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POSTER ABSTRACTS

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P001
Audit of Sickle Cell Disease (SCD) Patients in Sydney. (A Transfusion Laboratory Based Perspective)

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Aim
SCD patients in Sydney are increasing due to changes in migration patterns. We aimed to describe the characteristics of SCD patients in Sydney and demonstrate the phenotypic discrepancy between the red cells of SCD patients and the donor population.

Method
We conducted a retrospective analysis of all SCD patients who were referred to the Blood Service (ARCBS) or Westmead Hospital from 1984 to April 2011 for either supply of red cells or phenotyping. Data on age, sex, treating institution, blood group, RBC phenotyping, transfusion history and development of antibodies were collected from the ARCBS and computerised hospital blood bank records.

Results
84 patients were identified. Compared with the donor population, the phenotypic discrepancies are widest in the Rh, Kidd, Duffy and Kell systems. The frequency of the Dce phenotype was 21% compared to 1.7% in the donor population. Alloimmunization to the Kidd system is mainly against Jkb rather than Jka, likely due to the lower frequency of Jkb- of 26.3% compared to 73.6% JKb+ in the donor population. On the other hand, the Jkb- is higher at 36.5% in the SCD patient’s cohort than the donor population. Subsequently the chance of transfusing Jkb+ to Jkb- SCD patients is high. Another contributing factor is the practice of matching Jka rather than Jkb. In the Duffy Blood Group, the discrepancy is large, especially for Fy (a-b-). Fortunately SCD patients lacking a Duffy antigen usually express it on endothelial cells and so do not develop alloantibodies.

Conclusion
The phenotypic discrepancies of the Australian donor and the SCD patients revealed a very significant problem which is the relative shortage of donors with a red cell phenotype similar to the SCD population. Recruiting more donors from ethnic groups that mirror the SCD patients in Sydney is important, so there is phenotypically similar blood in the donor pool.

No conflict of interest to disclose
P002
Materno-Fetal ABO Incompatibility and the Risk of Maternal Alloimmunization to Non-D Fetal Antigens

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Background
Materno-fetal ABO incompatibility is known to reduce the risk of maternal alloimmunization to the fetal D antigen. We hypothesized that this might also reduce the risk of maternal alloimmunization to fetal non-D RBC and non-RBC antigens.

Methods
We performed a retrospective, cross-sectional, New Zealand-based study using convenience samples where the proportions of the ABO groups among parous D-negative women with anti-D, parous women with antibodies to non-D RBC antigens and males with RBC antibodies were compared. We also carried out a case-control study of HLA / HNA antibody presence or absence in parous women of groups O and A.

Results
Statistically significant differences were found between observed and expected ABO frequencies in parous women with anti-D alone (X², 11.27; P, 0.01) and in parous women with antibodies to non-D RBC antigens (X², 10.98; P, 0.01). This was not found in males with RBC antibodies (X², 6.53; P, 0.08). The proportions of groups O and A parous women among those with or without HLA / HNA antibodies were similar giving a non-significant OR of 0.85, (95% CI = 0.53 to 1.35) and the parity in these women was essentially similar (means of 2.59 and 2.85 respectively; SE = 0.20; z = 0.45; 2 sided P, 0.6).

Conclusions
As with pregnancy-related alloimmunization to the D antigen, in certain populations, group A women, because of the lesser likelihood of ABO incompatibility in the maternal-to-fetal direction, may also be more prone, than group O women, to alloimmunization to fetal non-D RBC antigens. This effect is not seen in males with RBC antibodies or in parous women with HLA / HNA antibodies.

No conflict of interest to disclose
P003
The Sticky Business of Compatibility Labels on Blood Bags

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During our last NATA inspection the transfusion auditor noted that we apply our compatibility label directly to the surface of the blood bags and enquired after the validation evidence for the adhesive – not an unreasonable request. We have been using the existing labels since we purchased and installed LabTrak LIS on December 31st, 1999. I was aware of the discussions and compliance requirements and appropriate validations at the time and understood that all of those requirements were met. However, the documentation was nowhere to be found!! – Dilemma!
We now had to embark on a revalidation of the labels – no problem/useful exercise! The exhaustive search for the actual requirements and then appropriately certified labels proved more challenging than we expected, but we eventually arrived at the desired outcome. We satisfied the NATA requirement and now have an approved and certified label which we can confidently apply to the surface of our bags.

No conflict of interest to disclose
P004
Counting the Cost of Platelet Transfusions

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Background
Platelet transfusion is a very common event in Australian hospitals, both for prophylactic and therapeutic indications. The complexity of the multi-step process contributes to the potential for problems and adverse events at each step. Comprehensive platelet transfusion-associated costs are not available in Australia.

Aims
(1) To examine the costs of the process involved in collecting and delivering a platelet transfusion to a patient. (2) To understand the complexities of laboratory and clinical transfusion processes and risks and inform development of strategies to manage these risks.

Method
This study is underway at Peter MacCallum Cancer Centre (PMCC) and Flinders Medical Centre/ SA Pathology (FMC), for adult non-trauma patients, using the transfusion process map methodology developed for the Australian red cell costing study. Maps were modified and validated by timing every aspect related to transfusion of a single unit (dose) of platelets. De-identified, aggregate transfusion episode data were extracted from clinical/laboratory sources and personnel/financial data provided by hospital administrators.

Results
Thirty nine major processes are being mapped for all aspects of platelet transfusion. These processes are complex, involving many steps and staff. During 2009-10 a total of 3734 platelets were transfused (1581 FMC & 2153 PMCC), with 133 being HLA-compatible units. Eleven transfusion reactions were reported. Additional processes included management of 101 product recalls, 34 of which related to bacterial screening results.

Conclusion
The transfusion process is complex, time-consuming and involves many individuals and resources. Preliminary financial data indicates the total cost of the transfusion event is substantially greater than currently the cost of production of the unit. Understanding the real costs related to the transfusion process can encourage better transfusion practice with the use of alternatives, where appropriate, to optimise platelet utilisation, improve patient outcomes, and reduce exposures, risks and costs of unnecessary transfusions.

No conflict of interest to disclose
P005
Update on Bacterial Pre-release Testing of Platelets - The Australian Red Cross Blood Service Clinical Experience

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Aims
To describe clinical and logistical aspects of routine bacterial contamination surveillance screening of platelets since introduction.

Methods
Five Blood Service testing laboratories use the BacT/ALERT® 3D automated microbial detection system. Closed system sampling (15-20mL) from each platelet component occurs at 24h; samples are inoculated for aerobic and anaerobic culture. Platelets are released ‘negative to date’ while culture continues over component shelf-life. Initial machine positive (IMP) and all follow up results are notified to transfusing laboratories. Communication and education was undertaken to support introduction. Following implementation (late April 2008 – October 2010) Blood Service transfusion medicine staff provided clinical follow up of cases where transfusion had occurred prior to IMP notification.

Results
Clinician feedback showed understanding of the clinical rationale for bacterial screening and follow up. Initial concerns related to clinical scenario management and potential workload for laboratories and clinical staff. Of 302 386 platelet components screened, there were 3207 (1.06%) IMP notifications of which 1041 (32.5%) had related components transfused; 1060 platelets or their associated components [red cells, plasma]. Of all screened platelets, 550 were confirmed positive/indeterminate (0.18%). In 154 (28%) of these the organisms were deemed clinically significant (not Propionibacterium species). Transfusion was prevented in 57% of these cases due to early notification. Sole anaerobic yield of significant organisms was 2% (64/3207) including two confirmed positive cases of Clostridium perfringens where transfusion was prevented. Transfusion occurred in 76% (300/396) of Propionibacterium species (confirmed/indeterminate), with a mean time to detection of 4.2 days. Cases of septic transfusion reaction have declined since the introduction of bacterial pre-release screening of platelets

Conclusion
Bacterial contamination screening of platelets continues to provide an important improvement in transfusion safety in Australia. A reduction in septic transfusion reactions has occurred, however screening has contributed to high workload in detection and notification to health care facilities.

No conflict of interest to disclose
P006
Multicolour Real Time PCR Genotyping of Human Platelet and Human Neutrophil Antigens

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Aim
Human platelet (HPA) and human neutrophil (HNA) antigen genotyping is valuable when investigating alloimmune thrombocytopenia and neutropenia, or transfusion reactions. We have used our existing platform (Corbett Rotor Gene 3000) to develop multicolour real time PCR (RT-PCR) assays for HPA and HNA genotyping using 5'-Nuclease (Taqman™) techniques. These assays are faster and more economical than our current RT and sequence-specific primer (SSP) PCR methods.

Method
Most HPA systems are biallelic, resulting from a single nucleotide polymorphism (SNP). By combining two PCR reactions utilising different probe reporter dyes, two HPA systems can be genotyped in one assay. Using this approach, we developed an assay that simultaneously genotypes HPA-2 and 3, and another for HPA-5 and 15, using Fam, Rox, Hex and Cy5 labelled probes. HNA-1 is a triallelic system which results from six SNPs located on the gene that encodes for the human neutrophil FcγRIIIb. Using probes which target three different SNPs, we developed a RT-PCR assay which simultaneously genotypes all three alleles in the HNA-1 system from one PCR product. This design overcomes homology that exists between the FcγRIIIb and FcγRIIIa genes, specifically at the nucleotides distinguishing the HNA-1 alleles.

Results
100 samples tested by each assay demonstrated 100% correlation with our validated, routine methods. The HPA multicolour RT-PCR assays reduced common reagent costs, assay run time and labour by approximately 50%. The HNA-1 multicolour RT-PCR assay reduced a procedure that required two separate SSP-PCR assays and gel electrophoresis, into one 2 hour assay. In addition, preliminary data indicates the HNA-1 assay has increased resolution, detecting hemizygotes as well as genotypes consisting of three FcγRIIIb gene copies.

Conclusion
Using different approaches of multicolour real time PCR, we have been able to design assays for HPA and HNA genotyping which are fast, economical, and sensitive.

No conflict of interest to disclose
P007
Effect of Whole Blood Hold Conditions Prior to Processing on the Accumulation of Microparticles in Fresh Frozen Plasma and Cryoprecipitate

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Aim
Microparticles (MPs) are small phospholipid vesicles shed into the plasma from all types of blood cells, namely platelets, red blood cells (RBCs), leucocytes and endothelial cells. MPs are markers of cell injury and apoptosis known to be involved in various biological activities, including thrombosis and inflammation. Fresh frozen plasma (FFP) and cryoprecipitate transfusion products are prepared from whole blood (WB) donations. WB-holding conditions prior to processing can vary both in time and temperature. It was hypothesised that variations in WB-holding conditions may influence the number and cell-derivation of MPs in FFP and cryoprecipitate, and may be of potential clinical relevance. This study aimed to investigate the effects of different WB-hold conditions on the numbers and types of MPs in FFP and cryoprecipitate.

Methods
Standard WB units from healthy donors were divided into four paediatric-size blood packs and held for nominated holding periods (6 h, 18 h and 24 h) at room temperature (RT) or refrigerated. Subsequently, FFP and cryoprecipitate were prepared according to standard blood bank protocols. The cellular source and the absolute number of MPs were determined by flow cytometric absolute bead count assay using cell-specific fluorochrome-labelled antibodies.

Results
Platelet-derived MPs were the most abundant in FFP and cryoprecipitate, followed by RBC-derived MPs. The total numbers of MPs in cryoprecipitate were significantly higher (p<0.05) than in FFP. Duration of WB-hold or WB storage temperature (i.e. RT or refrigerated) prior to processing had no statistically significant effect on the number of MPs.

Conclusions
WB holding time up to 24 h and WB storage temperature (i.e. RT or refrigerated) prior to processing did not have a significant effect on the number of MPs in FFP and cryoprecipitate. Further studies will determine the extent of donor variability on the numbers and types of MPs in FFP and cryoprecipitate transfusion products.

No conflict of interest to disclose
P008
How Appropriately is Blood Ordered in a Rural Hospital?

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Background and Aim
Blood products are a precious healthcare resource and appropriate use is important to maintain patient safety and minimise costs. However, unnecessary cross-matching and ordering of blood products still occurs. Such practice is of particular concern in a rural setting, where clinicians may have limited access to blood products. This study assessed the appropriateness of transfusion practices in a rural hospital.

Method
A retrospective audit of packed red blood cell (PRBC) use at Wimmera Base Hospital, a rural hospital 300km northwest of Melbourne was undertaken. All blood cross-match orders and PRBC transfusions from October 2010 to March 2011 were audited to assess the appropriateness of cross-match requests. Evidence-based hospital clinical pathways incorporating protocols on blood transfusion were also reviewed. Transfusion indices were calculated and statistical analysis was performed using the chi-squared test.

Results
Blood was cross-matched for 257 patients, and 657 PRBC units were cross-matched during the study period. 28.4% of these patients had pre-procedure (elective) cross-matches. 27.4% of elective cross-matches were inappropriate, compared with 16.1% of emergency cross-matches. The cross-match to transfusion ratio (C:T) for emergency requests was 1.59, compared with 5.96 for elective requests. This was notably the case in the surgical and obstetrics and gynaecology departments with a C:T ratio of 5.2 and 10.3 respectively. 16.3% of all transfusions were single-unit transfusions.

Conclusion
Emergency requests were predominantly appropriate and had a low C:T ratio. In contrast, a significant proportion of elective requests were inappropriate, particularly for certain surgical and obstetric procedures. These requests tended to correlate with a high C:T ratio, above the widely accepted level of ≤2:1. This may indicate the need for refinement of existing protocols governing elective crossmatch requests, and review of clinical education for healthcare professionals regarding ordering blood in a rural setting.

No conflict of interest to disclose.
P009
Red Cell Use in South Australia (SA) 2007-09: Trends, Patterns and a Focus on Haematology

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Aim
To review local red cell use, with a particular focus on Haematology Specialty-related groups (SRGs), to gain a greater understanding of the drivers of clinical usage and to assist in resource planning.

Methods
A linked electronic database developed for SA public sector containing clinical, epidemiological and transfusion data was used. Diagnostic-related group (DRG), principle diagnosis (ICD-10), admission details (including day cases) and red cell transfusions in patients admitted to SA public hospitals were analysed for three financial years (FY) up to 30/6/09.

Results
A total of 43,707 red cells units were transfused in SA in FY 2008-09, an 8% increase compared to 2006-07. The main contributors to the rise in blood use over the three year period (adjusted for activity and case mix) were haematology and gastroenterology; whilst surgical uses of red cells had decreased.
In FY 2008-09, the Haematology SRG was responsible for 27% of red cells transfused in the SA public sector, with a 15% increase in use (10.4% increase in malignant haematology and 6.5% in non-malignant haematology) compared to FY 2006-07.
Malignant haematological diagnoses used more than half of the red cells within the haematology SRG. The mean number of units per admitted patient was greatest for MDS (9.8), AML (9.2), ALL (5.2), and Multiple Myeloma (2.8). A principle diagnosis of iron deficiency anaemia (IDA) was the main source of red cell use in non-malignant haematology, however this is probably an underestimate as this condition may be coded as an additional rather than primary diagnosis or may go undetected.

Conclusion
Red cell use in SA is increasing in medical patients, both overall and in haematology. National Patient Blood Management Guidelines and programs for medical patients will help to ensure effective use of blood with an aging population and optimise patient outcomes.

No conflict of interest to disclose
A Retrospective Analysis of the Incidence of Platelet Specific Alloantibodies in Foetal Ventriculomegaly

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Aim
A retrospective analysis to determine the incidence of platelet specific alloantibodies in cases diagnosed with foetal ventriculomegaly on ultrasound/ MRI. Cases of hydrocephaly, porencephaly and intracranial cysts were included.

Methods
Cases of foetal ventriculomegaly investigated for platelet specific alloantibodies at the Sydney Processing Centre were studied over a period of 21yrs (1989-2010). The patients were referred from foeto-maternal medicine units at tertiary hospitals from NSW/ACT. Cases diagnosed with ventriculomegaly, hydrocephalus, porencephaly, cyst, or echogenic areas were identified from the FMAIT database. Cases with diagnosed intracranial haemorrhage and stillbirth were not included; however cases with doubtful but not confirmed cases of haemorrhage were considered in the study. Platelet specific antigens were studied using the Solid Phase Red Cell Adherence test and Monoclonal Antibody Immobilisation of Platelet Antigens (MAIPA). Platelet Genotyping and HLA typing for Class I and II were performed in most cases.

Results
A total of 120 cases were studied in the retrospective analysis. One case was diagnosed with the presence of anti-HPA-1a and another case with weak anti-HPA15a. A weak anti-HPA 3a reaction was seen in one case with platelet membrane glycoprotein (GP) IIb/IIIa isolated from HPA3aa and HPA 3bb panel platelets. Anti-HPA 5b could not be excluded in another instance as non-HPA specific maternal antibodies were detected to platelet membrane (GP) Ia/IIa isolated from panel and paternal platelets.

Discussion
Whilst some cases of ventriculomegaly were associated with platelet specific alloantibodies, there were no cases with Foetal-maternal Alloimmune Thrombocytopenia (FMAIT). The value of testing for platelet specific alloantibodies in cases of ventriculomegaly in the absence of a history of FMAIT or intracranial haemorrhage is now under review.

No conflict of interest to disclose
P011
Review One Year after Implementation of a Massive Transfusion Protocol (MTP)

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A number of studies involving trauma patients with massive bleeding have demonstrated lower mortality when a higher ratio (i.e. 1:1) of plasma to red cells is transfused. Severe trauma is not infrequently associated with a significant coagulopathy (prior to any intervention) and the earlier administration of plasma improves patient outcomes. In New Zealand many hospitals have implemented a massive transfusion protocol (MTP). Activation of the MTP prompts the blood bank to prepare components for a patient, with dispatch of blood components that provide a 1:1 ratio of resuspended red cells to fresh frozen plasma (FFP).

The MTP was implemented in Wellington Regional Hospital in May 2010. Over the first 12 month period the MTP was activated for 37 patients. Four patients died from haemorrhage: two from abdominal aortic aneurysm and two from gastrointestinal bleeding. Only 32% of patients received 10 or more units of red cells. The average number of red cells transfused was 8.6 units (range 1 – 30 units). The average number of plasma transfused was 6 units (range 0 – 24 units). The overall ratio of transfused FFP:red cells was 0.8 (range 0 – 1.7). Increased wastage of blood components was observed over the 12 month period. Wastage associated with MTP activations totalled 139 units (29% red cells, 48% FFP, 21% cryoprecipitate and 2% platelets).

The MTP is popular with clinicians. Aside from the increase in blood product expenditure, it poses a number of challenges. There is a need to promote education about patient assessment and utilizing the MTP appropriately. In addition better communication between the clinicians and the blood bank should be encouraged, in particular to notify when the MTP is ceased and promptly return any unused blood components to the blood bank, in order to minimise wastage.

No conflict of interest to disclose
Use of ROTEM® to assess Haemostatic function as part of a Comprehensive Patient Blood Management Program

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Aim
Management of surgical and non-surgical bleeding requires accurate, rapid detection of specific coagulation disturbances. Whole-blood rotational thromboelastometry (ROTEM®) is thought to provide a continuous qualitative haemostatic profile with rapid turn-around of results. ROTEM® was utilised at Fremantle Hospital to assess its effectiveness in directing blood product usage, in the setting of a Patient Blood Management Program.

Method
Thromboelastometry is a whole blood assay performed to evaluate the viscoelastic properties during blood clot formation and lysis. 150 bleeding patients were assessed using INTEM (tissue factor triggered intrinsic) pathway, EXTEM (Ellagic acid triggered contact factor pathway), and FIBTEM (Ellagic acid + Cytochalasin D), with a total of 368 ROTEM® profiles performed. Results were compared to the manufacturer’s suggested reference ranges.

