaker TBC



Wednesday 31 October  
APSTH/ASTH Free Communications 4: Haemophilia  
O126

0830-1000  
Room 210  
0830-0845

**Epidemiology, Demographics, Age at Diagnosis, and Mortality of Taiwanese Hemophiliacs: a Nationwide Study by National Health Insurance Research Database**

Chia-Yau Chang, Ta-Liang Chen, Yuan-Wen Lee, Ing-Jy Tseng, Shi-Yi Lin  
*Taipei Medical University Hospital, Department of Pediatrics, Hemophilia Center ;  
Taipei Medical University, Graduate Institute of Clinical Medicine, School of  
Medicine, Taipei, Taiwan*

**Aim**

The study aims to investigate the nationwide epidemiology, demographics, age at diagnosis and mortality of hemophilia in Taiwan, a society with 23-million people.

**Method**

The data of male patients with International Classification of Disease Ninth Revision code 286.0 and 286.1 retrieved from National Health Insurance Research Database and Ministry of Interior in Taiwan between 1997 and 2009 were analyzed. T-test was used for statistic methods.

**Results**

Annual prevalence rates of hemophilia A (HA) and hemophilia B (HB) increased over time from 4.95/100000 to 7.30/100000 male population (MP) and from 0.95/100000 to 1.34/100000 MP, respectively. Annual incidence rates of HA and HB varied from 5.63/100000 to 12.83/100000 male births and 0.73/100000 to 4.83/100000 male births, respectively. Ratio of HA to HB is about 5:1. Age distribution showed more young but gradually shifted to that of general MP. The proportion of pediatric (< age of 18) hemophiliacs reduced from 41.5% to 28.2%, with 1.85-time reducing velocity of general pediatric MP. The proportion of elder (> age of 60) hemophiliacs increased from 2.5% to 5.7%, with 1.39-time increasing velocity of general elder MP. In total 493 newly-diagnosed cases, with mean diagnosed age of 21.5, peak age of diagnosis was before age of 3 and age of 10-40. To compare data of the calendar periods of 1997-2000 and 2006-2009, age at diagnosis was earlier in 2006-2009. (p=0.035) In total 76 mortality cases, with mean mortality age of 44.4, peak age of mortality was between age of 18 and age of 60. Compared with mortality age before 2005, mortality age after 2005 became more delayed. (p=0.033) Before age of 80, average age-specific crude death rates of hemophiliacs were higher than that of general MP. Overall standardized crude death rate of hemophiliacs was 10.2/1000 population. Standard mortality ratio was 1.98.

**Conclusion**

To our best knowledge, this is the first population-based nationwide epidemiologic study of hemophilia in Asia. Advances of haemophilia care in Taiwan were evident.

**Keywords :** Hemophilia, Epidemiology, Taiwan

**Conflict of interest :** NO

Wednesday 31 October  
APSTH/ASTH Free Communications 4: Haemophilia  
O127

0830-1000  
Room 210  
0845-0900

**Tranexamic Acid Without Prophylactic Factor Replacement for Prevention of Bleeding in Patients With Hereditary Bleeding Disorders Undergoing Elective Endoscopy: Results of a Pilot Study**

Amanda Davis, Megan Walsh, Penny McCarthy, Huyen Tran, Gregor Brown, Stuart Roberts, Will Kemp.  
*The Alfred, Melbourne, Victoria, Australia*

**Aim**

Hereditary bleeding disorder (BD) patients undergoing elective endoscopy have not been well studied. Guidelines generally recommend correction of factor deficiency with concurrent use of tranexamic acid (TA). We propose that TA without prophylactic factor replacement or DDAVP is as safe as standard practice in these patients for prevention of bleeding complications.

**Method**

A prospective single-arm pilot study testing the feasibility of using TA, without prophylactic factor replacement or DDAVP pre-procedure, for the prevention of bleeding complications following elective standard risk endoscopic procedures in patients with BDs. The risk categories for specific endoscopic procedures were determined by investigators. Baseline factor levels, haemoglobin (Hb) and iron studies (IS) were measured pre procedure. Primary outcome of bleeding was undertaken by patient review and repeat Hb and IS on day 21. The NCI CTCAE bleeding scale was used. Secondary outcomes included cost of therapy.

**Results**

26 patients with diagnoses including severe haemophilia A/B (8), mild haemophilia A/B (12), factor XI deficiency (1), type 1 VWD (2), type 2 vWD (2) and Factor VII deficiency (1) underwent 30 endoscopic procedures from Sept 2010 until Jan 2012. 17 (56%) of the procedures required a biopsy, 7 (23%) of which included polypectomies. 1 patient received post procedure factor replacement according to the study protocol due to a high risk polypectomy (>1cm). 29 patients (97%) complied with TA use. Two patients experienced grade 1 bleeding and a single patient experienced grade 2 bleeding. All other patients had uncomplicated interventions and recovery.

**Conclusion**

In patients with hereditary bleeding disorders undergoing elective endoscopic procedures, the results of this pilot study suggest that TA without prophylactic factor replacement or DDAVP may be as safe as standard practice. Given the small numbers, a larger study is required to validate these findings.