Results
Samples were received from a variety of cases, predominantly Cardiac surgery, General surgery, Haematology, GI bleeds and trauma.
ROTEM® results were incorporated into the clinical decision algorithm with regard to prescription and dispensation of various blood products.
Data collected as part of the WA Patient Blood Management Program (WAPBM) has shown a significant decrease in use of FFP, red cells and platelets, with an increase in use of cryoprecipitate over this time.

Conclusion
Whilst the implementation of various strategies at Fremantle Hospital by the WAPBM has resulted in a reduction in blood product use, some of the shift in type of product used may be directly attributable to the use of ROTEM®.

This research was supported by HaemoView Diagnostics P/L and TEM International. The companies had no role in analysing the data or preparing the abstract.
P013
Seroprevalence of Ross River Virus and Barmah Forest Virus among Blood Donors during Favourable Climatic Conditions: Data for an Evaluation of Current Management Strategies

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Introduction
Ross River Virus (RRV) and Barmah Forest Virus (BFV) are two endemic Australian mosquito-transmitted viruses. The Australian Red Cross Blood Service (Blood Service) manages the risk of transfusion-transmission of RRV and BFV through a total restriction from individuals diagnosed with RRV for four weeks and BFV for two weeks, followed by a 12 month plasma-only restriction. For RRV and BFV infection can range from asymptomatic to a debilitating arthritis. Increased rainfall is associated with increased rates of infection for both viruses. During the first six months of 2011 there was high rainfall and flooding in Queensland. This study aimed to investigate the seroprevalence of RRV and BFV in blood donors from regions affected by the climatic events of 2011 that favoured RRV and BFV transmission.

Method
Samples were collected from donors in regions affected by high rainfall in early 2011. Theses samples were tested for anti-RRV and anti-BFV IgM antibodies, an indication of recent exposure to RRV or BFV, with ELISA-based assay kits.

Results
Analyses revealed that 30/1000 (3 %) (95% CI: 2.81-3.17 %) of donations collected from Townsville from the 7th February- 30th March were repeat reactive for anti-RRV IgM. Separate donations 3 out of 1000 donors (0.3%) (95% CI: 0-0.62 %) were repeat reactive for anti-BFV IgM. During this same February-March time period there were 148 cases of RRV and 112 cases of BFV reported in the Queensland Health database.

Conclusions
This study provided an estimate of the numbers of donors not deferred under current guidelines but with evidence of recent exposure to RRV or BFV. To fully define risk, nucleic acid amplification testing for viral RNA is required. Serological testing complemented by investigations for viral nucleic acid would allow the Blood Service to evaluate current RRV and BFV risk management strategies and transfusion transmission risk modelling.

No conflict of interest to disclose

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Background
In Australia, dengue fever is episodic in northern Queensland, where outbreaks occur seasonally. One of the largest epidemics in at least 50 years took place in 2008/2009, with over 1,000 confirmed clinical cases. This epidemic covered two separate outbreaks; one in Cairns (and surrounding regions; DENV-2,3,4 08-09) and a second in Townsville (DENV-1,3 09). This study examined the change in dengue virus IgG sero-prevalence in blood donors in North Queensland during this epidemic.

Methods
1,799 plasma samples collected at the beginning and end of the outbreak in Cairns and Townsville and 457 from Melbourne were tested for anti-dengue IgG by ELISA. Confirmatory testing was performed at a reference laboratory.

Results
The percentage of donations reactive for anti-dengue IgG antibodies in North Queensland was 10.1% (95% CI:8.7–11.5%), while the proportion of Melbourne donations reactive was 6.8% (95% CI:4.5–9.1%). The proportion of donations in North Queensland collected at the beginning of the outbreak (n=901) with anti-dengue IgG antibodies was 9.7% (95% CI:7.7–1.7%); the proportion at the end of the outbreak (n=898) was 10.6% (95% CI:8.6–12.6%). In both Cairns and Townsville, the proportion of IgG reactive donors increased with age, indicating cumulative exposure to DENV during this epidemic, or during previous outbreaks; this pattern was not observed in donors from Melbourne.

Summary/Conclusions
This study demonstrated that the 2008/2009 dengue epidemic did not raise the population prevalence of IgG in the North Queensland donor population and suggested that the epidemic may not have been as large as initially thought. This approach is likely to offer valuable insights on the impact of dengue epidemics on a population wide basis. Knowledge of prevalence of anti-dengue IgG antibodies in the population is important as it provides a measure of the population's vulnerability to Dengue Haemorrhagic Fever, which is more common in secondary infections.

No conflict of interest to disclose
P015
Preliminary Evaluation of a New Rapid Assay for Detection and Quantitation of Fetomaternal Haemorrhage

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Aim
Fetomaternal haemorrhage quantitation (FMH) is currently performed using Millipore anti-HbF FITC or Millipore anti-D FITC. The current anti-D FITC FMH quantitation method is rapid, however, weak D red cells with <1,000 RhD sites are not detectable but the FMH quantitation using anti-HbF FITC is labour intensive and time consuming. The objective was to evaluate the use of Trillium QuikQuant, an anti-HbF FITC based method which utilises propidium iodide for the exclusion of nucleated cells, and has a rapid assay time, for the establishment of a single platform for FMH quantitation in the antenatal and postpartum setting.

Method
A total of 48 antenatal and postpartum samples were used in the preliminary evaluation of the Trillium QuikQuant; 26 samples were compared with Millipore anti-HbF FITC and 22 samples with Millipore anti-D FITC. Blood from a male D-negative adult was spiked with varying amounts of D-positive fetal red cells between 0% and 7.8% and the samples analysed on the BD FacsCanto flow cytometer using 100,000 events.

Result
The correlation coefficient of the Trillium QuikQuant anti-HbF FITC with Millipore anti-HbF FITC and Millipore anti-D FITC was 0.99; the P value was 0.2 and 0.4, respectively, suggesting no significant departure from linearity.
The correlation coefficient of the spiked samples using Trillium QuikQuant anti-HbF was 0.99
In our hands the estimated assay time to analysis for the Trillium QuikQuant anti-HbF was approximately 35 minutes. This compares with approximately 90 minutes for the Millipore anti-HbF FITC.

Conclusion
To ensure adequate and timely prophylaxis, the detection and accurate quantitation of fetal red cells in maternal blood samples within 72 hours of a sensitising event, is essential. The rapid assay time to analysis, of the Trillium QuikQuant anti-HbF FITC, facilitates more frequent testing, improving the turn-around times for this assay. The preliminary evaluation indicates that this new method would be suitable, in our laboratory, as the single platform for quantitation of fetal cells in both the antenatal and postpartum setting.

No conflict of interest to disclose
P016
Blood Transfusion ‘WB1T’ Survey

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Background
There is an estimated frequency of 1 in 2000 ‘Wrong Blood in Tube’ (WB1T) incidents, resulting in possible incorrect therapy, including potentially fatal incompatible blood transfusion. National standards stipulate strict patient identification procedures and specimen labelling requirements, yet WB1Ts still occur. These errors are often detected in pathology laboratories when discrepancies with historical results are investigated. Many factors contribute to such errors, especially the failure to positively identify the patient and to label the tubes at the bedside. Many pathology laboratories have introduced additional requirements in an attempt to minimise WB1Ts.

Method
A survey was sent to a wide cross section of private and public laboratories in Australia, with the aim to analyse the approaches used in detection and prevention of WB1Ts and thereby to possibly identify potential improvement in practice in this area. The survey included questions to ascertain points of difference in practice, i.e. the use of Bradma labels, designated pathology collection staff, specific blood transfusion laboratory request forms, ‘zero tolerance’ practices and other novel strategies.

Results
22 respondents from both public and private pathologies gave an average of 2,123 specimens collected per month with a total of 56 WB1Ts in the last 2 years (1 in 910 samples) and 122 WB1Ts in the last 5 years (1 in 1044 samples).

No conflict of interest to disclose
P017
Iron Studies and Red Cell Transfusion in Cardiothoracic and Orthopaedic Surgical Patients: A Retrospective Audit at a Tertiary Hospital

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Aim
Preoperative diagnosis and treatment of anaemia is important to minimise adverse postoperative outcomes. This audit reviewed red cell transfusion practice, the degree of anaemia, the presence of iron deficiency anaemia (IDA) and anaemia of inflammation (AI) in cardiothoracic and orthopaedic surgical patients who had available iron studies.

Method
178 consecutive cardiothoracic and orthopaedic surgical patients with available iron studies were retrospectively reviewed.

Result
Only 36.5% patients had preoperative iron studies. However, 63.2% males and 45.3% females with postoperative iron studies presented with anaemia at admission. 38.5% of patients with preoperative iron studies had AI; 21.5% had IDA; 23.1% normal. For patients with iron studies requested within the first two postoperative intervals (≤5 days and 6 ≤10 days) 73.8% and 63.6%, respectively had AI; few had classical IDA or were normal. 51.5% patients transfused post-surgery had a discharge Hb ≥ 110g/L. Restricting the discharge Hb to 90g/L or 100g/L may have eliminated post-surgical transfusion in 14.8% to 42.6% patients.

Conclusion
Iron studies were more commonly requested postoperatively despite many being anaemic at admission. A higher proportion of patients with postoperative iron studies had AI and few had classical IDA or normal iron parameters, suggesting a transient inflammatory effect of surgery. This may mask underlying IDA or normal iron parameters and affect treatment. Preadmission assessment, including iron status, should be emphasised, allowing diagnosis and correction of pre-surgical anaemia with treatment modalities other than red cell transfusion. In the post-surgical setting consideration of a restrictive transfusion regimen sufficient to alleviate a patient’s clinical symptoms would ensure that this valuable resource is appropriately used.

No conflict of interest to disclose
P018
Outcome of Thrombotic Thrombocytopenic Purpura with Partial Exchange Transfusion in a Resource Limited Setting: A Single Center Experience

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Aim
Thrombotic thrombocytopenic purpura (TTP) is a medical emergency where prompt plasma exchange could reduce mortality. In Myanmar, because of economic sanctions, appropriate bags for apheresis machines cannot be imported and TTP has to be managed manually. This study is aimed to evaluate the efficacy of exchange transfusion in TTP for application in resource limited settings.

Method
Cases of TTP from the Clinical Hematology Department of Yangon General Hospital, the main tertiary center, during June 2009 to June 2011 were reviewed retrospectively for their outcomes. Diagnosis was made in the presence of microangiopathic hemolytic anemia having a minimum of three schistocytes in peripheral blood film under high power field with thrombocytopenia in adults without an apparent alternative cause. Once diagnosed, exchange transfusion was carried out through central venous catheter, removing 500-1000 ml of blood each time, replaced with an equal volume of fresh frozen plasma (FFP) in addition to crystalloid. Haemoglobin (Hb) was maintained above 7 g/dl by packed cell transfusion. The exchange was done twice a day if necessary. All cases received IV methyl-presnisolone 1000mg/day for 3 days followed by oral steroids.

Results
There were 5 cases at a mean age of 45.6 years (range 27-60) with a female to male ratio of 1.5:1. Two female patients had autoimmune disease, one male patient had a history of taking clopidogrel, and the rest were idiopathic. ADAMTS 13 activity was measured in 3 cases which were reduced to < 3% in two and 28% in one. Platelet response was observed between day 3 to 5 and all achieved complete platelet recovery within 3 weeks after an average of 16.3 (range 5-25) procedures. One patient developed exacerbation soon after stopping exchange-required cyclophosphamide.

Conclusion
Without equal volume plasma exchange recommended by guidelines, this partial manual exchange achieved comparable results and could be applied for the management of TTP in the less well-equipped hospital settings.

No conflict of interest to disclose
P019
Clinical Significance of Alloantibodies to Hybrid Glycophorins

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Background
MNS system antigens are expressed on Glycophorins A and B which are under the genetic control of two, similar genes. Genetic recombination events between these two genes frequently occur and a number of hybrid glycophorins bearing novel antigens have been described. The GP.Mur phenotype is the most common hybrid glycophorin and alloantibodies directed against neoantigens associated with this phenotype have been implicated in both Transfusion Reactions and Hemolytic Disease of the Fetus and Newborn (HDFN).

Aims
To examine the literature and conduct a meta-analysis of all published cases involving antibodies to MNS system hybrid glycophorins. Cases were analysed for antibody specificity, antibody class and severity of disease.

Results
60 years of case reports yielded 35 published cases. Cases of both Transfusion Reactions and Hemolytic Disease of the Fetus and Newborn were found in the literature. In all cases where antibody class and titre are known there is a strong association between the presence of high titre IgG antibodies and clinical disease. No cases were found which implicated IgM antibodies as being causative of morbidity. At least 40% of cases resulted in severe disease.

Conclusion
Strongly reactive IgG antibodies were present in all clinical cases and were always present in severe cases. In 89% of cases anti-Mi\textsuperscript{a}, anti-MUT and/or anti-Mur were reported. HDFN in some cases showed features of severe hyporegenerative anemia. Screening for these alloantibodies with cells expressing hybrid glycophorins is complicated by the frequent occurrence of clinically insignificant IgM examples, resulting in a considerable increase in laboratory workload. The recent description of kodecytes bearing two of the main GP.Mur neoantigens (MUT and Mur) and which are insensitive to IgM antibodies offers an alternative means of detecting clinically significant IgG examples of these alloantibodies.

This research was supported by CSL Limited. The company is a licensee of KODE™ technology.
P020
The Roll-out of a National Online Blood Ordering System (BloodNet) Across Australia

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Aim
The aim of BloodNet is to simplify and standardise the ordering and receipting of blood in public and private laboratories in Australia. This will assist to optimise product availability for patients and to improve inventory management and financial accountability by all players within the blood sector.

Method
The National Blood Authority (NBA) undertook a Proof of Concept Trial in 2010 to test BloodNet’s benefits including user feedback, technical enhancements required and strategies for implementation on a national scale. The trial was designed with the support of hospitals, state health departments and the Blood Service and was implemented in 79 hospital-based blood banks in Queensland, Tasmania, South Australia and Victoria.

Result
An evaluation of the trial found high user satisfaction, with 100% of participants wanting to retain BloodNet after the trial and 85% rating the system as either very good or good. In December 2010, the NBA was tasked with implementing BloodNet nationally. It is now operating in all facilities in Queensland, Tasmania, South Australia and the Northern Territory. Feedback from these sites is that BloodNet’s implementation was straight-forward and it has provided many benefits within laboratories. As at 30 June 2011, it was scheduled for implementation in WA, ACT and a large area health service in NSW. Discussions continue in Victoria to determine a timeframe for roll-out. New modules to capture fate and all other products are now being developed.

Conclusion
The national implementation of BloodNet replaces a myriad of systems previously used across laboratories for ordering and receipting blood. It has been proven to assist in better aligning demand with supply to ensure patients have access to required products. It also provides reliable and consistent data at a local, state and national level, meeting the original aims of the roll-out.

No conflict of interest to disclose.
P021
Inactivation of Transfusion-Transmitted Vector-Borne Pathogens

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Background
Several blood-borne pathogens are primarily transmitted to humans by insect vectors, including mosquitos, ticks, reduvid bugs, sandflies and mites. These pathogens are problematic in transfusion medicine because donors may be frequently exposed to the vectors and infections are frequently asymptomatic, rendering both donor screening questions and physical findings insensitive. The INTERCEPT Blood System™ (IBS) for pathogen inactivation of platelet and plasma components was developed to prevent transfusion-transmitted (TT) infections. This proactive approach inactivates high levels of a broad spectrum of viruses, bacteria, protozoa, and contaminating leukocytes. Treated components have demonstrated retention of therapeutic capacity in randomized controlled clinical trials and post-marketing surveillance studies.

This study evaluated published data to determine the effectiveness of IBS for inactivation of the important vector-borne pathogens West Nile virus (WNV), chikungunya virus, dengue virus, Plasmodium falciparum, Babesia microti, Trypanosoma cruzi, Leishmania sp, Borrelia burgdorferi, Anaplasma phagocytophilum and Orientia tsutsugamushi in platelet and/or plasma components.

Methods
In these studies plasma and platelet components were inoculated with pathogens to a titer of 10^6 viable organisms/mL whenever possible. Inoculated units were treated with 150 μM amotosalen and 3 J/cm² UVA. Infectivity titers were measured before and after treatment. WNV, chikungunya virus, and dengue virus infectivity was assayed in vero cells and P. falciparum was assayed in fresh RBC. Viability of B. burgdorferi, T. cruzi and L. mexicana (promastagote) was assessed by growth in culture medium, and that of A. phagocytophilum, B. microti, O. tsutsugamushi and L. major (amastagote) was determined by mouse infectivity.

Results
Pathogens that are transmitted to humans by a variety of insect vectors were effectively inactivated in platelets and plasma. All pathogens tested were inactivated to or below the limit of detection by treatment with IBS.

Conclusion
The INTERCEPT Blood System is highly effective for inactivation of a spectrum of TT vector-borne pathogens.

This research was supported by Cerus Corporation.
P022
An Active Hemovigilance Program Provides Safety Data for Transfusing INTERCEPT™ Platelet and Plasma Components in Routine Clinical Practice

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Background
The INTERCEPT Blood System™ inactivates pathogens and leukocytes in platelet and plasma components using amotosalen and UVA light.

Aim
Summarize safety outcomes from 41,276 transfusions of INTERCEPT platelet and plasma components in routine practice. The primary outcome measure was the incidence of acute transfusion reactions (ATR); defined as an adverse event assessed as “possibly related”, “probably related” or “related” to the transfusion by the Investigator.

Method
Centers (23) in 12 countries using the INTERCEPT Blood System participated in an active hemovigilance program to record basic patient demographic, transfusion, and safety data following each transfusion. There were no inclusion criteria for patients other than the need for a transfusion.

Results
Data were reported for 13,734 patients and 41,276 transfusions (>76,276 units). Transfusion recipients were <1-98 years old; recipients primary diagnoses included hematology-oncology (50.5% platelet, 18.0% plasma); surgery (18.4% platelet, 36.9% plasma); and “other” (30.6% platelet, 45.1% plasma). The mean exposure to platelets was 4.7 components (range 1-156); the mean exposure to plasma was 5.9 components (range 1-372).

Overall, the incidence of ATR was 0.4% of transfusions and 0.9% of patients. Most ATR were non-serious (Grade 1). The most frequently recorded events were chills, urticaria, rash, pruritus, and pyrexia. ATRs that met the “serious” criteria were very rare (8 patients; <0.1%) and included allergic, hypotensive, and respiratory symptoms. The “serious” criteria included Grade 2-4 events, classified as fatal, life-threatening, hospitalization, or other important medical events. Two of the cases were considered transfusion associated cardiac overload (TACO). No cases of TRALI, TA-GVHD, or transfusion transmitted infection were reported.

Conclusions
ATR following transfusion of INTERCEPT platelet and plasma components were infrequent and represented the types of events expected following transfusions. Components treated with the INTERCEPT Blood System were well tolerated in a wide range of patients.

This research was supported by Cerus Corporation. The company analyzed the data and prepared the abstract.
P023
In Vitro Evaluation of Pathogen Inactivated RBC Using the S-303 Treatment System

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2 Institute of Transfusion Medicine, DRK-Blutspendedienst Baden-Württemberg - Hessen, Frankfurt, Germany
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Aim
A new pathogen inactivation (PI) system for red blood cells (RBCs) has been developed using S-303 to crosslink nucleic acids and prevent replication of contaminating pathogens and leukocytes. Glutathione (GSH) is included to quench non-specific reactions. In vivo 24-hour recovery per the FDA criteria and PI efficacy has been evaluated using various bacteria and viruses selected for their relevance to RBC transfusion. The purpose of this study was to demonstrate the quality of stored PI RBC in a European blood center.