**Keywords** Hereditary Bleeding disorders, Tranexamic acid, Endoscopy

**Conflict of interest** No

Wednesday 31 October  
APSTH/ASTH Free Communications 4: Haemophilia  
O128

0830-1000  
Room 210  
0900-0915

# **The Incidence of Factor VIII Inhibitor in Chinese Patients with Hemophilia A: A Four-year Prospective Follow-up Study**

Xuan Zhou<sup>1</sup>, Qiang Li<sup>2</sup>, Yang Liu<sup>1</sup>, Jing Sun<sup>1</sup>

<sup>1</sup> Department of Hematology & <sup>2</sup> Department of Laboratory, Nanfang Hospital, Southern Medical University, Guangzhou, China

## **Aim**

In order to assess inhibitor development in 215 Chinese patients with hemophilia A, a prospective study was initiated in 2007 in hemophilia treatment Centre at Nanfang Hospital.

## **Methods**

During the four-year study period, 215 Chinese hemophiliacs were observed. Factor VIII activity level and inhibitor testing were performed on the first follow-up day and at regular intervals (3-6 months) thereafter.

## **Results**

The cumulative incidence of Factor VIII inhibitor was found to be 15.3% (33/215), 17.0% in severe, 10.7% in moderate and mild forms. 34 previously untreated patients seemed more easy to develop inhibitors, compared to 181 previously treated patients (38.2% vs 11.0%,  $P < 0.01$ ). Of the 33 hemophilia patients with inhibitors, 12 (36.4%) were high titer, and the remaining 21 (63.6%) were low titer. Thirteen new inhibitors developed during our follow-up period, with 8 (61.5%) high titers and 5 (38.5%) low titers. The occurrence of inhibitors in prior inhibitor negative patients probably due to their previous low exposure (median 20 exposure days). Comparing the patients receiving plasma derived products or/and recombinant products ( $n = 197$ ) with those treated with recombinant concentrate exclusively ( $n = 18$ ), 14.2% of the plasma derived or/and recombinant group developed inhibitors and 27.8% of the recombinant group ( $P = 0.126$ ). Of the thirteen new inhibitors cases, 6 were treated with recombinant concentrate exclusively.

## **Conclusion**

These findings showed that the incidence of Factor VIII inhibitor in Chinese patients with hemophilia A was lower than many western countries, and differ in previously untreated patients and previously treated patients. Our results do not support the notion that the concentrate type is associated with the risk of developing inhibitors.

**Keywords** hemophilia A ; inhibitor ; incidence ; Chinese patients

Wednesday 31 October  
APSTH/ASTH Free Communications 4: Haemophilia  
O129

0830-1000  
Room 210  
0845-0900

### Health-Related Quality of Life and Musculoskeletal Complications in Adult Hemophilia in Indonesia

Findy Prasetyawaty<sup>1</sup>, Lugyanti Sukrisman<sup>2</sup>, Bambang Setyohadi<sup>1</sup>, Siti Setiati<sup>1</sup>, Marcel Prasetyo<sup>3</sup>

<sup>1</sup> Department of Internal Medicine, <sup>2</sup> Division of Hematology – Medical Oncology, <sup>3</sup> Faculty of Medicine, University of Indonesia / Cipto Mangunkusumo Hospital Jakarta Indonesia

#### Aim

Health-related quality of life is an important goal in hemophilia management. Due to financial constraints, F.VIII substitution is used only on demand in Indonesia, which might contribute to musculoskeletal complications and affect the quality of life of adult hemophilia patients. This study's aim is to assess health-related quality of life and its relation to musculoskeletal complications in adult hemophilia patients in Indonesia.

#### Results

In 66 subjects aged 18-57 (median 28) years old, the scores of the SF-36 ranged from 42.1 (role physical) to 60.9 (vitality). The physical and mental component summary scored 40.0 and 57.7. Clinical severity influenced the SF-36 scores in all domains. Forty-seven subjects (71%) had severe arthropathy which affected the physical but not mental areas.

#### Conclusion

Health-related quality of life in adult hemophilia showed poor results in physical components which was affected by the number of bleeding episodes and severity of arthropathy, while the mental components were comparable to the normal population.

**Keywords** health-related quality of life, musculoskeletal complications, adult hemophilia

**Conflict of interest** No

Wednesday 31 October  
APSTH/ASTH Free Communications 4: Haemophilia  
O130

0830-1000  
Room 210  
0930-0945

# **Studies on Molecular Mechanism of Severe Hemophilia A Caused by Large Deletion of F8 Gene**

Guoling You, Yeling Lu, Jing Dai, Xuefeng Wang, Qiulan Ding, Hongli Wang  
*State Key Laboratory of Medical Genomics, Shanghai Institute of Hematology; Ruijin Hospital of Shanghai Jiaotong University School of Medicine, Shanghai, China*

## **Aim**

To identify the molecular mechanism of the deletion of F8 in a severe hemophilia A patient, and to screen the potential deletion carrier of the family members.

## **Method**

All the exons of F8 gene and its flanking sequences were amplified and sequenced directly. Multiplex fluorescence competitive PCR was used to detect the copy number variations of F8 gene. Deletion breakpoints were identified by primer walking strategy. Deletion-specific PCR was established for the identification of carriers. Multiplex PCR was used to test the polymorphism of 6 STR locus (F8Up226, F8Up146, F8Int13, F8Int25, F8Down48 and DXS1073) for gene linkage analysis.

## **Results**

Exons 4-7 of F8 gene were repeatedly amplified by PCR, electrophoresis showed no corresponding bands, other exons showed no mutations by sequenced directly, indicating the presence of large deletion occurred in the patient. The deletion of exons 4-7 were confirmed by Multiplex fluorescence competitive PCR. The patient had a deletion of 27685 bp comprising exons 4-7, the 5'breakpoint was located between g.32406\_32407 in intron 3, the 3'breakpoint was located between g.60090\_60091 in intron 7, a 2-bp microhomology and an insertion of 7 nucleotides were observed at the breakpoint site. The results of deletion-specific PCR showed that the fetal and the proband's daughter were deletion carriers, and the proband's cousin was normal, consistent with the results of the genetic linkage analysis.