Methods
Leukocyte-depleted (LD) SAGM RBCs were prepared from whole blood (500mL) which was held overnight at room temperature (RT) and buffy coat depleted. ABO matched RBCs were pooled and divided into units of approximately 280 mL (N=3). For each replicate, units were untreated and stored at 1-4°C (Control) or treated with the S-303 (Test). The S-303 PI process involved combining RBC with GSH and a custom diluent solution followed by the addition of S-303 for a final concentration of 20 mM GSH and 0.2 mM S-303. After an 18h RT incubation, Test units were centrifuged and the treatment solution was replaced with SAGM. Units were stored at 4°C for 35 days and sampled periodically throughout storage.

Results
All Test units had >40 g of hemoglobin (Hb) after PI treatment. After 35 days of storage MCV, MCHC, Hct, ATP, potassium (K+), and glucose were not different between Test and Control. Lactate and pH were higher in Control, whereas hemolysis was lower.

Conclusions
The S-303 PI process is compatible with buffy-coat depleted RBCs in SAG-M, met EU and AABB guidelines for LD RBCs with respect to Hct, Hb, and hemolysis and can be efficiently managed within blood center workflow. These data support further clinical development of the S-303 PI process and evaluations in other blood centers.

This research was supported by Cerus Corporation
Preparation and Characterisation of Granulocyte Concentrates for Transfusion

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Aim
Buffy-coats, as a source of neutrophils, have been used to successfully treat severe neutropenic sepsis. However, to obtain a suitable yield of neutrophils (>1x10^{10}), at least 10 buffy-coats may be required. Transfusion of buffy-coats may lead to adverse events due to the large volume and contamination with red cells and platelets. This study aimed to optimise a robust protocol for the production of a semi-purified, volume-reduced, pooled granulocyte concentrate (PGC) from whole blood-derived buffy-coats.

Method
PGCs (n=6) were prepared by pooling 12 ABO-matched buffy-coats with 400 mL of plasmalyte-A. Excess plasma, red cells and platelets were removed by centrifugation (1800 x g; 7 mins) and expression using an Optipress II. The granulocyte rich layer was then suspended in 70 mL of ABO-matched plasma. The final PGC was irradiated (25-50 Gy) and stored at 20-24°C for up to 24 hours. The cellular composition of the PGC was assessed and compared to cells present in an average buffy-coat (n=19). Neutrophil viability and function was also examined.

Results
The average volume of the PGCs was 158.14 ± 5.90 mL, compared to 706.80 mL if given as a dose of 12 individual buffy-coats. The neutrophil yield in PGCs was also higher than the dose present in 12 buffy-coats (1.06x10^{10}/unit versus 0.96x10^{10}/unit), whilst red cell contamination was less than 10%. Importantly, neutrophils in the PGC maintained >90% viability and were capable of phagocytosis, chemotaxis and generating oxidative burst for up to 24 hours post-irradiation. In addition, neutrophils were capable of secreting IL-1β, IL-6, IL-8 and IL-10 in response to LPS.

Conclusion
The PGC had a higher neutrophil concentration with less volume and red cell contamination compared to the average number of cells present if 12 individual buffy-coats were transfused. Importantly, neutrophil function in the PGC was preserved for up to 24 hours.

No conflict of interest to disclose
Red Cell Alloantibody Formation in Patients with Myelodysplasia: A Single Centre Retrospective Audit

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Aim
There is a paucity of data regarding the formation of red blood cell (RBC) alloantibodies in patients with myelodysplasia (MDS) and chronic myelomonocytic leukaemia (CMML). The aim of this study was to determine the rate of RBC alloantibody formation in transfused patients with MDS/CMML.

Method
A single centre retrospective audit of RBC alloantibody formation in patients with newly diagnosed MDS/CMML at PeterMac from January 2005-April 2011. Of 140 MDS/CMML patients, 47 received RBC transfusions over >1 episode. As a comparative group, alloantibody formation in patients who received ≥1 cycle of Hyper-CVAD chemotherapy in the same time period was assessed. Of the 101 patients in this group, 96 received RBC transfusions over >1 episode. Fisher’s exact test was performed with a p value of <0.05 considered significant.

Results
8/47 (17%) MDS/CMML patients (6/38 males; 2/9 females) and 4/96 (4%) patients receiving Hyper-CVAD (2/71 males; 2/25 females) had ≥1 RBC alloantibodies. Fisher’s exact test showed a two sided p-value of 0.020 strongly suggesting transfused patients with MDS are more likely to form alloantibodies than patients with high grade B and T lymphoid malignancies receiving Hyper-CVAD.

The eight MDS/CMML patients had in total 15 distinct alloantibodies with 13/15 alloantibodies detected at the time of referral to PeterMac. In the Hyper-CVAD group, five alloantibodies were found with three present at baseline. All subsequent alloantibodies were detected after ≤7 RBC transfusions. All four women had prior pregnancies; three had alloantibodies at baseline, only one had a definite prior RBC transfusion.

Conclusion
Despite a higher number of alloantibodies in patients with MDS/CMML, provision of appropriate red cell units was not problematic. Phenotyping patients can be costly and time consuming. Additionally, this is not possible if recently transfused. In our centre it appears appropriate to forgo phenotyping and instead provide RhD compatible, Kell negative red cells.

No conflict of interest to disclose
Pre-Operative Hb and Likelihood of Transfusion in Elective Orthopaedic Joint Replacement

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Aim
Peer review literature suggests that elective surgical orthopaedic patients have a higher rate of peri-operative transfusion if pre-operative Hb levels are low. We recorded Hb levels and likelihood of transfusion in elective joint replacement procedures over a three month period at RPAH.

Method
All patients undergoing elective joint replacement had Hb levels measured at the time of their pre-operative assessment three weeks prior to surgery.

Results
172 patients were reviewed. Females (n=113) had mean pre-op Hb 132 ±11.3g/L; with 10/113 (9%) having Hb <120g/L pre-operatively. The lowest recorded female pre-op Hb was 110g/L.
Males (n=59) had mean pre-op Hb 139 ± 11.4g/L; with 9/59 (15%) having Hb <130g/L pre-operatively. The lowest recorded pre-op Male Hb was 114g/L.
Post-operative transfusion rates for knee replacements were 6% for females (n=4/62) of whom none of the four women transfused had pre-op Hb <120g/L; and 18% for males (n=6/32) of whom three of the six transfused had pre-op Hb <130g/L. Transfusion following hip replacement was 18% for both females (n=8/44) of whom two had pre-op Hb <120g/L; and males (4/22), of whom three had Hb <130g/L. Revision knee and hip operations had higher rates of transfusion ~33% (n=4/12 male/female data combined).

Conclusion
This data shows that fewer patients undergoing elective joint replacement are pre-operatively anaemic than has previously been reported. The transfusion rates of 6% for females having knee replacement approximate world’s best practice as does the 18% transfusion rates post hip replacement. A transfusion rate of 18% for males undergoing knee replacement may be attributable to the higher rate of pre-operative anaemia in this group.

No conflict of interest to disclose
P027
A Five Year Comparison of Hb Levels of Non-Haematology Patients Who Are Admitted and Subsequently Transfused in SLHD and SWSLHD (SSWAHS)

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Aim
Patients with pre-existing anaemia prior to hospitalisation are significantly more likely to require transfusion and the Hb threshold at which transfusion should be commenced remains uncertain.

Method
We have undertaken a review of patients who were transfused in March each year for the past five years in SLHD and SWSLHS (SSWAHS). Inclusion criteria include adults with normal bone marrow whose Hb was recorded on admission and peri-transfusion. Medical and elective surgical patients are included in the cohort.

Results
Five Year Comparison of Hb Levels in Patients who are Subsequently Transfused in SLHD and SWSLHD

<table>
<thead>
<tr>
<th></th>
<th>2007 (n=572)</th>
<th>2008 (n=540)</th>
<th>2009 (n=1060)</th>
<th>2010 (n=1103)</th>
<th>2011 (n=1200)</th>
<th>p=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission Hb (g/L)</td>
<td>101 ± 0.9</td>
<td>103 ± 1.5</td>
<td>99 ± 2.8</td>
<td>98 ± 2</td>
<td>98 ± 2.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pre-transfusion Hb (g/L)</td>
<td>79.1 ± 0.4</td>
<td>77.2 ± 0.4</td>
<td>77 ± 1.1</td>
<td>76 ± 1.2</td>
<td>75 ± 0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Post-transfusion Hb (g/L)</td>
<td>103.6 ± 0.5</td>
<td>95.9 ± 1</td>
<td>93 ± 1.2</td>
<td>94 ± 0.9</td>
<td>92 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data expressed as mean and standard error of the mean

Conclusion
Patients requiring transfusion during hospitalisation are anaemic at admission. The mean Hb level at which transfusion is instigated is decreasing over time. Post-transfusion haemoglobin levels are significantly lower indicating a significant trend both in lower pre-transfusion thresholds and post transfusion levels.

No conflict of interest to disclose
Devolution of Blood Budgets: An Evaluation

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Aim
To examine the effects of devolution of blood budgets in New South Wales and Tasmania, and provide insights that may assist other jurisdictions when making decisions about financial management of blood.

Method
Data provided by the National Blood Authority on the supply of blood were analysed to determine if an intervention effect could be observed, and whether there were differences in use of blood between those jurisdictions with devolved blood budgets and other jurisdictions. The study also employed qualitative stakeholder interviews analysed using nVivo software.

Result
Data analysis did not identify a significant intervention effect attributable to the devolution of blood budgets, although implementation of other policies that may have resulted in changes in demand for blood may have masked the intervention effect.

Awareness of the policy varied between participants, suggesting that price signals may not be evident to all those responsible for product ordering. Funding models were based on historical usage patterns. Positive outcomes included increased focus on management of the blood, transparency of costs and reduction in wastage. Unintended consequences included changes in prescribing practice resulting from differential pricing of alternative products, increased just-in-time ordering practices, and perceived inequities resulting from devolution of blood budgets to the public but not the private sector.

Conclusion
Devolved blood budgets, when coupled with strategies to improve management of blood, can be an important component of any blood management framework. It is recommended that devolution of the blood budgets be supported by data systems to allow development of an appropriate funding model, development of a national pricing strategy, decisions relating to inventory holdings, and provision of funding for initiatives to reduce demand for blood. Greater equity and reduced duplication of effort may be achieved through adoption of consistent policies for jurisdictional and national administration and governance of blood.

No conflict of interest to disclose
P029
Informed Consent for Blood Products: Do Our Patients Understand Why They Are Having A Transfusion?

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Introduction
Informed consent for transfusion implies a conversation has occurred between a patient and doctor. It permits the patient to participate more fully in treatment decisions. As part of the consent process patients should be given a clear explanation of the indication, benefits, potential risks and side effects, and available alternatives. The process of obtaining consent for transfusion at Peter Mac requires a doctor to sign a statement which confirms the patient has verbally consented to receive blood products, and the above process has been completed. The consent is electronically logged through the pathology information system and is able to be viewed in the patient's electronic medical record.

Aim
To assess the transfusion consent process for patient’s, their recall of information provided and their level of understanding of the discussion.

Method
A questionnaire was undertaken for all new patients that received their first transfusion at Peter Mac from 1st March - 30th June 2011. The questionnaire was completed after the commencement of the transfusion episode.

Results
Seventy three patients were interviewed, 36 male. The median age was 60 years (range 18 - 87). The consent process was documented for 59 (80%) patients. Of these 83% remembered discussing with a doctor the need for a possible transfusion. Of the 83% that remembered the discussion, 86% understood the information given and 84% agreed they were given enough information before the transfusion despite only 50% reading the “Blood Transfusion Patient Information” brochure, which is routinely given to new transfusion recipients. The questionnaire was completed by 14 patients who did not have consent documented with 64% remembering discussing the need for a possible transfusion.

Conclusion
The consent process appears to be routinely performed, understood and informative for the majority of patients, although documentation of this process requires further review and improvement.

No conflict of interest to disclose
P030
Review of Cross-Matching Policies at the Royal Hobart Hospital

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A review was undertaken into ordering of blood products at this hospital, and the information was then used to try and reduce blood product wastage. Hospital transfusion records from November 1\(^{st}\) 2010 to January 31\(^{st}\) 2011 were examined to determine how many blood units were cross-matched but not used during this time.

Throughout this period, 796 unused cross-matches were recorded. Of these, 202 units were from cardiac patients and were excluded due to hospital protocol which dictates that all patients having cardiac surgery must have a pre-operative cross-match.

Out of the 594 unused units, 338 were cross-matched for patients with a haemoglobin level greater than 100g/L; this amounted to 56.90% of unused units cross-matched for patients with haemoglobin above the hospital transfusion limits. A total of 335 of the 594 units did not leave the transfusion fridge.

Recommendations:
- Pathology staff to follow transfusion protocols when issuing blood products
- Only cardiac patients should have blood cross-matched pre-operatively
- Non-cardiac patients should only have pre-operative cross-matches if detailed clinical notes are provided
- Blood shouldn’t be cross-matched for patients with a haemoglobin >80g/L without relevant clinical information
- Blood should only be cross-matched for patients with a haemoglobin >100g/L with approval from a haematologist
- Staff not to use haemoglobin as the sole decider as to whether a cross-match is needed, for example, iron therapy may be more appropriate if the patient is stable
- Limit the number of units to be issued to a patient at any one time
- Review FBE and Coagulation results before further cross-matches occur
- Staff education sessions on appropriate ordering of blood products

No conflict of interest to disclose
P031
Case Study - Severe Common Variable Immunodeficiency Presenting With Loss of ABO Backgroup

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Common Variable Immunodeficiency (CVID) is the most common form of humoral immunodeficiency in adults. Patients with CVID have significantly reduced serum IgG levels and can have reduced IgA and/or IgM levels; as a result they experience recurrent sino-pulmonary disease and poor responses to vaccination. During the course of the disease they can develop chronic lung disease, reactive lymphadenopathy and splenomegaly, autoimmune disorders and gastrointestinal disease; they are also at increased risk of lymphoma, and other malignancies.

Standard treatment for patients with CVID is supportive care with regular IVIg therapy and surveillance for and treatment of infection and other complications.

A 42 year old female presented to our hospital with a decades-long history of recurrent chest and ear infection and mediastinal lymphadenopathy and was found to have profound hypogammaglobulinaemia [serum IgG <0.3g/L, IgM <0.1g/L and IgA <0.1 g/L] and non-caseating granulomatous reactive change on lymph node biopsy. Her ABO/Rh blood grouping results were as follows; indicating the absence of the expected ABO backgroup result:

<table>
<thead>
<tr>
<th>Anti- A</th>
<th>Anti B</th>
<th>Anti D</th>
<th>A1 Cells</th>
<th>B Cells</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>O Pos (no reverse group)</td>
</tr>
</tbody>
</table>

There was concern regarding possible anaphylaxis to IVIg, given the severe IgA deficiency, this led to the measurement of anti-IgA Ab, which was undetectable. She tolerated a 50% dose reduction of Intragam P with close clinical monitoring and has gone on to receive full dose monthly IVIg replacement without incident.

The lack of IgM isohaemagglutinins in CVID as seen has been reported but is relatively rare and in this patient presumably reflects the severity of her immunodeficiency.

No conflict of interest to disclose
P032
Special Platelet Support – Engaging the Team Through Education.

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Aim
To increase awareness and understanding of health professionals regarding Special Platelet Support (SPS) provided by the Australian Red Cross Blood Service. The SPS program primarily coordinates requests for HLA-compatible platelets; HPA-compatible, less common ABO groups and IgA deficient platelets may also be requested. Close clinical coordination between the Blood Service and treating hospitals is imperative to ensure timely patient support and minimisation of wastage by appropriate reallocation. The service is optimal when all participants understand and engage in the process, ensuring best use of precious gifts from volunteer donors.

Method
A pilot package of educational tools including power point presentation and resource folder was developed with adaptable sections of the presentation that can be added or removed in order to suit the target audience. The package was presented to 15 nursing staff at a major metropolitan hospital, identified as one of the top consumers of SPS product. Participants assessed their own knowledge before and after the education session using a structured evaluation tool.

Result
66% of the attendees reported that they were previously unaware of the existence of the SPS program. 85% of participants reported a greater understanding of the appropriate use of SPS, with 71% indicating an increase in the understanding of the resources involved.

Conclusion
Post-session evaluation indicates an increased awareness of why SPS support is required. Assessment of interventions aids our understanding of baseline knowledge and the impact of educational tools. This assessment enables the tailoring of interventions to meet the educational requirements of the target audience. The education package will be introduced to additional Victorian treating centres using SPS, and further refined following ongoing evaluation and monitoring of the level of engagement from clinical teams. Future potential includes on-line availability of the package for self-directed learning.

No conflict of interest to disclose
P033
Assessing Clinical Urgency Of Red Blood Cell Supply: A Comparison of Two Methods

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Background
Contingency plans for blood shortages anticipate triage of available stocks based on clinical urgency. However, the optimal method for determining urgency has not been defined. In the BloodHound (BH) study of red cell (RBC) use, urgency was determined by pathology staff at time of RBC issue from the hospital blood bank, using information available at the time.

Aim
To audit the urgency of supply category assigned in the BH study.

Method
Random selection of 5% RBCs from the BH study issued to 3 hospitals (which accounted for 24% of overall BH sample). Data regarding indication for transfusion, clinical context and laboratory results were collected from medical records. Two haematologists independently assigned urgency of supply category according to BH criteria. Where there was disagreement, the case was reviewed and a consensus reached. Paired urgency of supply data were examined for agreement using weighted kappa statistics.

Results
278 RBCs (133 from hospital 1, 118 hospital 2 and 27 hospital 3) were identified. BH urgency of supply category was available for 227 of these units. There was agreement for 72/227 (31.8%). The difference in urgency category assigned was asymmetrical, with BH more likely to assign a higher urgency category (p-value <0.001). Kappa values are shown in the table:

<table>
<thead>
<tr>
<th></th>
<th>Observed agreement</th>
<th>Chance agreement</th>
<th>Kappa value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH vs. H consensus</td>
<td>85.7%</td>
<td>82.9%</td>
<td>0.16* (0.10-0.19)</td>
</tr>
<tr>
<td>BH vs. H 1</td>
<td>85.9%</td>
<td>82.7%</td>
<td>0.18* (0.11-0.26)</td>
</tr>
<tr>
<td>BH vs. H 2</td>
<td>85.9%</td>
<td>82.7%</td>
<td>0.14* (0.03-0.24)</td>
</tr>
<tr>
<td>H 1 vs. H 2</td>
<td>93.0%</td>
<td>84.1%</td>
<td>0.56** (0.51-0.65)</td>
</tr>
</tbody>
</table>

*Kappa value <0.4 represents poor agreement, **kappa value 0.4-0.75 fair to good agreement and ***kappa value >0.75 excellent agreement. H=haematologist.

Conclusions
There was fair to good agreement between the two haematologists but poor agreement between the haematologists and BH urgency of supply category assigned by the laboratory scientists. This study demonstrates the complexities in determining urgency of clinical need, and some of the likely challenges that may occur when triaging of available supplies is required during blood shortages.

No conflict of interest to disclose
Anti-A Antibody Mediated Severe Haemolysis After High Dose Intravenous Immunoglobulin Therapy – A Case Report

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Background
High-dose Intravenous immunoglobulin (IVIG) is used in the management of many immune disorders. High-dose IVIG is generally well tolerated, but there are a few reports of haemolytic anaemia induced by antibodies present in IVIG against blood group antigens. We report a case of severe haemolysis caused by high dose IVIG given for ITP.

Case Report
A 58 year old female known to have ITP underwent emergency cholecystectomy. Post-operatively her platelet count dropped to 15x10^9/L. IVIG (Intragam) was administered at 1g/kg over 2 days. The platelet count normalized in 2 days. However after 24 hours of second dose of IVIG, the patient complained of reddish urine with significant drop in Hb from 123g/L to 80g/L. Severe intravascular haemolysis was evidenced by reticulocytosis, high LDH, low haptoglobin, spherocytes in blood film, strongly positive DAT, haemoglobinemia and haemoglobinuria. Patient’s blood group was A Rh positive. With corticosteroid therapy, Hb and haemolytic parameters started to improve within the first week and normalized in 4 weeks, without need for blood transfusions. After the haemolysis settled completely, a small aliquot from the same batch of IVIG was cross-matched against patient red cells, with pooled A, B and O cells as controls. There was strong agglutination with patient’s red cells and control A cells up to 1:256 dilution, suggesting Anti-A antibody in IVIG as the cause for haemolysis.