## **Conclusion**

The deletion of exons 4-7 in F8 gene resulted in the severe hemophilia A. Microhomology-mediated end-joining may mediate the formation of detected deletion. The identification of exact deletion breakpoints provided a reliable diagnostic tool for carrier identification by means of a deletion-specific PCR.

**Keywords** Hemophilia A; Copy number variation; Carrier diagnosis

**Conflict of interest** No conflict of interest to disclose.

Wednesday 31 October

APSTH/ASTH Free Communications 4: Haemophilia

O131

0830-1000

Room 210

0945-1000

# **Loss of the Anamnestic Response of a High Titre FVIII Inhibitor During Immune Tolerisation Following *In Vivo* Induction of Regulatory T Cells**

SA Brown<sup>1,2</sup>, D Gillis<sup>3</sup>, A McKinlay<sup>3</sup>, N Williams<sup>3</sup>, J Robertson<sup>1,2</sup>, W Poulsen<sup>2</sup>, M Chong<sup>2</sup>, J McCosker<sup>2</sup>, P Higgins<sup>2</sup>

<sup>1</sup>Department of Haematology, Pathology Queensland, Royal Brisbane & Women's Hospitals, Brisbane, Australia; <sup>2</sup>Haemophilia Centre, Haematology & Oncology, Royal Children's Hospital, Brisbane, Australia; <sup>3</sup>Department of Immunology, Pathology Queensland, Royal Brisbane & Women's Hospitals, Brisbane, Australia

## **Aim**

FVIII inhibitors remain the most significant complication of severe haemophilia A. A minority of patients who undergo immune tolerisation induction (ITI) fail to eradicate the inhibitor. Strategies to tolerate refractory inhibitors include the use of plasma derived FVIII concentrate (pdFVIII) and the addition of immunosuppression (IS). The *in vivo* induction of regulatory T cells (Tregs) may help promote tolerisation in patients with refractory FVIII inhibitors.

## **Case report**

We report the case of a 17 year old man who failed ITI after 32 months of a combination of high dose pdFVIII and IS (Rituximab<sup>®</sup> and vincristine). After ceasing ITI the patient suffered 2 intra-cranial haemorrhages (ICH) within 4 months; in total he has suffered 5 ICH. A further attempt at ITI with pdFVIII and IS was commenced with the addition of 4 weekly cycles of a combination of intravenous immunoglobulin (IVIg), sirolimus and a histone deacetylase inhibitors (HDACi) in an attempt to induce Tregs.

## **Results**

The FVIII titre was 35.6 BU/mL prior to commencement of the modified ITI and was 0 BU/mL 3 weeks after commencing ITI (this compares with figures of 12 BU/mL and >1600 BU/mL with the prior course of ITI); no anamnestic response was observed. A transient induction of Tregs relative to total CD4+ T cells was observed, as determined by immunostaining for CD4/CD25/FoxP3 and exclusion of CD127 positive cells. The increase in Tregs was observed up to a week after completion of the 4 week cycle of sirolimus and HDACi.

## **Conclusion**

The addition of specific IS, HDACi and IVIg to induce Tregs to an ITI regimen resulted in loss of the immediate anamnestic response and evidence of a transient *in vivo* induction of Tregs. To date this patient has achieved a good partial response with negative FVIII inhibitor titres and measurable plasma FVIII levels.

**Keywords:** FVIII inhibitor, regulatory T cells, immune tolerisation

**Conflict of interest:** no conflict of interest

Wednesday 31 October

APSTH/ASTH Free Communications 5: Clinical Thrombosis

O132

0830-1000

Room 211

0830-0845

## The Utility of the Wells Clinical Prediction Model and Ventilation-Perfusion Scanning for Pulmonary Embolism in Pregnancy

Briony A Cutts<sup>1,4</sup>, Eileen Merriman<sup>1</sup>, Dee Nandurkar<sup>2</sup>, Geoffrey Soo<sup>2</sup>, Huyen Tran<sup>1</sup>  
Dhruba Dasgupta<sup>3</sup>, Nita Prassannan<sup>4</sup>, Beverley Hunt<sup>4</sup>

<sup>1</sup>Clinical Haematology Department, Monash Medical Centre, Melbourne, Victoria, Australia. <sup>2</sup>Radiology Department, Monash Medical Centre, Melbourne, Victoria, Australia. <sup>3</sup>Radiology Department, Guys and St Thomas Trust, London, United Kingdom. <sup>4</sup>Department of Thrombosis and Haemostasis, Guys and St Thomas Trust, United Kingdom

### Aim

To retrospectively evaluate the Wells clinical prediction model and ventilation-perfusion scanning (VQ) for Pulmonary Embolism (PE) in pregnancy.

### Methods

A retrospective study was performed on all pregnant women who presented with suspected PE and underwent VQ scanning at both institutions from 2007 until 2010. The clinical pretest probability (PTP) was determined as low, intermediate or high by two independent retrospectively using Wells Modified Criteria. VQ scans were determined as normal, non-diagnostic or high probability for PE independently by two experienced radiologists. Disagreements were resolved by a third assessor independently.