Discussion
Haemolytic anaemia is a rare adverse event of IVIG therapy, reported most often in patients with blood group A or AB, commonly due to Anti-A antibody in IVIG. Haemolysis is more common in patients receiving high dose of IVIG and occurs typically within 10 days after IVIG infusion. This condition may mimic Evan’s syndrome which is a chronic auto-immune haemolytic anaemia associated with ITP. Generally IVIG induced haemolysis will be mild and self-limiting. This patient unusually had a severe haemolysis requiring steroid therapy. In such patients, to prevent recurrence, cross matching of patient’s red cells against IVIG products may be useful to find the appropriate product. In summary all patients on high dose IVIG should be closely monitored for haemolysis.

No conflict of interest to disclose
P035
Bridging the Biology of the Ovine TRALI model – Human Inflammatory Cell Responses to TRALI Inducing Supernatants

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Background
Transfusion-related acute lung injury (TRALI) is a serious and potentially fatal consequence of transfusion. A two-event TRALI model demonstrated date-of-expiry-day (D) 5 platelet (PLT) and D42 packed red blood cell (PRBC) supernatants (SN) induced TRALI in LPS-treated sheep. We have adapted a whole blood transfusion culture model as an investigative bridge between the ovine TRALI model human responses to transfusion.

Methods
A whole blood transfusion model was adapted to replicate the ovine model - specifically +/- 0.23 µg/mL LPS as the first event and 10% SN volume (transfusion) as the second event. Four pooled SN from blood products, previously used in the TRALI ovine model, were investigated: D1-PLT, D5-PLT, D1-PRBC, and D42-PRBC. Fresh human whole blood (recipient) was mixed with combinations of LPS and BP-SN stimuli and incubated in vitro for 6 hrs. Addition of golgi plug enabled measurement of monocyte cytokine production (IL-6, IL-8, IL-1α, IL-12, TNF-α, IL-1α, CXCL-5, IP-10, MIP-1α, MCP-1) using multi-colour flow cytometry. Responses for 6 recipients were assessed.

Results
In the presence of LPS, D42-PRBC-SN significantly increased monocyte IL-6 (P=0.031), IL-8 (P=0.016) and IL-1α (P=0.008) production compared to D1-PRBC-SN. This response to D42-PRBC-SN was LPS-dependent, and was not evident in non-LPS-stimulated controls. This response was also specific to D42-PRBC-SN, as similar changes were not evident for the D5-PLT-SN, compared to the D1-PLT-SN, regardless of the presence of LPS. D5-PLT-SN significantly increased IL-12 production (P=0.024) compared to D1-PLT-SN. This response was again LPS-dependent.

Conclusions
These data demonstrate a novel two-event mechanism of monocyte inflammatory response that was dependent upon both the presence of date-of-expiry blood product SN and LPS. Further, these results demonstrate different cytokines responses induced by date-of-expiry PLT-SN and PRBC-SN. These data are consistent with the evidence from the ovine TRALI model, and enhancing its relevance to transfusion related changes in humans.

No conflict of interest to disclose
P036
Age of Blood at Transfusion and Sepsis Sequalae

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Aim
It has been suggested that the transfusion of old blood affects clinical outcomes, especially the incidence of sepsis. We examined our transfusion data looking at the affect of old blood on positive blood cultures.

Results
In 2009/2010 16,188 units of blood were transfused to 2,604 patients at Royal Prince Alfred Hospital. More than 50% of units were transfused within 2 weeks of collection; 2% of units (383 units) were > 5 weeks of age at time of transfusion. We classified each patient according to the age of oldest unit received over the 12 month period. 271 patients received a unit > 5 weeks of age, the majority receiving only 1 unit of old blood.

We looked at transfused patients with a positive blood culture between 2 and 16 days following transfusion. Patients with a positive blood cultures in the 3 months prior to transfusion were excluded. After exclusion of Haematology patients 2.1% of patients overall had a positive blood culture (see table below).

<table>
<thead>
<tr>
<th>Oldest Blood Transfused</th>
<th>No Patients</th>
<th>Pos Cultures</th>
<th>% Pos Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>71</td>
<td>1</td>
<td>1.4%</td>
</tr>
<tr>
<td>Week 2</td>
<td>230</td>
<td>8</td>
<td>3.5%</td>
</tr>
<tr>
<td>Week 3</td>
<td>460</td>
<td>8</td>
<td>1.7%</td>
</tr>
<tr>
<td>Week 4</td>
<td>712</td>
<td>15</td>
<td>2.1%</td>
</tr>
<tr>
<td>Week 5</td>
<td>574</td>
<td>11</td>
<td>1.9%</td>
</tr>
<tr>
<td>Week 6</td>
<td>269</td>
<td>6</td>
<td>2.2%</td>
</tr>
<tr>
<td>Total</td>
<td>2316</td>
<td>49</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

The medical records of 46 of the 49 patients with a positive culture were examined. Of these, 38/46 (83%) were taking antibiotics prior to transfusion; and 21/46 (46%) had signs of infection prior to transfusion. Sixteen of the 46 patients (35%) have since died.

Conclusion
Results failed to show an association between age of oldest unit transfused and sepsis. A significant proportion of positive cultures post-transfusion occur in the presence of prior and concurrent antibiotic treatment; and an existing septic profile. These results may reflect local practice where patients predominately receive only one unit of old blood in a transfusion episode.

No conflict of interest to disclose
P037 Flower Robert  
Genotyping Di\(^\text{a}\) and GP.Mur in Australian Donors

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**Aim**  
Over 8% of the contemporary Australian population are of Asian ethnicity however, there are few reports of frequency of the GP.Mur hybrid glycophorin or the Di\(^\text{a}\) single nucleotide polymorphism found in Asian ethnic groups, in Australian donors. Antibodies to the Di\(^\text{a}\) polymorphism and to neoantigens (Mut, Mur, and Hil) found on GP.Mur can cause transfusion reactions and haemolytic disease of the foetus and newborn. Di\(^\text{a}\) and GP.Mur are found in 5-8% in East Asian populations. The aim of the study was to establish and apply high resolution melt (HRM) genotyping procedures to type Australian donors for these polymorphisms, found at higher frequency in Asian ethnic groups.

**Method**  
Di\(^\text{a}\) and GP.Mur were typed using standard serological procedures. DNA from donors that were serology-positive (n=13) and serology-negative (n=10) for GP.Mur were investigated to validate HRM genotyping, using primers from previously published methods. Amplification and analysis was performed using an HRM kit (QIAGEN). A similar characterisation was carried out for Di\(^\text{a}\). Genotyping was carried out for collections when Donor Mobile units were at locations with a high frequency of blood donors of East Asian background.

**Results**  
HRM genotyping showed 100% concordance with serology. GP.Mur was detected in 3 out of 100 donor samples by both serology and HRM genotyping. For all GP.Mur samples no variant types were found (a full profile of neoantigens was detected). Some samples (54) were also investigated by Di\(^\text{a}\)/Di\(^\text{b}\) HRM typing with 1 Di\(^\text{a}\) homozygote (1.8%) detected and 7 Di\(^\text{a}\)/Di\(^\text{b}\) heterozygotes (13%).

**Conclusion**  
HRM genotyping reliably detected Di\(^\text{a}\) and GP.Mur polymorphisms. In this selected study cohort, the antigen frequency of GP.Mur was 3% and for Di\(^\text{a}\), the frequency surprisingly high, almost 15%. With changes in the demographics of the Australian population, this is evidence that the frequency of these phenotypes in some Australian donor cohorts is higher than previously reported.

No conflict of interest to disclose
P038
Patient Blood Management Guidelines

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Aim
The National Blood Authority is managing the production of Patient Blood Management (PBM) Guidelines to replace the product-focused 2001 ASBT/NHMRC Clinical Practice Guidelines for the use of Blood Components. Six modules are being developed for specific populations: Critical Bleeding/Massive Transfusion, Perioperative, Critical Care, Medical, Obstetric and Paediatric/Neonatal. A PBM approach seeks to optimise the use of donor blood and obviate or reduce the need for transfusion.

Method
An Expert Working Group, with representation from clinical colleges and societies, defined the scope of the new Guidelines and constructed generic questions, to be applied to each module’s population. Clinical Reference Groups (CRG) have been established for each module. CRGs develop additional questions specific to their population. A research protocol is developed for each module. Results of the systematic review are synthesised by the CRG to produce a series of Evidence Statements and Evidence-based Recommendations. In situations where guidance is necessary but good quality evidence is insufficient, Practice Points, based upon CRG consensus, are developed. A comprehensive communication strategy has been developed to inform and involve the clinical community in guideline development and to facilitate dissemination and implementation. Quality assurance and approval is controlled by the National Health and Medical Research Council (NHMRC).

Results
Over 100,000 citations have been screened to date. The Critical Bleeding/Massive Transfusion module was released in March 2011, and is available at www.nba.gov.au. Public consultation for the Perioperative module is complete. The systematic literature reviews for the Medical and Critical Care modules are in progress. Aspects of the content and development process of the initial two Guideline modules will be presented.

Conclusion
High quality evidence is often lacking to guide transfusion practice.

guidelines@nba.gov.au
No conflict of interest to disclose.
P039
Gap Analysis of Pre-operative Anaemia Rates in Elective Surgery in SA Hospitals

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2. Australian Red Cross Blood Service, South Australia, Australia.
3. BloodSafe Program, Adelaide, South Australia, Australia

Aim
To retrospectively examine the frequency of pre-operative anaemia in elective surgical patients in major SA public hospitals.

Method
A linked electronic database was developed for SA public hospitals using clinical, epidemiological and red cell transfusion data. Elective admissions for a range of major surgical procedures over 2 financial years were included (2009 & 2010). Pre-operative laboratory data (up to 8 weeks prior to admission but the closest result to surgery) were assessed. Anaemia was defined as Hb <115 g/L for females and <135 g/L for males.

Results
A total of 2821 elective surgical admissions including arthroplasty of hip [THA] (530), arthropalsty of knee [TKA] (643), right-sided colorectal procedures (332), left-sided colorectal procedures (305), coronary artery bypass [CABG] (621) and on-bypass valve surgery (390) were included. The overall frequency of anaemia was 28% (778/2871), 19% in THA, 13% in TKA, 55% in right colorectal, 35% in left colorectal procedures, 29% in CABG and 31% in valve surgery. The frequency of microcytosis (MCV ≤ 80fl) and hypochromia (MCH ≤ 27pg) in anaemic admissions was 14% and 23% respectively suggesting a significant proportion of patients had iron restricted erythropoiesis and would benefit from assessment of and optimisation of iron stores to reduce the risk of transfusion. In anaemic admissions impaired renal function was common (eGFR < 15ml/min in 2%, eGFR of 15-29 in 4.5%, eGFR of 30-44 in 11%, eGFR of 45-60 in 19%). Rates of transfusion were higher in admissions with pre-operative anaemia.

Conclusion
Uncorrected and potentially reversible anaemia prior to elective surgery is common. Data linkage is a useful tool to allow planning of Patient Blood Management initiatives.

No conflict of interest to disclose
P040
Serological Results in Patients with Autoantibodies - A Single Institution Retrospective Audit

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Background
Transfusion management of patients with autoantibodies is problematic. The major concern is that clinically significant allo-antibodies are being masked by the autoantibody activity. Our clinical impression, in contrast to the literature, is that our rate of allo-antibodies in this group of patients is low.

Aim
To retrospectively determine the rate of alloimmunisation in patients with a detectable free autoantibody.

Method
The Southern Health network transfusion records for January 2006 to March 2011 were reviewed for the patients who had red cell absorption studies performed for an autoantibody. The transfusion histories of the patients identified were then examined. Either an autoabsorption or allogeneic absorption was performed using RAM PEG (Rapid Antibody Medium with Polyethylene Glycol LISS Additive). The allogeneic absorption was performed using R1R1, R2R2 and rr K neg cells (including cells that were negative for Fya Fyb Jka Jkb Ss). An allogeneic absorption was performed: if the patient had been recently transfused, had an unknown transfusion history, a heavy coating of IgG on the cells, or insufficient autologous cells due to anaemia.

Results
46 patients had an allogeneic or autoabsorption performed, encompassing 97 studies. 12 had only autoadsorptions performed, 23 had alloabsorptions and 11 patients had both techniques at different times. In nine patients underlying allo-antibodies were identified but for 7 patients this was newly indentified antibody. These were anti-E (3 patients), anti-S (2 patients) and one case each of anti-Jka and anti-C. The other two patients had mixtures of antibodies: anti-M, c and Jkb in one and anti-K, C, e and Fya in the other.

Conclusion
Consistent with established literature, 20% of our patients who had an adsorption performed for a free autoantibody had a clinically significant allo-antibody. This confirms the importance of testing for an allo-antibody in these patients.

No conflict of interest to disclose
P041
Issues in the Serial Titration of Clinically Significant Antibodies in an Antenatal Patient

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Background
Titration of antibodies in the antenatal patient has an important role in decision making in the alloimmunised pregnancy. A case is described where changing obstetric practices over time have led to problems in clearly communicating the significance of titration results.

Method
A 39 year old female patient has a long history of alloimmunisation to C which was first detected in 2000. She has been monitored through multiple pregnancies by serial titrations. However the use of Rh D immunoglobulin prophylaxis at 28 and 34 weeks has added complexity to the monitoring and reporting of her titration results.

Results
The anti-C at first presentation in the current pregnancy was titrated using a validated column agglutination method (1). The sample was tested against pooled cells containing a homozygous dose of the antigen, as is standard practice. R₁R₁ (CDe/CDe) cells have been used to monitor the previous pregnancies, and they were again selected for testing. When the rr (cde/cde) patient was administered prophylactic Rh D Immunoglobulin, the R₁R₁ cells could no longer be used, and heterozygous r'r (Cde/cde) cells were selected. A change in the anti-C titre was noted with the change of testing cell. The Standard Operating Procedure in use at the time reported the titre but not the test cells, so the information reported to the clinical team was confusing.

Conclusion
The use of prophylactic Rh D Immunoglobulin adds complications to the monitoring of antibodies during alloimmunised pregnancies. Our Standard Operating Procedures have been updated to ensure that appropriate cells are selected in the first trimester so that serial titrations can be performed on the same phenotype of cells throughout the entire pregnancy. Also the reporting of results to the clinician has been updated to include the phenotype of the testing cells used to remove any ambiguity of results.


No conflict of interest to disclose
P042
Frequency, Clinical Profile and Outcome of Rh D Alloimmunised Pregnancies in a Tertiary Care Centre in India

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Introduction
Development of anti D immunoglobulin. Better facilities for early detection and treatment and better neonatal care have brought down the frequency and magnitude of HDFN. But these advantages are limited to places with good access to health care making it prudent to have studies from areas with less access to health care.

Aim
1. To find out the frequency of Rh D alloimmunisation in antenatal cases and severity and treatment of HDFN in their offspring. 2. To correlate the antenatal anti Rh D titre and cord blood values with severity of HDFN

Materials and Methods
A longitudinal cross-sectional study was done in Dept of Transfusion Medicine on 64 antenatal cases positive for anti Rh D antibodies by ICT by Gel method and followed up with serial titres and ultrasound. Peak titre levels were correlated with severity of disease and cord blood values. Data were analysed in SPSS ver.17.correlations done by pearson correlation

Results
Of 2496 RhD negative women tested with ICT,78 (3.12%)were positive. Failure to administer RhIg lead to alloimmunisation in 42 (65.6%) cases. Frequency of HDFN was 57/ 64 cases. 54 RhD positive newborns were DCT positive (93.1%) and 4 were negative (6.9%). Male:female ratio 1.46:1.50. 9% cases were unaffected or mild and 10% were severe. Majority (50%) received no treatment and phototherapy was the major modality of treatment. Maternal titres correlated with severity of disease, cord Hb (negative correlation r=-.310, p=.016) cord bilirubin (r=.591, p.000). Cord Hb had a negative correlation (p.04) and cord Bilirubin (p.004) had positive correlation with intensity of treatment.DCT grade did not correlate with treatment (p=.39). Overall survival rate of affected newborns was 92.18%. Of 6 hydropic babies, four died in utero.

Conclusion
Frequency of Rh D alloimmunisation is higher in the institution compared to global standards but Survival rate in newborns is >90%. Hydropic babies have a higher death rate. Serological tests correlates acceptably with the disease. Better strategies to prevent RhD alloimmunisation and introduction of interventions like IUT are warranted.

No conflict of interest to disclose
**P043**

**Regeneration of Glycorex AB Columns for Blood Group Incompatible Kidney Transplants**

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ABO mismatched kidney transplants can enhance the live kidney donation program. Blood group A or B (ABO) antibodies must be removed from the recipient's blood immediately pre and post transplant transplant. Glycorex AB columns are a licensed device that are both safe and effective in removing these antibodies during plasmapheresis, with the lowest risk of clinical complications.

**Aims**
The aim of this study was to demonstrate that Glycorex AB columns can be regenerated to absorb blood group A or B antibodies.

**Methods**
Both _in vitro_ and _in vivo_ studies were performed. The regeneration protocol was according to Kumlien _et al_ (2006). The capacity of regenerated columns to re-absorb ABO antibodies was evaluated _in vitro_ for 4 columns by mimicking the apheresis procedure with pooled plasma and monitoring ABO antibody titre by the anti haemaglutinin assay. The columns were then regenerated a second time and the regeneration validation repeated. Following _in vitro_ validation, 5 patients, undergoing an ABO mismatched kidney transplant, underwent between 3-9 plasmaphereses, using the Glycorex AB columns. Columns were dedicated to a single patient. Following the initial plasmapheresis, columns were regenerated and used for a second and third plasmapheresis procedure before disposal. Blood samples obtained prior to the commencement and at the completion of the plasmapheresis procedure were tested for blood group A or B antibody titres.

**Results**
For 5 patients undergoing 32 plasmapheresis procedures, 21 procedures involved the use of regenerated columns. All patient blood group antibody titres were reduced by the Glycorex column plasmapheresis, except where the titres were already low. Up to a fourfold reduction in antibody titres was seen for both new and regenerated columns. Sterility was maintained throughout processing and no adverse reactions or events were observed for the regenerated columns. Reduction in titre of regenerated columns compared to new columns.

**Conclusions**
This study validated the regeneration of the Glycorex AB columns for multiple re-use in the same patient. Regeneration of columns can only be performed under strictly controlled conditions of a GMP facility.

**References**
Kumilien G _et al_. Clinical experience with a new apheresis filter that specifically depletes ABO blood group antibodies. Transfusion 2006; 46: 1568-1575

No conflict of interest to disclose
P044
The Role of External Quality Assurance in Identifying Laboratory Performance

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Aim
In 2010 RCPA Quality Assurance Programs, received Quality Use of Pathology Program funding to determine if regular external quality assurance that laboratories perform could be used to identify unacceptable performance earlier than the usual 3 year NATA accreditation cycle, to help minimise risk to patients.

Method
Criteria for acceptable performance have been set to identify laboratories of concern. The criteria are set on the following principles:
- Critical nature of the test
- Frequency of surveys
- Outside the allowable limits of performance
- Not obtaining the target result
- Outside of consensus
- Nonparticipation
- Results returned late or amended
- Exclusion criteria for anomalies (e.g. change of method, participation for education).

A new framework is proposed for managing participants falling outside levels of acceptable performance to proactively notify and assist them early.

Results
When unacceptable performance is identified, according to the set criteria, a letter will be sent to the participant outlining the result(s) falling outside the criteria set for acceptable performance and offering assistance to review the QAP results. Persistant unacceptable performance will be referred to a Committee for review and results referred to NATA for follow-up.

Conclusion
The RCPA QAP has established a performance monitoring system using the EQA results. The early warning system will identify laboratories of concern and assist them to obtain their accreditation.

This research was supported by the Department of Health and Aging. The Department had no role in analysing the data or preparing the abstract
P045
Improving Sample Labelling and Request Form Error Rates at Palmerston North Hospital

Liz Thrift
New Zealand Blood Service (NZBS)

Background
Pre-transfusion samples and forms must meet stringent labelling standards. A review showed Palmerston North Hospital Blood Bank’s error rate was higher than any other NZBS Blood Bank using the same standards.

Aim
To investigate which professional groups were making the errors and to develop and implement strategies to decrease the sample labelling error rate.

Method
All errors were entered into a database. The TNS retrospectively reviewed errors from April 2010 to March 2011. The distribution of errors per clinical area and per professional group was identified. The number of samples collected by each group over a two week period was used as the denominator for deriving their errors rates.

With hospital transfusion committee (HTC) support, a strategy was developed to reduce the error rate. The TNS and Transfusion Medicine Specialist (TMS) provided education on the rationale for labelling requirements. Anyone submitting more than two errors per month received a letter from the TMS, copied to their clinical manager.

Result
In the six months prior to the management strategy, the error rate was 5.2% (224/4324 samples). Following the strategy, the error rate fell to 3.8% (164/4341 samples) (p=0.02). This significant and sustained improvement has reduced the samples discard rate (3.4% vs 2.4%, p=0.01) and may have reduced the risk of wrong blood in tube. The calculated error rate of doctors was 1:35, Nurses 1:42, Midwives 0:44, and phlebotomists 0:15 (p=#).

Conclusion
Monitoring errors was straightforward but improving practice was more challenging. Error rates by professional group provided information required for focussed education. This and the TMS letter have reduced the error rate. Staff were surprised they could be identified individually, in itself a deterrent. Staff are now aware there is a patient safety problem that needs addressing. Errors will continue to be monitored and reported to the HTC.

No conflict of interest to disclose
P046
Outcome of a Pre-Operative Anaemia Clinic

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Aim
To identify and treat anaemic patients on the waiting list for joint replacement surgery (JRS).

Background
Pre-operative anaemia increases the likelihood of transfusion which is an independent risk factor for poorer clinical outcomes including increased length of stay.

Method
Joint replacement nurses ensured that all patients on the waiting list for JRS had a haemoglobin check within the previous three months. Patients found to be anaemic (male Hb < 135 g/L, female Hb < 115 g/L) were referred to the Pre-operative Anaemia Clinic. The anaemia was investigated for each patient and treated prior to JRS. Surgery was delayed if deemed necessary by the physician.

Results
385 patients were screened between February 2010 and May 2011 with 33 (9.4%) patients referred to the Clinic. Iron-deficiency anaemia was identified in 14 patients (42%), one secondary to malignant and two to non-malignant GI loss, two to menstrual loss, one to operative loss, one to non-GI malignant loss, one to non-GI non-malignant loss and six with no identifiable blood loss. Of the iron-replete patients, the causes included hypogonadism, thalassemia, probable myelodysplasia and chronic disease. Appropriate therapy was instituted for all patients. After the underlying cause was identified, one patient chose not to proceed with JRS.

Conclusion
The incidence of anaemia in this population is lower than that reported in other studies. However, the proportion of IDA within the anaemic population is comparable. This clinic has identified patients with treatable causes of anaemia. The next step is to establish whether such an intervention reduces blood product use, avoids last minute cancellation of JRS and influences clinical outcome. The use of orthopaedic nursing staff to initiate the simple screening process is an effective method of identifying anaemic patients as part of routine care.

No conflict of interest to disclose
P047
Supernatant from Stored Blood Components Does Not Affect Trans-Endothelial Migration of Allogeneic Neutrophils.

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Aim
Blood transfusion has been linked to certain adverse immunological responses, such as Transfusion-Related Acute Lung Injury (TRALI), and the broader concept of Transfusion-Related Immunomodulation (TRIM). In some circumstances, including TRALI, these adverse responses involve endothelial dysfunction. Older stored blood components have been more strongly associated in some studies. The nature of the responsible factors within blood components remains unclear. The study’s aim was to determine the effect of supernatant from stored blood components on the migration behaviour of allogeneic neutrophils (representing the patient’s cells) through an endothelial layer (representing the patient’s blood vessel wall), using a trans-endothelial migration assay as an in vitro model of transfusion.

Method
Leucocyte-reduced blood components [red blood cells (RBC) and platelets] were prepared according to standard blood bank procedures. Blood component supernatant was collected at nominated storage times. Human umbilical vein endothelial cells (HUVECs) were grown to confluence on transwell membranes inserts. Fresh allogeneic neutrophils from healthy volunteers were isolated and fluorescently labelled with calcein vital dye. For the migration assay, neutrophils were placed in the upper chamber, with or without various stimuli (blood component supernatant, TNF-α) and chemoattractants were placed in the lower chamber. After incubation, the amount of fluorescence in the upper chamber (unmigrated neutrophils), within the HUVEC layer (adhered/partly migrated neutrophils) and the lower chamber (migrated neutrophils) were measured on a fluorescence plate reader.

Results
Blood component supernatant did not induce any statistically significant effect on the trans-endothelial migration behaviour of allogeneic neutrophils, either in the presence or absence of stimulation with TNF-α. Blood component supernatant was not by itself a chemoattractant to neutrophils.

Conclusion
Supernatant from leucocyte-reduced blood components does not affect the trans-endothelial migratory behaviour of allogeneic neutrophils. Further studies are required to identify the factors within stored RBC and platelet components that contribute to TRALI and TRIM.

No conflict of interest to disclose
P048
Hemovigilance Data of INTERCEPT Blood System™ for Platelets Demonstrate Reduced Adverse Transfusion Reactions and Ability to Protect Against TA-GVHD and Transfusion Related Sepsis

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Background
The INTERCEPT Blood System™ (IBS) for platelets received CE Mark in 2002 for pathogen inactivation (PI) of platelets. IBS platelets have been in routine use for 8 years without patient exclusions. IBS has been approved to replace gamma irradiation and due to robust PI of bacteria, can replace bacterial screening. Australia has yet to implement a pathogen inactivation strategy.

Methods
This study consists of a review of information from several sources; the French National Hemovigilance system for data on the rate of acute transfusion reaction (ATR) and transfusion related sepsis (TRS) cases between 2006 and 2010, 8 years of experience from clinical trials, hemovigilance data and routine use of INTERCEPT platelets transfused to a broad patient population, and Australia’s experience of bacterial detection.

Results
A statistically significant reduction of ATR was measured for platelet transfusions in Alsace (utilising INTERCEPT) compared to the remainder of the French National system. Data from 24 centers in 12 countries showed no cases of TA-GVHD for patients transfused with INTERCEPT platelets in place of gamma irradiation. Patient populations represented included hematology-oncology, HSCT recipients and pediatric patients. Most blood centers using INTERCEPT have discontinued gamma irradiation and rely on IBS to protect against TA-GVHD. Whilst Australia’s bacterial detection strategy prevented some cases of sepsis, there have been reported cases of sepsis since implementation of this strategy. No cases of sepsis from contaminated platelets have been reported in Alsace since the introduction of INTERCEPT.

Conclusion
The use of INTERCEPT is associated with a reduction in adverse transfusion reactions and transfusion related sepsis. Clinical experience supports use of INTERCEPT treatment in place of gamma irradiation to prevent TA-GVHD in at-risk patients, which when implemented in Australia would result in a safer blood supply.

Cerus is the manufacturer of the INTERCEPT Blood System
CSL is the distributor of the INTERCEPT Blood System for the Australian region.
A Review of Crossmatching Practice for RhD Negative Patients in the Antenatal Setting: Maximizing Resources and Improving Red Cell Inventory Management

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Introduction
Blood group and antibody testing in pregnancy has as its primary aim to minimise the incidence and severity of Haemolytic Disease of the Newborn (HDN). Laboratory identification of RhD negative females and assistance in appropriate management of a RhD-Ig immunoprophylaxis program, requires close adherence to established guidelines. Current best practice with regard to anti-RhD detection in the setting of confirmed RhD-Ig antenatal prophylaxis program requires performance of a standard three cell screening set followed by a RhD-negative screening set if the initial screen is positive. The results of these investigations in conjunction with clinical history can provide clear confirmation of antibody screening positivity due to passive acquisition of antibodies via the RhD-Ig immunoprophylaxis program. In line with current guidelines for the selection of red cells when the antibody screen is positive, an abbreviated crossmatching procedure is not recommended. In our laboratory three donor units are manually crossmatched via an indirect antiglobulin test (IAT) and quarantined for use for a period of up to 72 hours. RhD negative red cells are a scarce resource both because of the frequency (a maximum of 16% in Australia) and its quarantine for and use in emergencies.

Method
Austin Pathology provides a blood banking service to two busy obstetric hospitals. We reviewed antenatal blood banking data from the period 2001 to 2010. The frequency of a positive antibody screen in a RhD-negative prenatal female unambiguously attributed to passive acquisition of antibodies was established.

Results
The monthly average for 2010 is presented here. Each month 572 antenatal blood bank samples were processed. Of these 38% (216 samples) were RhD negative and 15% (85 samples) returned a positive antibody screen. Positive antibody screens attributed to passive acquisition of antibodies accounted for 78% (66 samples) of all samples with a positive antibody screen.

Conclusion
As a result of the high frequency of unambiguous confirmation of antibody screening positivity due to RhD-Ig immunoprophylaxis, we propose a computer crossmatching procedure should be adopted where appropriate. We are confident that the current screening procedures ensure no compromise to safety. Removing the restrictive practice of quarantining cross matched units will contribute to strategies to maintain adequate supply of blood products for example O Rh negative blood in emergencies. The proposed changes in laboratory practice remain in line with established guidelines.

No conflict of interest to disclose
Development of Multiplex PCR method for the Analysis of Glutathione S-transferase Polymorphisms

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Aim

Busulfan (BU) is a key compound in myeloablative chemotherapy before hematopoietic stem cell transplantation (HSCT) for children. Genetic polymorphisms of glutathione S-transferase (GST), involved in the metabolism of BU, have been implicated in the interindividual variability in BU pharmacokinetics. Development of rapid and simplified method for polygenic analysis of GST may facilitate large pharmacogenetic studies and clinical application of individualized BU dose adjustment. We previously introduced an effective PCR method of analyzing multiple genes using a small amount of DNA, termed as TotalPlex amplification. The aim of this study was to extend the application of the TotalPlex method to the specific GST gene families (A1, P1, M1, and T1) which are related to BU metabolism and thereby facilitate pharmacogenetic analysis of GST polymorphisms.

Method

Seven single nucleotide polymorphisms (SNPs) (GSTA1 promoter -52G>A, -69C>T, -567T>G, -631T>G, GSTP1 313A>G, GSTM1 deletion, GSTT1 deletion) were analyzed by multiplex PCR and genotyping, and the genotyping results from TotalPlex were verified with those from uniplex PCR.

Result

Seven specific gene fragments were successfully amplified using 5 pairs of specific bulging specific primers by multiplex amplification coupled to a multiplexed bead array detection system with smaller amounts of DNA and shorter process times compared to conventional approaches. The genotypes of 7 loci from 30 different genomic DNA samples derived using the multiplex system were consistent with the results of standard genotyping methods.

Conclusion

Our multiplex system provides a fast, inexpensive and accurate method to detect multiple GST polymorphisms (GSTA1, GSTP1, GSTM1, and GSTT1).

No conflict of interest to disclose
Surveys Assessing the Need for Viable CD34 Quality Assurance Program (QAP) for Haemopoietic Progenitor Cell Transplantation

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Aim
The Australian National Pathology Accreditation Advisory Council (NPAAC 3rd edition, 2009) requires viability testing of haemopoietic progenitor cell (HPC) products for transplantation. The aim of this study is to ascertain how transplant centres are addressing this requirement. Response was also sought on their readiness to enrol in a viable CD34 (vCD34) QAP.

Method
During 2008-2009, participants in the RCPA CD34⁺ QAP were sent 2 questionnaires to determine their readiness to enrol in a vCD34 QAP if one was offered. In 2011, 60 centres were asked to nominate their current assay and preferred future assay for viability testing of HPC.

Result
In the 2008 survey to 56 centers, 21 centres wanted to enrol in a vCD34 QAP, and in the 2009 survey, 16 of these 21 centres confirmed their positive response. Among these 16 centres, 14 were from Australia, with 8 from NSW.
In the 2011 survey, the assays used by the 40 respondents to determine viability of HPC are vCD34 (n = 32), and CFU (n = 11), with 11 centres performing both assays. Aldefluor assay was not used at all, and 8 centres did not test for viability. The preferred future assays were very similar, and 2 centers intend to use Aldefluor.

Conclusion
These surveys indicate that the majority of the transplant centres use the vCD34 assay for viability testing of HPC products. Since the Australian centres need to comply with the NPAAC requirement, the RCPA is determining the feasibility of offering a vCD34 QAP, and a study is underway to examine the various issues involved in offering such a program including transportation of cryopreserved samples, standardization and costs.

No conflict of interest to disclose
P052
A Strategy for Enhancing the Engraftment of Human Hematopoietic Stem Cells in NOD/SCID Mice: Methodology for HSC Transplantation and Application of Human MSCs

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Aim
Human mesenchymal stem cells (MSCs) enhance the engraftment of hematopoietic stem cells (HSCs) when they are co-transplanted in animal and human studies. However, the type of MSCs that preferentially facilitates the engraftment of HSCs is largely unknown. Thus, we conducted a study to identify a strategy for enhancing the engraftment of HSCs.

Method
Co-transplantation with MSCs derived from adult human tissues, including bone marrow (BM), adipose tissues (AT) and umbilical cord blood (CB), and intra-bone marrow transplantation (IBMT) of HSCs was conducted. We categorized CB-MSCs as either the least-effective MSCs or most-effective MSCs at enhancing the engraftment of HSCs, and compared the characteristics including proliferation rate and the gene expression profiles in the MSC populations.

Result
We showed that AT-MSCs and CB-MSCs enhanced the engraftment of HSCs as effectively as BM-MSCs in NOD/SCID mice, suggesting that AT-MSCs and CB-MSCs can be used as alternative stem cell sources for enhancing the engraftment of HSCs. In addition, IBMT with HSCs alone enhanced the engraftment of HSCs as effectively as co-transplantation with MSCs. Moreover, CB-MSCs derived from different donors showed different degrees of efficacy in enhancing the engraftment of CB-HSCs. The most effective CB-MSCs showed higher proliferation rates and secreted higher levels of several factors, including CXCL12, RANTES, EGF, and SCF, which are required for the engraftment of HSCs. By contrast, levels of GRO, IGFBP1, and IL-8, which are associated with immune-inflammation, were secreted at higher levels in the most effective UCB-MSCs.

Conclusion
This study suggests that AT-MSCs and CB-MSCs could be alternative stem cell sources for co-transplantation in HSC transplantation. Furthermore, in terms of MSCs’ heterogeneity, characteristics of each population of MSCs are considerable factors for selecting MSCs suitable for co-transplantation with HSCs. In addition, IBMT of HSCs may be a useful method for enhancing the engraftment and homing of HSC. No conflict of interest to disclose
P053
Similar Outcome of Matched Unrelated Versus Sibling Donor Hematopoietic Stem Cell Transplant for Children with Aplastic Anemia

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Objective
It is difficult to find matched sibling donors (MSD) in our country because of the policy “one family, one child”. To evaluate the outcome of matched unrelated donors (MUD) hematopoietic stem cell transplant (HSCT) and to analysis the influence factors for children with aplastic anemia (AA) received MUD HSCT in our hospital.

Method
Retrospectively analysis the clinical data for pediatric AA received MUD HSCT from Oct.2003 to Oct.2010 in our hospital. All patients received conditioning with CY120mg/kg+ATG (15-30mg/kg, Fresenius)+Fludarabine 150-200mg/m2. 2-3GY TBI was added for those heavy transfused (transfusion more than 20 times) or 1-2/10 high resolution mismatched MUD HSCT. Except CSA and MTX, MMF was added for the GVHD prophylaxis for patients with 2/10 mismatched unrelated donor HSCT.

Results
The median age of these 29 children (12 VSAA, 14 SAA and 3 NSAA) was 8.5 (3-16) years old. 9/29 with 10/10 MUD, 12/29 with 1/10 mismatched and another 8/29 with 2/10 mismatched unrelated donor HSCT, after the median fellow up 18 (5-89) months 25/29 (86.2%) survived. 4 patients died of transplant related mortality (2 with infection and another 2 with severe aGVHD). Except 2 died before hematopoietic reconstruction, 2/27 suffered graft failure (1 with primary graft failure and another 1 with secondary graft failure). 8 of these 29 patients received HSCT without previously ATG for IST because of active infection (n=4) or the parents refused to ATG first (n=4). For the overall survival there was no any significant difference between different type of AA, different courses of the disease and patients with different degree (0-2/10) mismatched unrelated donor or received different conditioning regimen HSCT. The only influence factor was patients older than 10 years got significant poor outcome (66.7% versus 100%, p=0.021). Compared with the data of AA patient received MSD (n=9) and MMRD (n=9) HSCT during the same period in our center, except more severe aGVHD than MSD (68.9%, 11.1% vs 71.4%, p=0.06), there was no any significant difference between disease free survival (86.2%, 100% vs 85.7%, p=0.637) and graft failure (7.4%, 11.1% vs 37.5%. p=0.07).

Conclusion
Even with higher risk of aGVHD, 0-2/10 mismatched unrelated donor HSCT in China can still got similar outcome with MSD after received individualized conditioning regimen and GVHD prophylaxis. It is reasonable to consider the high resolution well matched unrelated donor HSCT as the first line treatment for children with VSAA in China.

No conflict of interest to disclose
P054
Incidence, Risk Factors and Clinical Outcome of Late-Onset Noninfectious Pulmonary Complications after Allogeneic Stem Cell Transplantation from HLA-identical Sibling Donors

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Aim
Pulmonary complications develop in 40-60% of allo-transplant recipients and cause 10-40% of transplant-related death. Late-onset non-infectious pulmonary complications (LONIPCs) occurring beyond 3 months after transplantation are recognized as life-threatening events affecting patients' quality of life. In this study we retrospectively evaluated the incidence, risk factors and prognosis of LONIPCs in patients receiving allo-SCT from HLA-identical sibling donors.

Method
Two hundred and seven adult patients who received first allo-SCT from HLA-identical sibling donors and survived ≥ 3 months at our institute between Jan 2000 and Jan 2009 were enrolled. We reviewed the clinical parameters, radiologic findings, pulmonary function tests and pathology reports to identify patients who fulfilled the diagnostic criteria of LONIPCs. Patients were further subclassified as bronchiolitis obliterans (BO), bronchiolitis obliterans with organizing pneumonia (BOOP) and idiopathic pneumonia syndrome (IPS).

Result
Of the 207 recruited patients, 34(16.4%) developed LONIPCs at a median interval of 224 days post-transplant (range, 90-729 days). 34 patients were subclassified as having BO(16 patients), BOOP(4 patients), and IPS(14 patients). Sex mismatch, conditioning regimens, GVHD prophylaxis, pulmonary function tests preallo-SCT, acute GVHD or CMV antigenemia were not associated with the occurrence of LONIPCs. On the contrary, several factors were strongly associated with the development of LONIPCs in multivariate analysis, including advanced stage of disease (HR 2.43, 95% CI, 1.04-5.68, P=0.041) and extensive chronic GVHD (HR 4.0, 95% CI, 1.82-8.91, P=0.001). Twenty-two of the 34 patients with LONIPCs died(64.7%), including 11 with BO. The major cause of death was progressive respiratory failure(77.3%). In addition, the 5-year overall survival rate was significantly lower among patients with, than without LONIPCs (36.1% vs. 58.6%, P=0.032). Patients with LONIPCs had lower relapse rate. Nevertheless, the potential survival advantage from less relapse was offset by their high non-relapse morality rate.