### Results

In 183 pregnant women the pretest probability (PTP) was determined as 'PE likely' in 76 (42%), and 'PE unlikely' in 107 (58%) of women. VQ scans were high probability in 4 (2%), non-diagnostic in 6 (3%) and normal in 173 (95%) of women. This gives the PTP using Wells modified criteria a sensitivity of 100% (CI: 0.4-1.0) and a negative predictive value of 100% (CI: 0.96-1.0).

### Conclusions

A structured clinical model such as modified Wells criteria may be useful in pregnancy but further prospective evaluation is required.

### Keywords

Pregnancy, pulmonary embolism, Wells Criteria

### Conflict of interest

No

Wednesday 31 October

APSTH/ASTH Free Communications 5: Clinical Thrombosis

O133

0830-1000

Room 211

0845-0900

## Review of Management and Outcome of Women with Thrombophilia-Risk during Pregnancy at a Single Institution

Alhossain Khalafallah,<sup>1,2</sup> A Al-Brazan,<sup>1</sup> Abdul-Rauf O Ibraheem,<sup>1</sup> Qiong Yue Teo,<sup>1</sup> Ramanathan Parameswaran,<sup>1</sup> Emily Hooper,<sup>1</sup> Toly Pavlov<sup>1</sup>, Amanda Dennis<sup>1,2</sup>  
<sup>1</sup>Launceston General Hospital; <sup>2</sup>School of Human Life Sciences, UTAS, Australia

### Background and Aims

Pregnancy is a hypercoagulable state that is associated with an increased risk of venous thromboembolic disease (VTE). The application of different anticoagulants remains challenging in this cohort of patients. We conducted this study in order to examine pregnant women at risk of thrombophilia who received anticoagulation therapy according to the RCOG-British Guidelines and compare their obstetric outcomes with a matched cohort of women without thrombophilia-risk.

### Patents and Methods

At a single institution, we retrospectively analysed 38 Caucasian women with thrombophilia-risk, who received active anticoagulation (34/38) for their pregnancies during the period between Jan 2007 to Dec 2010. The patients received different anticoagulant regimens with aspirin only (6 cases), enoxaparin (27 cases) and warfarin (1 case). Patients with inherited risks included: Methyltetrahydrofolate reductase (MTHFR) gene mutation in 10 cases, Factor V Leiden (FVL) in 2 cases, Prothrombin Gene Mutation (PGM) in 6 cases, Antithrombin III deficiency in 1 case, Protein C and S-deficiency in 5 cases. Acquired thrombophilia-risk was: 7 anticardiolipin syndrome and 1 lupus anticoagulant. Patients, who presented with recurrent unprovoked VTE, were also considered as high-risk (6).

### Results

Twenty-six out of thirty-eight pregnant women (68.4%) with an increased risk of thrombophilia experienced one or more obstetric complications defined as hypertension, pre-eclampsia, placenta abruption, VTE, oligohydramnios, intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR) and Stillbirth, compared with 15 of 40 (37.5%) pregnant women in the control group (OR 3.6; 95% CI 1.42, 9.21,  $p < 0.001$ ). The incidence of obstetric complications was significantly higher in the thrombophilia group compared to the controls. However, these complications were lowest among patients who received full-dose anticoagulation with enoxaparin throughout pregnancy. There was no statistical difference between the two groups regarding the incidence of IUGR, IUFD and still birth.

### Conclusions

Our study suggests that the strict application of guidelines for the treatment of thrombophilia of pregnancy is associated with an improved pregnancy outcome.

**Keywords:** Thrombophilia, Pregnancy, anticoagulation, outcome, RCOG Guidelines

**Conflict of interest** No



Wednesday 31 October

APSTH/ASTH Free Communications 5: Clinical Thrombosis

O134

0830-1000

Room 211

0900-0915

**Thrombosis in Japanese Patients with Multiple Myeloma Receiving Autologous Transplantation**

Kenji Yokoyama, Shinichiro Okamoto

*Keio University School of Medicine, Department of Medicine, Division of Hematology, Japan***Aim**

Patients with multiple myeloma (MM) are at increased risk of developing both venous thromboembolism (VTE) and arterial thrombosis (AT). In Western countries, the incidence of VTE was up to 5% with conventional chemotherapy and it increased to 10-30% if treated with thalidomide (Thal) or lenalidomide (LEN). AT occurred in 11 (5.6%) of 195 patients during a period of 522 patient-years in one report. However, the incidence of VTE/AT in Asian patients with MM has not been fully documented. Therefore, we retrospectively assessed the incidence of VTE/AT in Japanese patients with MM.

**Results**

Incidence of VTE/AT was assessed in patients with MM who were treated with induction chemotherapy with or without Thal/LEN, followed by single or double autologous peripheral blood stem cell transplantation (ASCT) conditioning with high-dose melphalan from January 2000 to December 2010 at Keio University Hospital. Ninety-eight patients were analyzed. Median age at diagnosis was 54 years (range 28-65) and median follow-up period was 44.5 months (range 10-147). Induction therapy included vincristine, doxorubicin, and dexamethasone (VAD) in 94 patients, high-dose dexamaethasone in 2, and vincristine, melphalan, cyclophosphamide, and prednisolone in one. No patients were placed on prophylactic antithrombotic agents. 4 patients received Thal after VAD. Single and double ASCT was performed in 48 and 50 patients, respectively. 44 received Thal/LEN, and 19 received allogeneic stem cell transplantation after ASCT. VTE occurred in 7 patients (3 during induction chemotherapy, 2 at ASCT and 2 after ASCT). 5 patients with VTE occurred until ASCT did not receive Thal/LEN before the occurrence of VTE. AT occurred in only one patient 9 years after the diagnosis of MM.

**Conclusion**

The incidence of VTE in transplantation-eligible Japanese patients with MM was comparable to those previously reported in Western countries, whereas AT was rare in this cohort. VTE occurred especially early in the course of the treatment. Our observation suggests that VTE prophylaxis might be beneficial in some of patients with MM without receiving Thal/LEN.

**Keywords** multiple myeloma, venous thrombosis, arterial thrombosis**Conflict of interest** No conflict of Interest

Wednesday 31 October

APSTH/ASTH Free Communications 5: Clinical Thrombosis

O135

0830-1000

Room 211

0915-0930

## Non-Catheter Related Proximal Upper Extremity Deep Vein Thrombosis

Noel Chan<sup>1</sup>, Eileen Merriman<sup>2</sup>, Huyen Tran<sup>1</sup>, Sanjeev Chunilal<sup>1</sup>

<sup>1</sup>Department of Haematology, Monash Medical Centre, Clayton, Vic, Australia

<sup>2</sup>Department of Haematology, North Shore Hospital, Auckland City, NZ

### Aim

To explore the risk factors and management of upper extremity DVT in patients without central venous catheter (non-CVC UEDVT) at two major hospitals.

### Method

We identified patients with objectively diagnosed proximal (axillary/subclavian) UEDVT through a search of the radiology database at Monash Medical Centre and through a pre-existing database at North Shore hospital (NZ) in the period of 01/2007 to 03/2012. Central venous catheter, or pacemaker UEDVT were excluded.

### Results

We identified 70 patients with non-CVC UEDVT from a total of 138 patients with UEDVT after excluding 68 patients with CVC or pacemaker related UEDVT. Among these non-CVC UEDVT, 26 (36%) patients were diagnosed with cancer at the time or preceding the diagnosis of UEDVT. Forty four (76%) patients were classified in the non-CVC, non-overt cancer UEDVT, and of those, two patients had a concurrent diagnosis of PE. These 44 patients (M:F=19 :25) with non-CVC, non overt cancer UEDVT had a younger mean age compared to the 26 patients (M:F=16:10) in the cancer group (44.9yrs vs 62.9yrs,  $p < 0.001$ ). The non-CVC, non overt cancer UEDVT group was composed of six (14%) patients with effort related UEDVT, nine (20%) patients with a preceding history of either surgery or trauma to the upper limb, and the majority 29 (66%) of patients with no clinically identifiable risk factor, apart from two patients on cOCP, and one pregnant woman. Dynamic venography was performed in 13 patients (4 effort thrombosis, 9 no risk factor group). Evidence of a significant thoracic outlet syndrome defined by occlusion to flow >90% on abduction was observed in 3 patients belonging to the effort related group only. Most patients in the non-CVC, non-cancer UEDVT group were successfully managed with anticoagulation with one recurrence at two months of cessation.

### Conclusion

The non-CVC, non cancer group was younger than the non-CVC, cancer UEDVT group. Positional venography was infrequently used but showed significant occlusion in 3/13(23%) of the patients. Anticoagulation therapy was successful in managing most patients without the need for lytic therapy or first rib resection.

**Keywords** UEDVT, anticoagulation, upper limb vein thrombosis.

**Conflict of interest** No

Wednesday 31 October

APSTH/ASTH Free Communications 5: Clinical Thrombosis

O136

0830-1000

Room 211

0930-0945

**Anti-Human VWF Monoclonal Antibody SZ-123 Prevents Arterial Thrombus Formation by Inhibiting VWF–Collagen and VWF–Platelet Interactions in Rhesus Monkeys**

Yiming Zhao, Miao Jiang, Shundong Ji, Yang He, Changgeng Ruan  
*Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, China*

**Aim**

The interactions between collagen, von Willebrand factor (VWF), and glycoprotein Ib (GPIb) are crucial for hemostasis and thrombosis. The aim of this study is to investigate the in vivo antithrombotic efficacy of an anti-VWF-A3 monoclonal antibody SZ-123 and its potential underlying mechanism.

**Methods**

Cyclic flow reductions (CFRs), an indicator of arterial thrombosis, were measured in the femoral artery of anesthetized monkeys before and after intravenous administration of SZ-123. Ex vivo VWF binding to collagen, platelet aggregation, platelet count and template bleeding time were performed as measurements of antithrombotic activity. In addition, plasma VWF, SZ-123 levels, and VWF occupancy were measured by ELISA. The effects of SZ-123 on the interaction of VWFA1 with A3 and on the binding of VWFA1 to a recombinant glycoprotein Iba (rGPIba) fragment (H1-V289) were detected using radioimmunoassay.

**Results**

Administration of 0.1, 0.3, and 0.6 mg/kg SZ-123 resulted in 45.3%, 78.2%, and 100% reduction in CFRs, respectively. When 0.3 and 0.6 mg/kg SZ-123 were administered, 100% of VWF was occupied by the antibody. Moreover, 100% ex vivo inhibition of VWF-collagen binding and 60-95% inhibition of platelet aggregation were observed from 15 min to 1h. None of the doses resulted in significant prolongation of bleeding time. In vitro experiment also revealed that SZ-123, but not 82D6A3 (a anti-VWF-A3 monoclonal antibody), significantly enhanced the binding of <sup>125</sup>I-rVWF A3 to rVWF A1 in a dose dependent manner. SZ-123 not only blocks collagen-VWF A3 interaction but also inhibits indirectly VWF A1 binding to GPIb induced by ristocetin.