Conclusion
LONIPCs is an important factor of posttransplantation morbidity and mortality. The development of LONIPCs were strongly related to the presence of extensive chronic GVHD and high-risk stage of disease at transplant. The clinical outcome of patients with LONIPCs was significantly worse. Owing to the lack of effective therapy to LONIPCs, strategies aimed at prevention of LONIPCs should be sought, such as better control of chronic GVHD.

No conflict of interest to disclose
P055
Flag Re-Induction and Peripheral Blood Progenitor Cell (Pbpc) Re-Infusion for Relapsed Acute Leukaemia Post Allogeneic Haematopoietic Stem Cell Transplantation (HSCT): A Single Centre Retrospective Study

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Aim
To assess the outcome of patients with relapsed acute leukaemia post allogeneic HSCT treated at our institution with FLAG re-induction followed by allogeneic donor PBPC re-infusion.

Methods
All patients with relapsed acute leukaemia post allogeneic HSCT were identified from an institutional data-base. Two groups of patients were defined: those treated with FLAG re-induction followed by allogeneic donor PBPC re-infusion (similar to JCO 2002; 20: 405) and those actively treated with post-relapse chemotherapy (including re-induction chemotherapy) but who did not receive subsequent allogeneic donor PBPC re-infusion. Individual medical records were then retrospectively reviewed to determine overall survival (OS) from time of relapse in both groups.

Results
19 patients were identified between January 2003 and January 2011 who had received FLAG re-induction and subsequent PBPC re-infusion, and 16 control patients were identified who had received active therapy but without PBPC re-infusion. Median time post-initial transplant to 1st relapse was 227days (range 40-1945) for patients in the FLAG PBPC re-infusion group and 391days (range 30-991) in the control group (p=NS). At median follow-up of 44mths post relapse (range 6-99mths) for the FLAG-PBPC re-infusion group and 21mths (range 8-92mths) for the control group, median OS was 8mths vs 7mths respectively (P=0.6993). 3/19 patients (16%) in the FLAG-PBPC re-infusion group died of non-relapse mortality (2 due to sepsis and 1 due to progressive encephalopathy) and 13/19 died (68%) from relapsed disease. Three patients (16%) remain alive and in remission post FLAG-PBPC at 5, 19 and 80mths post relapse respectively, 2 of whom have developed extensive stage chronic GVHD.

Conclusion
Although FLAG re-induction followed by PBPC re-infusion as treatment of relapsed acute leukaemia post allogeneic HSCT may result in long term survival in a minority of patients, overall prognosis appears similar to active therapy without further donor PBPC infusion. No conflicts of interest to disclose.
P056
Tumorablative Allogeneic Hematopoietic Cell Transplantation for High Risk and Refractory Leukemia - New Concept and Clinical Practice

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Aim
The traditional allogeneic myeloablative can cure or improve outcome of acute leukemia, however, the disease relapse after transplant for acute leukemia with high risk and refractory is 40% to 80%. Moreover the leukemic cell in the majority relapsed cases originates from inceptive leukemic cells at initial diagnosis, which strongly indicated that the standard myeloablative conditioning regimen could remove the normal lymphohematopoietic system of the recipients and make grafts successfully ecize and proliferate, but could not always kill the residual leukemic stem cells in vivo, particularly the those in extramedullary sites. Those residual leukemic stem cells are the crime for the disease recurrence. We pioneered the tumorablative allo-HCT to treat the high-risk, refractory, even advanced acute leukemia, and observe the effect of tumorablative allo-HCT in those patients.

Method
The tumorablative regimen included HD Ara-C+Bu/Cy, G-CSF primed HD Ara-C+Bu/Cy and FLAG/RIT (FLAG/reduced intensive transplantation, according to their constitutional state before transplantation. Cyclosporine A, short courses of Methotrexate and mycophenolate mofetil (FFM) were used to prevent GVHD. ATG was added to HLA-haploidentical or unrelated transplantation.

Results
In the median follow-up 17.5 months (2-34 months), 56 patients attained durable engraftment after transplantation. Total survival rate was 74.7±6.1%, total disease free survival rate was 62.4±6.7%, and leukemia relapse was 24.57±6.37%. The cumulative incidences of grades II to IV and III to IV acute graft-versus-host disease (aGVHD) were 12.3±0.04% and 8.8±0.04%, respectively. The incidence of cGVHD was 72.89±7.91%. During the period of neutropenia, thirty-eight patients developed bacterial infections, one of CMV pneumonia, 2 of CMV enteritis, 9 of CMV antigenaemia and 11 of virus cystitis. Of all the patients, 15 died from infection (5 cases), relapse (6) and cGVHD (4). The transplantation related mortality was 15.79%.

Conclusion
These results demonstrated that tumorablative allogeneic hematopoietic stem cell transplantation is safe and effective choice for the treatment of high-risk and refractory leukemia.

No conflict of interest to disclose
A Case of Poor Mobilized Relapsed Multiple Myeloma Successfully Transplanted Using Plerixafor

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Aim
Some heavily treated multiple myeloma patients had difficulties in collecting enough stem cells for autologous transplantation. Plerixafor is a novel CXCR4 chemokine-receptor antagonist for autologous hematopoietic stem cell (HSC) mobilization. Here is a case of successful myeloma transplant using plerixafor.

Method
A forty-nine year old woman was diagnosed multiple myeloma in 2007. She received VAD chemotherapy for 10 cycles in 2007. Myeloma recurred in August 2009, and was treated with melphalan/thalidomide/prednisone chemotherapy for 7 cycles. Myeloma relapsed in July 2010 and she was treated with bortezomib for 3 cycles and regimen was changed to VAD again because of the cost. VAD was maintained until February 2011. Initial mobilization chemotherapy with cyclophosphamide was done at the end of March. HSC harvest with G-CSF was done in 4 apheresis sessions. Total dose of CD34 positive cells was 1.9x10⁶ cells/kg which unmet the minimal cell dose of autotransplant. There were compassionate use program (CUP) of plerixafor. The patient’s disease status was sufficient for the CUP criteria of plerixafor. HSC mobilization and apheresis schedule for plerixafor in conjunction with G-CSF as described in phase III trials.

Result
Three apheresis were done and each apheresis yielded 1.46x10⁶, 1.66 x10⁶ and 0.98 x10⁶ CD34 positive cells/kg respectively. Autologous transplantations were done in June 2011. The number of infused CD34 positive cells was 3.12x10⁶ cells/kg. Engraftment was confirmed 10days after transplantation. Follow up bone marrow aspiration, serum protein electrophoresis and serum-free light results will be presented.

Conclusion
Plerixafor gained Food and Drug Administration (FDA) approval in 2008 and European Medicines Agency (EMEA) approval in 2009 for autologous HSC mobilization in non-Hodgkin lymphoma and multiple myeloma. In Korea, plerixafor can be purchased via orphan drug centres and the price of plerixafor is quite expensive. Plerixafor is a very useful drug for the poor mobilizer in non-Hodgkin lymphoma and multiple myeloma

No conflict of interest to disclose
P058  
Haploidentical Hematopoietic Stem Cell Transplantation Without T Cell Depletion for Treatment of Hematological Malignancies

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Aim  
Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) provides an opportunity for nearly all patients to benefit from HSCT when an HLA-identical sibling donor is not available. Here, we report the results of analysis in 28 patients who underwent haplo-HSCT at our center.

Method  
From 2007-2011, 28 patients with different hematologic malignancies underwent haploidentical hematopoietic stem cell transplantation without T-cell depletion. Of 28 patient/donor pairs, 26 were mismatched at 2 or more HLA loci and 2 at 1 locus. 21 patients received busulfan/cyclophosphamide-based conditioning regimens plus antithymocyte globulin (ATG), whereas other cases were preconditioned with ATG and fludarabine with or without melphalan, regarding primary diseases. Cyclosporine and methotrexate were used for graft-versus-host disease (GVHD) prophylaxis.

Result  
Haplo-HSCT was performed in 21 cases of acute leukemia (there were 3 patients in CR1, 16 in more than or equal to CR2, and 2 in non-remission), 2 of Fanconi anemia, 2 of osteopetrosis, and 3 other disorders. The median age was 16.5 years (range, 5 months to 47.5 years) and 57% of the transplants performed in adults (aged>15 years). Engraftment was successfully established in 24 patients (85.7%) and graft rejection occurred in only four patients (14.3%). The median times for neutrophil and platelet recovery were 14 and 19 days, respectively. Among the 25 engrafted patients, only 6 (21.4%) developed severe aGVHD (grade III or IV). The median follow-up period was 3.5 months. The one-year overall survival (OS) was 43.4%. Out of 28 patients, 15 (53.6%) died of relapse, infections and GVHD.

Conclusion  
Haplo-HSCT can be an alternative option for the treatment of patients who need urgent allogeneic transplantation but lack HLA-identical family donors.

No conflict of interest to disclose
P059
Outcomes of Unrelated Donor Peripheral Hematopoietic Stem Cell Transplantation for Children with Hematopoietic Diseases

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Objective
To evaluate the efficacy of matched and mismatched unrelated donor peripheral hematopoietic stem cell transplantation (URD-PBSCT) and influencing factors in children with hematopoietic diseases.

Method
Retrospective analysis was performed on clinical data about 15 consecutive children who received URD-PBSCT between June 2003 and December 2010. Patients were divided into two groups according to the HLA matched or mismatched. Myeloablative conditioning regimen based on Busulphan and CTX was applied to all the children except 1 pure red cell aplasia (PRCA) and 1 acute lymphoblastic leukemia (ALL) patient, Fludarabine was given to the other patients with ALL or severe thalassemia. ATG 7.5-22.5 mg/kg + cyclospofine A [CSA, 3 mg/(kg·d) with serum trough levels 150-300ng/ml + methotrexate (MTX) were administered at graft versus host disease (GVHD) prophylaxis. Mycophenolate mofetil [MMF, 30 mg/(kg·d)] was added for 2 patients.

Results
The median age was 12 (2-18) years with the median follow up period of 15 (6-96) months. The estimated 3 years overall survival (OS) was 89%; no patients died of transplant related mortality. One (6.67%) patient died of leukemia relapse. Four patients (26.67%) experience hemorrhagic cystitis. The development of grade III~IV acute GVHD (aGVHD) was significantly higher in the HLA mismatched group than that in the HLA matched group (55.56% vs 0). The re is no significantly difference in chronic GVHD development between the two groups (33.3% vs 44.4%).

Conclusion
The outcome of URD-PBSCT for Chinese children with hematopoietic disease is encouraging. For URD-PBSCT, single mismatches at HLA-A or HLA-C appear tolerated with apropriate conditioning regimens and is associated with higher rates of severe aGVHD.

No conflict of interest to disclose
P060
Outcome Analysis of Haematopoietic Stem Cell Transplantation in Idiopathic Severe Aplastic Anaemia and of Predictive Factors Associated With It: 10 Years Data from a Single Centre in Pakistan

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Aim
Hematopoietic stem cell transplantation (HSCT) has a curative potential for the treatment of severe aplastic anemia. The aim of this data analysis is to assess the outcome of children and adult patients who underwent HSCT in the previous 10 years and to analyze the predictive factors which are associated with survival.

Method
From 2000 to 2010, 80 patients with idiopathic acquired aplastic anemia received allogeneic HSCT from their matched sibling donors. Cyclophosphamide 50 mg/kg/day for 4 days was given to every patient for conditioning. Six patients who previously failed immunosuppressive treatment and were heavily transfused received ATG 5 mg/kg/day for four days as well. In 70 patients, the source of stem cells was mobilized peripheral blood, six patients received bone marrow, while four received both PBSC and bone marrow. Statistical analysis was done by using SPSS version 17 and outcome analysis based on Kaplan Meier. Variables analysis was done, relating to patients, disease and transplant in order to revealed predictors of outcome.

Results
With 7 years follow-up, the actual 1, 2, 3, 4 and 5 years survival was 61%, 54%, 49%, 47% and 41% respectively. Survival is better in children as compared to adults. Multivariate and univariate analysis was done. Three variables: i.e. time period between diagnosis and transplant and graft versus host disease and regimen related toxicity associated with poor outcome in multivariate analysis. Univariate analysis recognized 6 predictive factors associated with poor outcome ie older age (age cut off 15 yrs), number of blood and platelet transfusions during transplant, chronic GvHD, prolonged time period between diagnosis and transplant, number of platelet transfusions before HSCT and secondary graft failure. Leading cause of death was relapse of disease (13) in 28.3 patients.

Conclusion
There is a need to improve the results of HSCT in severe aplastic anaemia by recognizing factors which are predictive of poor survival and selecting those patients who have comparatively better outcomes.

No conflict of interest to disclose
P061
Comparative Effect of Antithymocyte Globulin versus Antilymphocyte Globulin for the Prevention of Graft versus Host Disease Prophylaxis after Allogeneic Hematopoietic Stem Cell Transplantation

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Aim
To compare the therapeutic effects and side effects of the rabbit antithymocyte (ATG) versus swine antilymphocyte globulin (ALG) within the preparative regimen of allogeneic hematopoietic transplantation (allo-HSCT) for graft versus host disease (GVHD) prophylaxis.

Method
102 patients admitted to our hospital and treated with allo-HSCT with the preparative regimen including ATG (10mg/kg)/ALG (45 mg/kg) were followed up from June 2002 to June 2008. They were divided into ATG group and ALG group. The allergic reaction, effect of GVHD prophylaxis, transplantation-related mortality (TRM), disease-free survival (DFS) and relapse rate (RR) between the two groups were retrospectively analyzed. Cumulative rates were analyzed by the Kaplan-Meier method and the factors associated with the III~IV aGVHD were analyzed with the COX regression model.

Results
ALG group had more allergic reaction than ATG group, but less bacteremia and cytomegalovirus (CMV) antigenaemia. The haematopoiesis reconstitution was comparable in the two groups. The III~IV aGVHD, two-year TRM, DFS and RR were 40% vs 21% (P=0.028), 54% vs 29% (P=0.039), 41% vs 53% (P=0.174), 10% vs 24% (P=0.306), respectively in ATG and ALG groups. In multivariate analysis, ATG was as a protective variable to III~IV GVHD occurrence (RR=0.53; 95%CI: 0.38 ~ 0.71). The CD3+ cell counts of administration was associated with an increased risk for III~IV GVHD occurrence (RR=4.43; 95%CI: 3.87 ~ 4.95).

Conclusion
Compared with ALG, 10mg/kg ATG significantly decreased the risks for III~IV AGVHD and extensive chronic GVHD (ecGVHD). The lethal infections became the most important cause of death in the ATG group, but the increased risk for infection did not neutralize the reduction of TRM induced by the decrease of severe GVHD.

No conflict of interest to disclose
P062
Ophthalmological Examination before Hematopoietic Stem Cell Transplantation

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Background
Systemic workup before hematopoietic stem cell transplantation (HSCT) is routinely performed in order to prevent regimen-related toxicities and infectious diseases. However, the necessity of ophthalmological examination remains to be established.

Methods
We retrospectively reviewed the medical records of 29 patients with various hematological malignancies including AML (n=9), ALL (n=4), MDS (n=5), and lymphoma (n=11). They underwent HSCT (allogeneic, n=21; autologous, n=8) in our hospital between April 2010 and June 2011.

Results
Ophthalmological examination was performed for 18 patients (allogeneic, n=13; autologous, n=5). Thirteen patients had some findings including cataract and myopia which were not clinically problematic, whereas five patients had significant findings which could lead to complications in performing HSCT (fundus hemorrhage, n=2; fungal endophthalmitis, n=1; dacycystitis, n=1; glaucoma, n=1). Among these five patients, only one patient with dacycystitis had ocular symptoms. Dacycystitis was treated with ocular instillation of moxifloxacin hydrochloride, and it was cured before the initiation of a conditioning regimen. For two patients with fundus hemorrhage, we could safely perform HSCT by close monitoring and appropriate transfusion of platelet concentrates. Fungal endophthalmitis was treated with systemic and intravitreal voriconazole before HSCT, and its exacerbation was not observed during HSCT. Although one patient was diagnosed as having mild glaucoma, only close observation was recommended by the ophthalmologist. Thus, all patients received appropriate therapy and had no serious complications.

Conclusion
Our results suggest that ophthalmological examination before HSCT may be considered as a routine work up even for patients without ocular symptoms.

No conflict of interest to disclose
P063
Twenty Years Experience of Stem Cell Transplantation in Iran

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Aim
From March 1991 through June 2011, a total of 3299 hematopoietic stem cell transplantations (HSCTs) were performed in the Hematology-Oncology and Stem Cell Transplantation Research Center, affiliated to Tehran University of Medical Sciences. We report here 20 years of experience with HSCT.

Method
Totally, 3299 patients underwent HSCT. Of these, 2250 patients received allogeneic stem cell transplantation, 1032 autologous-HSCT, and 18 syngeneic. Among 2250 patients who underwent allogeneic-HSCT, 34 received cord blood units. It is important to point out that the cord blood bank at our center provided facilities for patients' treatment.

Result
Stem cell transplantation was performed for various diseases. The main indications were acute myelogenous leukemia (AML, n=840), thalassemia major (n=512), Acute lymphoblastic leukemia (ALL, n=473), lymphoma (n=444), chronic myelogenous leukemia (CML, n=240), severe aplastic anemia (SAA, n=186), inherited abnormalities of RBC (n=77) and solid tumors (n=70). There were also 220 cellular therapies for postmyocardial infarction, multiple sclerosis, cirrhosis, head of femur necrosis, diabetes mellitus and GVHD treatment. 47 patients were retransplanted in this center. 2801 patients were treated with peripheral blood hematopoietic stem cell transplantation. The donor types for 2250 allogeneic patients were 2092 (93%) Human Leukocyte Antigen (HLA) matched-identical siblings, 56 (2.5%), HLA mismatched sibling/other relatives, 79 (3.5%) HLA matched other relatives and 23(1%), unrelated. About 78.1% of the patients (2624 of 3299) remained alive between one to 211 months after stem cell transplantation. Nearly, 21.9% (722) of our patients died after stem cell transplantation. The causes of deaths were relapse, infections, hemorrhagic cystitis, graft-versus-host disease, and others.

Conclusion
In Iran, HSCT has been successfully adapted in routine clinical care. New methods such as double cord blood and haploidentical transplantation are currently being used at our center.

No conflict of interest to disclose
P064
The Role of Pre-Treatment Comorbidities Index in Defining Outcomes in Patients Undergoing Allogeneic Transplantation

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Hypotheses
1. Patients with greater comorbidities (haematopoietic cell transplantation-specific comorbidity index [HCT-CI] score ≥2) have favourable non-relapse mortality [NRM] when conditioned with reduced-intensity Fludarabine / 2 Gy Total Body Irradiation [Flu/TBI] compared to more intensive regimens.
2. Reduced-intensity (Flu/TBI) transplantation abrogates the impact that comorbidities leading into transplantation have on non-relapse mortality.
3. Outcome of patients that underwent allogeneic transplantation at The Alfred is comparable to published data.

Method
Retrospective cohort analysis using the allogeneic HCT-CI database compiled at The Alfred Hospital, Victoria, Australia for patients that underwent allogeneic HCT between January 2008 and December 2010. Subset analysis performed according to transplant conditioning intensity. Data analysed with GraphPad Prism software to assess Kaplan-Meier survival curves and comparison using Log-rank (Mantel-Cox) test.