**Conclusion**

SZ-123 prevents in vivo arterial thrombus formation under high shear conditions by inhibiting VWFA3–collagen and VWFA1–platelet interactions and does not prolong bleeding time. SZ-123 inhibits the binding of VWF A1 to GPIb by interfering in the interaction between VWF A1 and A3 domain.

**Keywords** Thrombosis; collagen; VWF; platelet; platelet adhesion; SZ-123

**Conflict of interest** No conflict of interest to disclose

Wednesday 31 October

APSTH/ASTH Free Communications 5: Clinical Thrombosis

O137

0830-1000

Room 211

0945-1000

## Long Term Follow Up of Homozygote Protein C Deficiency Following Multi Modal Therapy

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<sup>1</sup>The University of Melbourne, Department of Paediatrics, <sup>2</sup>Murdoch Children's Research Institute, <sup>3</sup>Department of Clinical Haematology, Royal Children's Hospital, <sup>4</sup>The University of Melbourne, Department of Nursing, <sup>5</sup>Department of Gastroenterology, Royal Children's Hospital, Melbourne, Victoria, Australia.

### Aim

Homozygous protein C deficiency is an extremely rare condition presenting in the neonatal period with purpura fulminans, ophthalmologic complications, as well as cerebral and renal thrombosis and is associated with very high rates of morbidity and mortality. Optimal treatment for this condition is highly complex, poorly understood, and, in many countries, still difficult to obtain for cost and supply reasons.

### Method

We report a clinical case report.

### Results

The child was born at term to consanguineous parents, who presented 2 days after birth with purpura fulminans, on both of his feet and buttocks. Further examination revealed bilateral severe retinal damage but normal brain imaging. Until protein C concentrate became available he was treated with fresh frozen plasma and unfractionated heparin, resulting in fluid overload. After surgical management of the original skin purpura, he was managed with a regime of daily warfarin (maintaining an INR 4-5). Clexane and protein C were used as salvage therapy, for recurrent episodes of purpura fulminans. During this time the patient's progress was complicated by glaucoma requiring bilateral lensectomies, and recurrent episodes of otitis media. This was continued until 2 years of age when daily subcutaneous protein C replacement therapy was used, before his liver transplant at 3 years of age. The patient is now 12 years of age, well, with blindness as his only long term deficit.

### Conclusion

This study is the first to report the sequential use of multimodal therapy, and follow up of a patient 10 years post successful liver transplant with normal levels of protein C antigen and activity. Furthermore, we describe here our use of lab parameters to guide the multimodal therapy.

**Keywords** Homozygote Protein C deficiency, multi modal therapy.

**Conflict of interest** No

Wednesday 31 October  
HSANZ Symposium 9: Scanning the Lymphoid Horizon  
New Treatments in MM

1100-1200  
Rooms 212/213

Philip McCarthy  
*Roswell Park Cancer Institute, Buffalo, NY, USA*

### Aim

New Treatments for Multiple Myeloma patients

### Results

There are several treatment options for multiple myeloma (MM) patients. Staging by LDH, albumin and  $\beta$ -2 microglobulin ( $\beta$ -2M), bone marrow metaphase karyotyping and fluorescence *in situ* hybridization allows for risk stratification for determining treatment. Radiographic imaging by skeletal survey +/- PET and MRI identifies bone and extramedullary involvement. Molecular tests such as BM gene expression profiling help with further risk stratification. Treatment is based on clinical factors such as age, performance status, organ involvement, disease risk and consideration for intensive therapy such as autologous hematopoietic stem cell transplant (HSCT). In the USA, MM induction regimens include older agents: melphalan (M) and glucocorticoids (prednisone (P) or dexamethasone (D)) and cyclophosphamide (Cy). Incorporation of novel agents into induction regimens has improved response rates and duration. Novel agents including the proteasome inhibitor (PI) bortezomib (B, V or P) and the immunomodulatory drugs (IMiDs) thalidomide (T) and lenalidomide (R or L) improved responses for transplant-eligible before HSCT and HSCT-ineligible patients. Older doublet therapies such as MP or TD may be replaced by newer doublets such as VD or Rd (low dose D). Triplet regimens such as RVD, PAD, CyBorD and VTD are appropriate regimens for HSCT-eligible patients. For the non-HSCT eligible patients, MPT, MPR, Rd and VD are appropriate treatments. Maintenance therapy with L and B are new standards to maintain response. There are several promising agents for relapsed and refractory (RR) disease. A new PI, carfilzomib has been approved for use in the US with activity especially in combination with R/D. Bendamustine combined with R/D is active in RR patients. Another PI, MLN 9708 is undergoing clinical testing. The new IMiD pomalidomide alone or combined with D is active for RR patients failing prior IMiD therapy. Antibody therapy against the MM marker CS-1, elotuzimab is undergoing clinical trial. The AKT inhibitor perifosine and the HDAC inhibitors panobinostat and vorinostat are being evaluated in RR MM patients.

### Conclusion

There are several approaches for MM therapy. The role of HSCT, single versus tandem HSCT, consolidation and maintenance have undergone and are undergoing clinical evaluation. Inducing and maintaining a complete response remains a long term goal of MM treatment.

**Keywords** Continuum of therapy for multiple myeloma

**Conflict of interest** Advisory board for Celgene, Speaker Janssen Pharm.

**Wednesday 31 October**  
**HSANZ Symposium 9: Scanning the Lymphoid Horizon**

**1100-1200**  
**Rooms 212/213**

## **New Developments in CLL Therapy**

Constantine S Tam

*St Vincent's Hospital and Peter MacCallum Cancer Centre, Melbourne, Vic,  
Australia*

Chemoimmunotherapy is currently standard treatment for CLL, and achieves excellent outcomes in younger patients. However, the outcomes for older patients and patients with high-risk genetic features remain inadequate, giving impetus to the development of novel treatment strategies tailored to the biology of the disease. In this session, I will cover the following "burning questions":

- 1) Which novel prognostic markers are really needed in real life ?
- 2) How do we salvage patients who relapse following frontline FCR ?
- 3) How do we deal with older and/or less fit patients ?
- 4) Should we be using MRD in clinical practice ?
- 5) How do we treat patients with 17p deletion ?
- 6) What promising immunotherapy approaches are on the horizon ?
- 7) What are BCR pathway inhibitors, and why will they revolutionize the way we treat CLL ?

**Keywords** Immunotherapy, novel agents, Bruton's tyrosine kinase

**Conflict of interest** Dr Tam receives honorarium from Roche Australia

Wednesday 31 October  
ASTH/APSTH Symposium 11: Haemophilia Update

1100-1200  
Room 211

## Phenotypic Heterogeneity in Severe Haemophilia - Implications for Individualised Therapy

Alok Srivastava  
*Christian Medical College, Vellore, India*

It has been long recognized that 10-15% of patients with severe hemophilia A (<1% FVIII activity) have clinically mild disease. The reasons for these differences have not been completely understood. Conventional markers associated with variations of phenotype include the level of clotting factor activity below 1%, type of mutation in the FVIII gene, presence of prothrombotic genetic markers and longer T<sub>1/2</sub> of administered clotting factor concentrates (CFC). The overall balance of haemostasis factors as reflected in the tests of global haemostasis have also been correlated with clinical phenotype. All these markers however pertain only to the haemostasis aspect of joint damage. We have observed in large numbers of patients receiving minimal quantities of CFC replacement therapy that phenotypic differences are not confined to the frequency of bleeding but also in the response within the joint to such bleeding in terms of synovial hypertrophy and cartilage damage. Some show extensive joint destruction with a few bleeds (5-10) while others maintain good joint space and surface even after many bleeds (>20). These differences in the extent of joint damage are likely to be related to variations in the inflammatory response to blood in the joint. Early data indicates that various cytokines involved in inflammation and angiogenesis such as MYC, MDM2, HIF1, VEGF, MMP9, SDF1, TNF $\alpha$ , IL1 $\beta$  and IL10 impact on these responses in joints. Polymorphisms in these genes may account for the inter-individual differences. Further work is needed in large numbers of patients to confirm these observations. However such studies are challenging for several reasons. There is no consensus on the definition of the mild phenotype. Most patients with factor levels <1% are initiated on prophylaxis early in their lives altering the natural history in a way that makes it difficult to study this phenomenon. The main aim of such a study though would be to develop a model of biological parameters that can predict the phenotype of an individual with severe haemophilia. With that it should be possible to move away from the approach of factor replacement therapy with uniform protocols for all people with severe haemophilia to a more individualized approach based on the personal requirements of each person. However, till such algorithms are worked out, an approach based on bleeding patterns and T<sub>1/2</sub> of CFC in each individual could be a good option.

**Keywords:** hemophilia, phenotype heterogeneity, replacement therapy.

**Conflict of interest:** No

**Wednesday 31 October**  
**ASTH/APSTH Symposium 11: Haemophilia Update**

**1100-1200**  
**Room 211**

**Update on Haemophilia Practice**

Chris Barnes

Abstract not available at time of going to print



Wednesday 31 October  
ASTH/APSTH Symposium 12: Trauma and DIC

1100-1200  
Room 210

## Trauma Bleeding Management: The Concept of an Early Goal Directed Coagulation Therapy

Herbert Schöchl  
*AUVA Trauma Centre Salzburg, Austria*

Coagulopathy resulting from bleeding causes approximately 40% of all trauma-related deaths and the need for effective treatment strategies to address this burden is clear. Recent military and civilian experience has increased the understanding of the pathophysiology of trauma-induced coagulopathy. The “blind” transfusion of allogeneic blood products is associated with the risk of inappropriate over- or under-transfusion and in potentially adverse outcomes. The usefulness of standard coagulation tests to both diagnose trauma-induced coagulopathy (TIC) and to subsequently guide hemostatic therapy has been recently challenged. There is increasing evidence that point-of-care (POC) viscoelastic monitors like rotational thromboelastometry [ROTEM®] or thrombelastography [TEG®], which assess whole-blood coagulation, provide a more comprehensive and rapid overview of the hemostatic capacity of trauma patients than standard coagulation tests. Consequently, some trauma centers now routinely use viscoelastic tests to guide coagulation therapy.

The early and aggressive high-volume administration of fresh frozen plasma (FFP), platelet concentrates (PCs), and red blood cells (RBCs), using ratio-driven massive transfusion protocols (MTPs), has been adopted by many for the treatment of TIC and hemorrhagic shock. However, the optimal ratio of RBC:FFP and RBC:PC is still under investigation. In some European trauma centers, hemostatic agents such as fibrinogen concentrate, prothrombin complex concentrates, and antifibrinolytics are integral parts of goal-directed MTPs. Both a ratio-driven coagulation therapy and a POC-guided coagulation management based on coagulation factor concentrate aim for the same target: the rapid prevention and treatment of shock and coagulopathy to prevent death from traumatic hemorrhage. This review compares the evidence relating to the efficacy and safety of the ratio-driven and goal-directed approaches to TIC, in order to draw attention to the potential benefits and draw backs associated with these management strategies.