Result
110 patients included; 17 died of relapsed disease and 18 of NRM. Forty patients had HCT-CI scores ≥2; 9 underwent Flu/TBI conditioning (NRM=1), and 31 underwent more intensive regimens (NRM=6). Twenty-nine patients underwent Flu/TBI conditioning; 20 scored HCT-CI 0-1 (NRM=1), and 9 scored HCT-CI ≥2 (NRM=1). Sub-analysis of the entire cohort showed comparable overall survival and NRM to published data.

Conclusion
1. Patients with a greater number of comorbidities (HCT-CI ≥2) showed a trend toward improved NRM with reduced-intensity Flu/TBI conditioning.
2. Reduced-intensity Flu/TBI conditioning abrogates the impact that comorbidities have on NRM.
3. Overall survival and NRM of patients that underwent allogeneic HCT at The Alfred between 2008 and 2010 are comparable to published data.

No conflict of interest to disclose
P065
Hematopoietic Stem Cell Transplantation in Pediatrics: Report from the Hematology-Oncology and Stem Cell Transplantation Research Center, Iran 1991-2011

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Aim
Hematopoietic stem cell transplantation (HSCT) is a curative treatment for many malignant and non-malignant diseases. This is a follow-up report of pediatric patients who were treated with transplantation therapy in Hematology-Oncology and Stem Cell Transplantation Research Center affiliated to Tehran University of Medical Sciences, Tehran, Iran.

Method
A total of 915 pediatric patients (547 males/368 females) with a median age of 8 years (range: 4 month-15 years) underwent HSCT between 1991 and 2011. It has to be stressed that a separate pediatric unit officially opened in 2007. The most common indications for allogeneic HSCT were thalassemia (431, 53.6%), acute myeloid leukaemia (AML, 99, 12.3%) and acute lymphoblastic leukaemia (ALL, 90, 11.2%). Most patients who underwent autologous HSCT were diagnosed with AML (55, 50.9%) and Neuroblastoma (16, 14.8%).

Result
Of these, 804 (87.9%) received allogeneic, 108 (11.8%) autologous and 3 (0.3%) syngeneic transplants. Among allo-transplants, 474 (59%) were performed using peripheral blood (PB), whereas 290 (36%) were bone marrow (BM), 34 (4.2%) cord blood (CB) and 6 (0.8%) combined PB with BM; they were performed using HLA-identical sibling donors in 692 cases (86.1%) and alternative donors (other relative full match, partially-matched relatives or unrelated donors) in the remaining 112 (13.9%) cases. Of the auto-transplants, 25 (23.1%) were performed using BM, and 83 (76.9%) using PB. About 80.9% of the patients (740 of 915), remained alive between one to 195 months after HSCT. The most common causes of death were relapse, graft-versus-host disease and infections.

Conclusion
HSCT is an established therapy for children with a range of life-threatening illnesses. These data describe the development and current status of HSCT in our center. Moreover, new methods such as double cord blood and haploidentical transplant are used frequently.

No conflict of interest to disclose
P066
Treatment of Inborn Errors of Metabolism with Allogeneic Hematopoietic Stem Cell Transplantation

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Aim
Inborn errors of metabolism (IEM) are a group of inherited disorders that affect growth and development as well as neurologic and cognitive functions. Infants with metabolic disorders are usually normal at birth but clinical deterioration usually occurs shortly thereafter.

Methods
In this retrospective study, we analyzed the outcome of 19 patients (14 boys, 5 girls) with Osteopetrosis (n=13), Hurler syndrome (n=2), Maroteaux-Lamy (n=2), and Niemann–Pick type B (n=2) who had received HSCT between 2007 and 2011. The median age at transplantation was 23.3 months. Patients were transplanted from HLA-identical sibling (n=5), matched relative (n=7), HLA-haploidentical relative (n=3) and unrelated partially matched cord blood (n=4). The source of stem cell were bone marrow (n=8), peripheral blood (n=7) and cord blood (n=4). All patients received a conditioning regimen based on the use of busulfan in combination with cyclophosphamide. Cyclosporine with methotrexate was used as graft-versus-host disease (GVHD) prophylaxis regimen.

Results
At the present time, 16 patients with median follow up of 18 months are still alive and 14 patients are disease-free. Four out of 13 patients who achieved complete engraftment, had grade III-IV acute GVHD. They also had favorable response to therapy. There was no evidence of chronic GVHD in patients.

Conclusions
Although the results of this study indicate that transplant from HLA-identical sibling and matched other related donor results in long-term survival in all patients, transplantation from haploidentical related and unrelated partially matched cord blood requires more caution. It should be noted that in patients affected by genetic disorders (due to consanguineous marriage), donor selection among other related donors should be carefully considered.

No conflict of interest to disclose
P067
Non-Myeloablative Hematopoietic Stem Cell Transplantation for Patients With Hemophagocytic Lymphohistiocytosis

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Aim
Hemophagocytic lymphohistiocytosis (HLH) is a highly fatal disorder in children which represents the accelerated phase of some disorders such as chediak-Higashi syndrome, Griscelli syndrome and Familial Erythrophagocytic Lymphohistiocytosis (FEL). HSCT is the only curative treatment option for patients diagnosed with HLH. Since 2007, we have performed prospectively HLH transplantation with reduced-intensity conditioning (RIC) regimen which was due to their coexisting morbidity.

Methods
We prospectively analyzed the outcome of HSCT in eight children with Chediak-Higashi syndrome (n=1), FEL (n=3) and Griscelli syndrome (n=4) from June 2007 to January 2011. During this study, three patients were in accelerated phase at the time of transplantation. The median age at transplantation was 22.25 months. Seven patients underwent full matched related donor transplantation and one patient was transplanted from 2 locus mismatch unrelated cord blood donors. All of the patients received fludarabine/melephalan/ATG reduced-intensity conditioning. Cyclosporine and methylprednisolone were used as Graft-versus-host disease (GVHD) prophylaxis regimen.

Results
Engraftment occurred in all patients. At the present time, six patients with median follow-up of 25 months are still alive. Two patients died because of sepsis on days +16 and +165. They were in accelerated phase at the time of transplantation. Three patients who developed grade III-IV acute GVHD had a favorable response to therapy. Chronic GVHD occurred in one patient.

Conclusions
The results of this study show that use of RIC regimen with less toxicity instead of myeloablative conditioning regimens appear to be more reasonable. Early transplantation (in remission phase) and the use of reduced intensity conditioning regimen are suggested to improve patient satisfaction.

No conflict of interest to disclose
P068
Non-Oliguric Renal Failure Complicating Adult Dual Unrelated Cord Blood Transplant

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Introduction
The use of cord blood units as sources of haemopoietic stem cells in allogeneic bone marrow transplantation necessitates the addition of chemical cryoprotectants to preserve stem cell viability during the freezing process. Subsequent infusion of agents such as dimethylsulfoxide (DMSO) can be associated with adverse outcomes.

Case Report
A 17 year old female was diagnosed with Acute Myeloid Leukaemia (AML) with myelodysplasia related changes and a poor risk cytogenetic abnormality; t(6:9)(p23;q34) in addition to the poor prognostic molecular marker of FLT3-ITD. She demonstrated persistent cytogenetic disease, despite morphological remission post induction, and proceeded to dual unrelated cord blood transplantation in 1st remission with Fludarabine, Cyclophosphamide and TBI conditioning. Cyclosporin (CsA) and Mycophenolate Mofetil (MMF) was used as graft versus host disease (GVHD) prophylaxis. 1 of the cord blood units was red cell depleted and both had been cryopreserved with DMSO. 12 hours following infusion of the cord blood units, despite peri-infusion hydration, acute kidney injury (AKI) developed with creatinine rising from baseline values of 61µmol/L (NR 64-99 µmol/L) to 225µmol/L, and peaking at 823 µmol/L 5 days later. Although urine dipstick was positive for red cells, haemoglobin testing on urine several later was negative (myoglobin positive only). Potential contributors to AKI included intercurrent use of potential nephrotoxins acyclovir and CsA, as well as free haemoglobin from the cord blood units. The potential for renal toxicity related to the cryoprotectants in the cord blood units could also not be discounted. Although the AKI responded post institution of haemodialysis, GVHD prophylaxis was compromised due to interruption of calcineurin inhibitors. The patient developed stage IV gut and liver graft versus host disease unresponsive to steroids, ATGAM and etanercept and died 65 days post transplantation.

Conclusion
AKI complicating cord blood transplantation is infrequently reported and probably represents a multifactorial insult including contribution of nephrotoxic drugs and free haemoglobin as well as cryopreservation agents from the cord units. Clinicians should be aware of the potential for this complication in this setting.

No conflict of interest to disclose
P069
Comparison of the Yield and Outcomes According to Infused Stem cells Collecting from Each Consolidation Cycles in Patients with Acute Myeloid Leukemia in First Complete Remission who Underwent Autologous Stem Cell Transplantation

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Aim
Autologous peripheral blood stem cell transplantation (APBSCT) is increasingly being used in patients with AML. However, which consolidation chemotherapy cycles are the most compatible in terms of the yield of progenitor cells and outcomes after transplant has not yet been determined.

Methods
49 mobilization procedures were performed on 28 patients with AML who underwent APBSCT enrolled at three centers. The treatment protocols administered to patients with AML consisted of an induction chemotherapy in combination with cytarabine plus idarubicin, and consolidation chemotherapy with high dose cytarabine. In all patients, the collection of PBSC was performed during recovery after consolidation chemotherapy.

Results
First, second and third consolidation cycles were 17, 24 and 8, respectively. According to the total collected CD34+ cells, two groups were considered, that is, poor mobilizers (PM), with a collection of < 2 × 10^6/kg, and good mobilizers (GM), with a collection of ≥ 2 × 10^6/kg. Of 49 consolidation cycles, 15/17 (88.23%), 16/24 (66.66%) and 3/8 (37.5%) were GM after first, second and third consolidation cycles, respectively (P=0.012). When PBSC mobilization after first plus second and third consolidation chemotherapy was compared, the 2-year disease-free survival (DFS) rate was 36.1% and 64.3% (P=0.161), and the 2-year overall survival (OS) rate was 34.9% and 80% (P = 0.190). 2-year DFS was shorter in patients who were infused with < 2 × 10^6/kg CD34+ cells at the time of transplant compared to those with ≥ 2 × 10^6/kg CD34+ cells (25.0% vs 53.8%) (P = 0.169). Relapse rate after stem cells infusion which mobilized from first, second and third consolidation cycles were 60% (3/5), 50% (4/8) and 2/6 (33.3%), respectively.

Conclusion
If infused CD34+ cells are adequate, the stem cells harvested after third consolidation chemotherapy may be the best source for APBSCT in view of relapse rate and DFS.

No conflict of interest to disclose
P070
Highly Variable Pharmacokinetics of Once-daily Intravenous Busulfan When Combined with Fludarabine in Pediatric Patients: Phase I Clinical Study for Determination of Optimal Once-daily Busulfan Dose Using Pharmacokinetic Modeling

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Aim
We performed therapeutic drug monitoring (TDM) after once-daily IV busulfan combined with fludarabine to find out busulfan pharmacokinetics in hematopoietic stem cell transplantation (HSCT) using a targeted busulfan/fludarabine (+/- etoposide) regimen in children.

Method
IV busulfan was administered once daily for 4 consecutive days. The daily target area under the curve (AUC) was 18,125-20,000 ug*h/L/day, which was reduced to 18,000-19,000 ug*h/L/day after a high incidence of toxicity was observed. A total of 24 patients were enrolled and 20 patients were performed daily TDM.

Results
After infusion of busulfan on the first day, patients showed AUC that ranged from 12,079 to 31,660 ug*h/L/day (median 16,824 ug*h/L/day, percent coefficient of variation (%CV) =26.5%), with clearance of 1.74-6.94 mL/min/kg (median 4.03 mL/min/kg). During the daily TDM, the actual AUC ranged from 73-146% of the target AUC, showing high intra-individual variability. The %CV of busulfan clearance of each individual ranged from 7.7-38.7%. The total dose of busulfan administered for 4 days ranged from 287.3 mg/m² to 689.3 mg/m². Graft failure occurred in 3 patients with AUC less than 74,000 ug*h/L/day, and two patients with relatively high AUC experienced veno-occlusive disease.

Conclusion
Busulfan pharmacokinetics showed high inter- and intra-individual variability in HSCT using targeted busulfan/fludarabine regimen, which indicates the need for intensive monitoring and dose adjustment to improve the outcome of HSCT. Currently, we are performing a newly designed phase II study to decrease regimen-related toxicities and reduce graft failure by setting an optimal target AUC based on this study.

No conflict of interest to disclose
**P071**

**Second Generation TKI Nilotinib Inhibits Proliferation and Osteoblast Differentiation of Human Mesenchymal Stem Cells**

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**Aims**

Human bone marrow mesenchymal stem cells (hBM-MSCs) are multipotent cells found lining the bone marrow cavity supporting the growth and differentiation of hematologic progenitors. MSCs are considered therapeutically useful for hematopoietic stem cell transplantation. Nilotinib is one of second generation TKIs which is applied for the treatment of imatinib-resistant or -intolerant CML and Ph⁺ ALL. Whether the second-generation TKI nilotinib influences MSC proliferation and differentiation is still unclear. Here we examine the effects of nilotinib on the proliferation and osteoblast differentiation of hBM-MSCs.

**Methods**

Bone marrow was obtained from healthy adult donors and hBM-MSCs were cultured and identified as per previous report. MSCs were treated with nilotinib at therapeutic concentration (0-5.0µM). Cell proliferation was measured by Cell Counting Kit-8. To determine the effects of nilotinib on MSC differentiation, MSCs were cultured in osteoinductive medium. The mineralization was detected by Von Kossa and Alizarin-red S stain. ALP activity and Ca²⁺ contents were examined by commercially available kits. RNA expression levels of Osteoblast-specific genes were quantified by real-time PCR.

**Results**

In the range of 0.1 to 5.0µM, nilotinib inhibited hBM-MSC proliferation dose dependently. After 72h of incubation with nilotinib at the concentration of 0.1µM, 0.5µM, 1µM, and 5µM, the proliferation rate was reduced to 85.85±0.94%, 72.47±1.06%, 65.76±1.36%, and 56.53±1.60% respectively (P<0.05). Nilotinib dose dependently inhibited hBM-MSC early osteoblast differentiation by ALP activity detection and terminal differentiation of osteoblasts measured by reduced mineralization (Alizarin-red S stain and von Kossa's stain). Compared with control, the concentration of acid-solubilized calcium decreased significantly in presence of nilotinib at 14 days. In addition, nilotinib reduced the expression of both relative mRNA expression levels of osteoblast-specific transcription factors (Runx2 and Osterix) and osteoblastic markers (BSP and OPN).

**Conclusion**

Our data indicated that nilotinib inhibits the proliferation and osteoblast differentiation of hBM-MSCs in vitro.

No conflict of interest to disclose
P072
Derivation and Propagation of Human Induced Pluripotent Stem Cells on Human Bone Marrow Mesenchymal Stem Cells

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Aims
The generation of human induced pluripotent stem cells (hiPSCs) from adult human somatic cells by over expression of a few key transcription factors is greatly excited. It provides a new way to obtain pluripotent cells which have the potential to develop into every cell of the body without destruction human embryos. Derivation and propagation of hiPSCs are typically achieved by cultured on mouse embryonic fibroblasts (MEFs) as feeders. It is important to reduce xenogenic components for therapeutic usage of hiPSCs. The aim of this study is to investigate whether human bone marrow mesenchymal stem cells (hMSCs) can be used as feeder cells to support the derivation and propagation of human iPSCs.

Methods
We used passage 3-5 inactivated hMSCs as feeder cells. iPSCs were established from child foreskin fibroblasts by ectopic transcription “OKMS” factors using retroviral infection.

Results
We demonstrated that inactivated hMSCs can be used as feeder cells to support the established and maintained of human iPSCs. We tested three (two female, one male) independent hMSCs for the potential to maintain self-renewal and pluripotent of human iPSCs. All the hMSCs tested can support the generation and propagation of human iPSCs. The expansion efficiency was 2.32±0.05, and the growth of human iPSCs on hMSCs from various donors and different passages (p2-p5) was similar. After proliferation over 14 passages on these hMSCs, human iPSCs maintained the undifferentiated state and normal karyotypes. They expressed undifferentiated pluripotent cell markers and genes, and could differentiate into all three germ layers via embryoid body and teratoma formation.

Conclusions
These results suggested hMSCs can be used as feeder cells to derivate and maintain human iPSCs. Our results provide a feasible method to avoid using mouse feeder cells with human iPSCs, and make an important step toward the establishment of clinical grade iPS cells.

No conflict of interest to disclose
P073
Induction of Dendritic Cells With Multidrug Resistance Property From K562/MRD1 Cell Lines

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Aims
To investigate the influence of MDR1 gene transfection on the differentiation efficiency of K562 cell into dendritic cells (DC) and the multidrug resistance property of the induced K562/MDR1-DC cells.

Methods
K562/MDR1 cells and K562 cells were cultured in the presence of GM-CSF and IL-4 to generate DC and matured by TNF-α. On day 14, K562/MDR1-DC and K562-DC cells were harvested and the expression of CD1a, CD83, CD80, CD86, HLA-ABC, HLA-DR was assessed by flow cytometry. The antigen presentation function of K562/MDR1-DC and K562-DC was determined by allogenic mixed lymphocyte reaction (Allo-MLR). The expression of P-glycoprotein and the intracellular accumulation of daunorubicin (DNR) detected by FCM to assess the expression and function of MDR1 gene during differentiation; the sensitivity of K562/MDR1-DC and K562-DC cell to vincristine adriamycin were measured using MTT assay.

Results
Both of K562/MDR1 and K562 cells could differentiate into dendritic cells in the presence of cytokine cocktails, showing the morphologic and immunophenotypic characteristics of DC. K562/MDR1-DC induced more potent proliferation of allogenetic lymphocytes in MLR than K562-DC. High level expression of Pgp and efflux of DNR were demonstrated in K562/MDR1-DC. Meanwhile K562/MDR1-DC showed multidrug resistanc property, with much greater IC50 to VCR and ADM than that of K562-DCSs.

Conclusions
MDR1 gene transfection does not affect the differentiation efficiency of K562 cells into dendritic cells (DC). The induced K562/MDR1-DC cells express high level of Pgp and acquire the multidrug resistance property. Dendritic cells with antigen presenting function and multidrug resistance property provide promising tools for immunotherapy in leukemic relapse after chemotherapy or hematopoietic stem cell transplantation.

No conflict of interest to disclose
P074
Induction and Large-scale Expansion of Human Regulatory γδ T cells with Potent Suppression on Graft-versus-host Disease

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Aims
Graft-versus-host disease (GVHD) is a major cause of mortality and morbidity after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Regulatory γδ T cells (γδ Tregs) is a novel subset of cells with immunosuppressive function while the method for γδ Tregs induction and expansion is rarely introduced and its role in GVHD prevention remains unknown. The aim of this study is to establish a γδ Treg culture system and examine the ability to suppress GVHD.

Methods
Human peripheral blood mononuclear cells (PBMCs) were cultured with IL-2, IL-15, TGF-β1 and zoledronic acid. γδ Tregs were enriched by magnetic cell sorting system (MACS) and monitored for CD69, HLA-DR, CD45RA, CD45RO, CD25 and CTLA-4 expression by flow cytometry (FACS) 10 days later. They were tested for their ability to suppress proliferation of alloreactive PBMCs. Supernatant levels of IL-10, IL-4 and TGF-β1 were measured by ELISA. Using a chimeric humanized mouse system, we transplanted NOD/SCID mice with human PBMCs with or without human γδ Tregs. Survival time and GVHD manifestations of the transplanted mice were evaluated.