**Keywords** Coagulopathy, trauma, risk stratification

**Conflict of interest** HS received speakers fees from CSL Behring and TEM international

Wednesday 31 October  
ASTH/APSTH Symposium 12: Trauma and DIC

1100-1200  
Room 210

## The Changing Tides of Fluid Resuscitation and Haemorrhage Control

Russell Gruen

*National Trauma Research Institute, The Alfred and Monash University*

Haemorrhagic shock is responsible for about a third of all trauma deaths, or 2 million deaths per year. Half occur before patients reach hospital. The management of haemorrhagic shock has transformed in the past two decades, from aggressive crystalloid fluid resuscitation, to limited volume replacement, and prevention or early management of coagulopathy. This has changed the focus of surgery from damage control to definitive surgery during the first operation for many patients. Improvements in topical haemostatic agents and interventional radiological techniques make them increasingly useful adjuncts to surgical control of bleeding. Better understanding of trauma-induced coagulopathy is paving the way for replacing the current blind, unguided protocols for blood component therapy with systemic treatments targeting specific deficiencies in coagulation. Similarly, personalised broad-based therapies targeting the dysregulated inflammatory response to severe injury are being investigated. As point of care diagnostics become more suited to emergency environments, haemorrhage control will continue to improve, resulting in better patient outcomes and reduced demand for blood products.

**Keywords** Haemorrhagic shock, coagulopathy, trauma

**Conflict of interest** No

Wednesday 31 October

1400-1530

ANZSBT/ASTH Combined Symposium: Reversing the Novel Anticoagulants Rooms 219/220

## Clinical Trials Update

Raymond SM Wong

*Prince of Wales Hospital, Hong Kong*

Novel anticoagulants, such as rivaroxaban and dabigatran etexilate, have been developed for the prevention of stroke and systemic embolism as well as the prevention and treatment of thromboembolism. A major disadvantage of these anticoagulants is the absence of an antidote in case of serious bleeding or when an emergency intervention needs immediate correction of coagulation.

Prothrombin complex concentrate (PCC), activated PCC, such as FEIBA and recombinant activated factor VII (rFVIIa) such as Novoseven® have received attention as potential prohemostatic agents for the reversal of the new oral anticoagulants. A few studies have evaluated their effects on various hemostatic parameters in healthy volunteers.

Eerenberg ES et al evaluated the potential of prothrombin complex concentrate (PCC) to reverse the anticoagulant effect of dabigatran and rivaroxaban in a randomized, double-blind, placebo-controlled study including 12 healthy male volunteers. PCC immediately and completely reverses the anticoagulant effect of rivaroxaban measured by prothrombin time and endogenous thrombin potential. On the other hand, PCC has no influence on the anticoagulant action of dabigatran measured by the activated partial thromboplastin time, ecarin clotting time (ECT) and thrombin time.

In another study, Marlu et al demonstrated that PCC correct rivaroxaban-induced impaired thrombin generation in a dose-dependent fashion. The effect of PCC on reduced thrombin generation after administration of dabigatran was less pronounced. In this situation, however, activated PCC and recombinant factor (F)VIIa had some, albeit relatively modest effect.

The evidence that these agents will indeed reduce blood loss and improve outcome in patients with bleeding induced by the new anticoagulants are lacking. Further studies are needed to confirm their clinical effects.

## References

1. Eerenberg ES et al. Circulation. 2011 Oct 4;124(14):1573-9.
2. Marlu R et al. Thromb Haemost. 2012 Jul 25;108(2):217-24

**Keywords** Dabigatran, rivaroxaban, bleeding

**Conflict of interest** Consultant and speaker for Bayer, research fund from Bayer

Wednesday 31 October

1400-1530

ANZSBT/ASTH Combined Symposium: Reversing the Novel Anticoagulants Rooms 219/220

## Laboratory Testing for the Novel Anticoagulants

Joanne E Joseph

*St Vincent's Hospital and University of NSW, Sydney, Australia*

### Aim

Although the novel oral anticoagulants (i.e. dabigatran, rivaroxaban and apixaban) do not require monitoring for routine purposes, there are specific clinical situations (e.g. patients presenting with adverse complications such as bleeding or thrombosis) where specific laboratory testing would be indicated and useful for patient management. Wherever possible, laboratory testing should primarily utilise tests that are able to be performed quickly and routinely and which can be implemented out-of-hours and in non-specialised laboratories.

### Results

Global coagulation assays including the activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) display varying sensitivities to the novel anticoagulants, but may be helpful in ruling out the presence of drug, particularly if they are all normal. With the increasing use of novel anticoagulants in clinical practice, experience is being gained in relation to their effects on these routine assays and they could be considered as screening tests. In addition, their effects on other coagulation tests are also being characterised. When an exact concentration of drug is required to be determined, specific testing with assays including the Hemoclot® Thrombin Inhibitor (e.g. for dabigatran) or a specific anti-Factor Xa chromogenic assay (e.g. for rivaroxaban) should be performed. Clinical examples from recent practice will be presented to highlight the utility of laboratory testing for these agents.

### Conclusion

Although not routinely required, laboratory testing has a role to play in the management of patients receiving novel anticoagulants.

**Keywords** Testing, Novel, Anticoagulants

**Conflict of interest** Nil

Wednesday 31 October

1400-1530

ANZSBT/ASTH Combined Symposium: Reversing the Novel Anticoagulants Rooms 219/220

## **Guidelines for Anticoagulant Reversal**

Huyen Tran

Abstract not available at time of going to print