Results
γδ T cells containing 42.1% γδ Tregs were enriched. γδ Tregs expressed high levels of HLA-DR, CD69, CTLA-4 and CD45RA. Proliferation of alloreactive PBMCs was significantly reduced in the presence of enriched γδ Tregs. We also found that the suppression mechanism was cell-cell contact dependent and partially via TGF-β1. Transplantation of human PBMCs into NOD/SCID mice induced lethal GVHD with average survival time 25± 8 days, but the survival time was significantly prolonged (average 43± 5 days) and the clinical and histopathologic scores were reduced in mice co-transplanted with γδ Tregs in contrast to without γδ Tregs (P<0.05).

Conclusions
Human γδ Tregs could be induced and expanded in vitro from PBMCs. Expanded γδ Tregs retained suppressive activity and prevented lethal xenogeneic GVHD, revealing the therapeutic potential of expanded γδ Tregs for GVHD.

No conflict of interest
Twin Pregnancy Following Reduced-intensity Allogeneic Transplantation Combined with Imatinib Mesylate for Chronic Myeloid Leukemia

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Successful pregnancy and delivery after allogeneic hematopoietic stem cell transplantation (allo-HSCT) for chronic myeloid leukemia (CML) has rarely been documented. In the current study, we present the first report of a successful twin pregnancy and delivery after reduced-intensity conditioning stem cell transplantation (RIST) combined with imatinib mesylate (IM). A 19-year-old female was diagnosed with Philadelphia chromosome-positive CML in the chronic phase in June 2006. Imatinib was administered at a dose of 400 mg/day for about 6 months and obtained a cytogenetic response. The patient could not continue to use IM for economic reasons. RIST from her HLA-identical sister was performed in March 2007. The conditioning regimen was Flu+ Bu+ ATG.. Anti-graft-versus-host disease (GVHD) prophylaxis included CsA+MMF+MTX. Prophylactic IM at 400 mg/day was commenced on day +100 to prevent relapse and was discontinued at 24 months after transplantation. The patient did not present with obvious acute or chronic GVHD. Immunosuppressant was stopped one year after HSCT. Recovery of spontaneous menstruation occurred about 6 months after HSCT. She was found to be pregnant 2.5 years after HSCT and 6 months after withdrawal of IM, then delivered a pair of normal-weight healthy twins, after a full term pregnancy. There were no complications during pregnancy and no evidence of recurrence of CML after delivery. After a total follow-up of 4 years, the patient remains molecular response, and both children are in healthy growth without any physical defects. This case demonstrates that the RIST regimen may facilitate recovery of ovarian function. Combination therapy with short term IM may not have profound effects on female fertility.

No conflict of interest to disclose
**P076**  
**Favorable Long-Term Outcome of Nephrotic Syndrome Associated With Chronic Graft-Versus-Host Disease in Patients After Allogeneic Hematopoietic Stem Cell Transplantation**

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**Aims**  
Chronic graft-versus-host disease (cGVHD) is one of the most common late complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT) causing high morbidity and mortality. Renal involvement in patients with cGVHD presenting as nephrotic syndrome (NS) is a rare complication with few long-term outcome reports.

**Methods**  
348 patients with hematological diseases undergoing allo-HSCT were analyzed to evaluate the incidence and outcomes of NS from July 2005 to June 2011 in our single Center. They were conditioned with modified BU/CY. GVHD prophylaxis consisted of cyclosporine A (CsA), mycophenolate mofetil (MMF), and short-term methotrexate. The diagnosis of NS was based on clinical characteristics and pathological confirmation if possible.

**Results**  
Of 348 patients, 156 with a minimum follow-up of 100 days developed cGVHD while 6 developed NS. The cumulative incidence of NS in cGVHD was 3.85 %. Among the NS patients, 3 were female and 3 were male. They were diagnosed with AML(2), ALL(1), MM(1), NHL(1) and CML(1) respectively. 5 of them received HLA-matched allo-HSCT and 1 received HLA-mismatched allo-HSCT. All the 6 patients received peripheral blood stem cells. The median age at onset of NS was 33 years (range 18- 57), occurring at a median of 19 months (range 8 - 21) after HSCT. 5 patients had pathological examination and showed membranous glomerulonephritis. All 6 patients developed extensive cGVHD. Regarding therapeutic stratigies, tacrolimus and steroids were administered for 3 patients; additional MMF were administered for another patient while CsA and steroids were administered for the other patient. All patients achieved sustained remission of NS at a median time of 40 (range 27 - 50) months after transplantation.

**Conclusions**  
Patients with chronic graft-versus-host disease might be considered at risk for NS. Treatment with immunosuppression can reverse NS as well as cGVHD and achieve favorable sustained long-term remission.

No conflict of interest to disclose
P077
Central Neurological Complications Following Allogeneic Haematopoietic Stem Cell Transplantation

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Aims
Central neurological complications are big challenges in allogeneic hematopoietic stem cell transplantation (allo-HSCT) causing high morbidity and mortality. The aim of our study is to clarify the risk factors for NS and establish therapeutic strategies.

Methods
We retrospectively analyzed neurological complications (NC) in our single center. From August 2005 to June 2011, altogether 326 patients (range 10–54 years old) underwent allo-HSCT. 169 patients received matched unrelated donor (MUD) transplantation, 93 patients underwent sibling donor transplantation, and 64 patients received haploidentical transplantation. Risk factors and therapeutic strategies were analyzed.

Results
Central neurological complications post-HSCT were seen in 30 patients including 15 cases in MUD HSCT, 5 cases in sibling HSCT, and 10 cases in haploidentical HSCT. High incidence of NC was seen in haploidentical HSCT in contrast to MUD and sibling donor HSCT (15.6%, 8.9% and 5.4%, respectively). Most of the central neurological symptoms occurred in the early phase of transplantation. The frequency and type of neurologic complications depends on the type of HSCT, the underlying disease, and the case ascertainment. 8 cases were drug related encephalopathies and seizures (including 3 of Bu related, 4 of CSA related, and 1 of steroid associated). 5 cases suffered from encephalorrhagia, 6 cases suffered from relapse of original disease in CNS, 4 cases complicated with virus associated encephalitis/myelitis (2 of them were HHV-6 positive), 2 cases with demyelinating disease, two with TMA. Another 3 cases were undefined. 12 cases died of NC, including all encephalorrhagia or relapsed CNS-L patients and one TMA patient. Other patients were alived. High dose gamma globulin combined with methylprednisolone were administered in demyelinating disease. Antiepileptic agents were used in epilepsy. Cyanocobalamin was used in peripheral neuritis.

Conclusions
Haploidentical HSCT is a risk factor for central neurological complications. Encephalorrhagia and CNS-L caused high motality in HSCT recipients.

No conflict of interest to disclose
**P078**  
**Change in Quality of Life, Lean Body Mass and Nutritional Status following Haematopoietic Stem Cell Transplantation.**

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**Aim**  
The impact of haematopoietic stem cell transplantation (HSCT) on patients’ quality of life (QOL), body composition, and nutritional status are not well described in the literature despite reports of ongoing nutrition symptoms related to the treatment. Aim: The purpose of this study was to investigate changes in patients’ quality of life (QOL), lean body mass (LBM) and nutritional status after HSCT.

**Method**  
Patients undergoing HSCT (n=28; 64% male; mean age 56±12.3 years; 24 autologous-transplantation) were consecutively recruited from a private haematology clinic in Brisbane, Australia. Repeated measures for QOL, LBM, and nutritional status were assessed using EORTC QLQ-C30 questionnaire, air-displacement plethysmography (Bod Pod, COSMED USA, Inc.), and the patient-generated subjective global assessment (PG-SGA) respectively at 2 weeks pre-admission (T0), discharge (T1), and 100-days post-transplantation (T2). Change in outcome over time was assessed with pair-wise comparison or McNemar’s test.

**Result**  
At T0, 27/28 were well-nourished (mean PG-SGA score 3.0±3.3); no patient was underweight (BMI<18.5 kg/m²). At T1 all variables except global QOL were significantly different from T0. At T2, there were significant mean changes (95% CI) in weight -5.7 kg (-8.1, -3.2), p<0.001; LBM -1.3 kg (-2.3, -0.3), p=0.011; fat mass -4.2 kg (-6.4, -1.9), p=0.001 and nutritional status PG-SGA score 2.5 (0.8, 4.2), p=0.006 compared to baseline. Changes in global QOL scores did not reach statistical significance throughout the study. There were significant declines in all QOL subscales at T1 but only the emotional subscale was significant at T2 with a mean change of 14.7 (5.5, 23.8), p<0.001.

**Conclusion**  
Patients experience delayed recovery in weight and body composition up to 100-days post-HSCT. The findings suggest the need to explore the role of ongoing allied health support which may potentially lead to better outcomes in the long term.

*No conflict of interest to disclose*
P079
High Dose Etoposide as an Effective Mobilization Agent in NHL Previously Treated with CHOP±Rituximab

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Aim
Etoposide is one of the effective mobilizing agents in non-Hodgkin’s lymphoma (NHL), but safety and effectiveness of high dose etoposide followed by G-CSF is not well defined. We conducted a retrospective study to evaluate the efficacy of high dose etoposide plus G-CSF compared with other mobilizing agents in peripheral blood stem cell (PBSC) mobilization.

Method
A total of 46 patients with NHL who were treated only with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) ± Rituximab and sequentially underwent PBSC mobilization between 2006 and 2011 were analyzed. Twenty two patients received etoposide 500mg/m² at day 1, 2, 3 (VP16 group). Total 22 of other patients received ICE (ifosfamide, carboplatin, etoposide) or DHAP (cisplatin, cytarabine, dexamethasone) or CHOP ± Rituximab and 2 patients received G-CSF only (others group). All of chemo mobilization patients administered G-CSF 10µ/kg/day until apheresis completed.

Result
In the VP16 group, a median total CD34⁺ cells collected was 15.79x10⁶cells/kg (range 3.71-151.47), 77% of all patients having adequate (>2.0x10⁶ cells/kg) CD34⁺ collections after first day of apheresis compared with a median in the other group of 4.1x10⁶cells/kg (range 0.02-23.00) (P<0.001), with 41% having adequate collection after first day (P=0.015). None of 22 patients (0%) in VP16 group and 4 of 22 (19%) in others group failed to collect sufficient CD34⁺ cells (<2.0 x 10⁶ cells/kg) (P=0.038). Neutropenic fever developed in 14 patients (64%) in the VP16 groups, none of which were fatal, and 3 patients (14%) in the others group (P=0.05).

Conclusion
High dose etoposide improves the effectiveness of mobilization with more than threefold higher stem cell yield compared with other mobilizing agents. Considering no mortalities in neutropenic fever, high dose etoposide plus G-CSF is a highly effective mobilizing agent with acceptable toxicity in NHL who were previously treated with CHOP ± Rituximab.

No conflict of interest to disclose
P080
Non-myeloablative Transplantation With or Without T-cell Depletion in Unrelated Donor Stem Cell Transplantation

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Aim
For non-myeloablative stem cell transplantation (NST), graft-versus-host disease (GVHD) still remains a significant cause of morbidity and mortality. T-cell depletion has been used to reduce GVHD in unrelated donor stem cell transplantation. However, it may have an adverse impact on disease response, because NST has an antitumor activity via the graft-versus-tumor effect.

Method
To evaluate the efficacy of T-cell depletion, we analyzed 26 patients who underwent NST with or without T-cell depletion using unrelated stem cells. Twenty-five patients were acute leukemia and one patient was myelodysplastic syndrome. Conditioning regimens consisted of fludarabine based busulfex (n=20), melphalan (n=3), low dose TBI (n=1) and cyclophosphamide (n=2). Sixteen patients received alemtuzumab (n=12) or antithymocyte (n=4). The regimens for GVHD prophylaxis were FK506 with or without methotrexate (n=22) and cyclosporine with or without methotrexate (n=4).

Result
There were no significant differences in sex, age, status at transplantation and total infused CD34+ and CD3+ cells number between two groups. The group with T-cell depletion showed a tendency to increase in graft failure and rejection compared with the non-manipulated group (31% vs.10%, p=0.57). CMV disease after transplantation occurred in three patients, all of whom received T-cell depletion. There were no differences in the incidence of acute GVHD (≥ Gr II) and chronic GVHD (limited and extensive) between two groups (p=0.84, p=0.52 respectively). With a median follow-up of 175 days (range, 41-1618), event free survival at 1 year was 70% for non-manipulated group and 50% for T-cell depleted group (p=0.23). No differences were observed in overall survival at 1 year between two groups (65% vs. 39%, p=0.72)

Conclusion
In summary, T-cell depletion with NST using unrelated stem cells not only failed to show any benefits in the incidence of GVHD but was also associated with a higher incidence of CMV disease and graft rejection.

No conflict of interest to disclose
Follow-up Management Program of Unrelated Donor in Korea Marrow Donor Program (KMDP)

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Aim
The management of the unrelated donor after donation has not been properly conducted. We analyzed discomfort survey of donors to establish the efficient donor management program.

Materials and Methods
Eight hundred and thirty-eight donors who donated stem cells via KMDP from November 2007 to December 2010 were analyzed. Management of the donors was conducted by interview survey within 48 hours after donation and by telephone survey at 1 week, 4 weeks, and 4 months after donation.

Results
The ratio of male and female was 3.6:1 and the median age was 31.7 years old (range 19-53). Among 838 donors, 744 donated PBSC and 76 BM and 18 lymphocytes. Among BM donors, the change of hemoglobin level was -0.8 g/dL after donation. The main complaints during the donation were pain at puncture site, sore throat, and abdominal pain. The median visual analog scale (VAS) of discomfort of donation was 6.0/10 points, and limitation of daily activities 6.5/10. Among PBSC donors, mean WBC count before and after G-CSF administration was 3,570/mL and 41,500/mL, respectively, whereas mean platelet count 214,600/mL and 89,900/mL, respectively. 57.2% of donors required analgesics for control of G-CSF associated pain. The median VAS for discomfort by G-CSF injection was 4.8/10, and limit of daily activities 3.3/10. The median VAS for discomfort by PBSC collection was 3.7/10, and limit of daily activities 2.7/10. The 77% of all donors responded that they would donate again at the survey within 48 hours after donation, and 94% at 1 week after, 95% at 4 weeks and 4 months after donation.

Conclusion
The need for and the importance of unrelated donor management are increasing. BM donors felt more discomfort than PBSC donors. Based on these data, more effective and useful follow-up programs for unrelated donors should be established.

No conflict of interest to disclose
P082
The APBMT Activity Survey Over the Past 5 Years (2005-2009)

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Purpose
Over the past 5 years, the Asia-Pacific Blood and Marrow Transplantation Group (APBMT) has collected and accumulated hematopoietic stem cell transplantation (HSCT) data from the Asia-Pacific countries/regions. We demonstrate here the trend of HSCT in this area from 2005 to 2009.

Method
APBMT maintained statistics from 2005 based on the Activity Survey, which includes HSCT data by indication, stem cell source and donor type from the APBMT participating countries/regions every year.

Result
Fifteen countries/regions reported a total of 10,717 (as of June 30, 2011) HSCTs in 2009 with 6,506 (60.7%) allogeneic and 4,211 (39.3%) autologous. The main indications were leukemias (47.9%; 95.7% allogeneic), lymph-proliferative diseases (LPDs) (37.6%; 15.4% allogeneic), solid tumors (5.7%; 8.6% allogeneic), non-malignant hematological diseases (7.1%; 99.3% allogeneic) and non-hematological diseases (1.4%; 86.2% allogeneic). The ratio of related vs unrelated donors for allogeneic HSCT were 52.6:47.4, and the proportion of peripheral blood (PB) as stem cell source was 44.2% for allogeneic and 98.5% for autologous HSCT. In 2005, APBMT participants were 7 countries/regions and the total number of HSCTs was 4,598. Comparing the latest data with the initial data in 2005, the number of allogeneic HSCT was more increased (2.1 times) than autologous (2.9 times). Although the proportion of underlying disease did not change in these 5 years, the percentage of PB increased from 49.6% (2005) to 65.5% (2009).

Conclusion
With the increase of participating countries/regions to the APBMT survey and the progress of the HSCT technique, the trends have remarkably changed over past 5 years in this area. These results show the future importance of the role of data accumulation and analysis.

No conflict of interest to disclose
P083
Pandemic H1N1 Influenza Infections in Hematopoietic Stem Cell Transplant Recipients: Clinical Outcome and Emergence of Resistant Strain with H275Y

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Aim
There have been only limited reports of H1N1pdm in the HSCT recipients. The aim of the present study was to evaluate the characterization of H1N1pdm in five HSCT recipients and to emphasize the need for the optimal anti-viral management.

Method
We conducted a retrospective study in a series of five HSCT recipients with H1N1pdm as of February 2011 at our institution. The main analyses are clinical manifestation, in-hospital mortality, duration of viral shedding and emergence of resistant strain with H275Y.

Result
Among HSCT recipients, H1N1 positivity has been confirmed in 5 patients (3 allo-HSCT, 2 auto-HSCT), using real-time PCR. All patients presented with upper respiratory symptoms and 4 of 5 patients had also complicated lower respiratory tract disease and pneumonia at the onset of influenza. 2 patients have responded to the anti-viral treatment and recovered clinically, the remaining 3 patients died due to respiratory failure with severe pneumonia and steroid-refractory diffuse alveolar damage. Oseltamivir resistance with mutation H275Y was documented in 2 patients, who received oseltamivir treatment after diagnosis and post-exposure Oseltamivir prophylaxis respectively. They also displayed prolonged viral shedding over 23 days.

Discussion
These cases highlight the potentially serious effect of the H1N1pdm infection in HSCT recipients. The absence of an adequate immune response and possibly suboptimal drug levels may have contributed to the fatal clinical outcomes and the emergence of the H275Y mutant. Our experience provides the urgent need for novel and intensified approaches to influenza management. If HSCT recipients have severe lower respiratory tract disease or have received recent Oseltamivir prescription, we advocate considering combination therapy when possible, given the poor outcome and potential for the development of resistance, acknowledging that supporting data from randomized trials are still lacking.

No conflict of interest to declare
P084
Allogeneic Stem Cell Transplantation for Korean PNH Patients

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Introduction
PNH is a debilitating and life-threatening hematopoietic stem cell disorder characterized by chronic hemolysis leading to significant morbidities and mortality. Even though there are different therapeutic approaches for PNH, allotransplantation can cure the disease but cause several complications. We report here 33 Korean PNH patients who underwent allotransplantation.

Results
Patient ages ranged from 15 to 56 years (median 29 years, male 14, female 19). Classic PNH were 12 patients and PNH with bone marrow disease were 21. Median LDH Fold at the time of diagnosis was 3.36 Fold (0.74 – 16.04). The source of stem cells was bone marrow in 13 patients and peripheral blood in 20 patients. There were 23 sibling donors and 10 unrelated donors. Conditioning regimens consisted of BuCy or Cy/TBI (n=14), fludarabine containing reduced intensity regimen (n=18), or other. All patients attained successful leukocyte engraftment (median 13 days, range 10~30 days) and platelet engraftment (median 17 days, range 11~48 days). Grade II~IV acute GVHD were in 19 patients (skin 13, liver 3, gut 3). Grade II~IV chronic GVHD were in 13 patients (limited 10, extensive 3). With a median follow-up of 82.6 months (range, 19.5~160.2 months), 26 patients are alive with CR. Seven patients died after transplantation due to pulmonary hemorrhage (n=2), sepsis (n=2), GVHD/multi organ failure n=2), or massive thromboembolism (n=1). Patients with reduced intensity conditioning regimen had a better survival rate than patients with conventional conditioning regimen (p=0.027). There were no survival differences between classic PNH patients and PNH patients with BMD. There were also no differences between two groups categorized by PNH clone sizes, stem cell sources, and PNH related symptoms.

Conclusion
Our data demonstrate that reduced intensity conditioning regimen is suitable for allogeneic stem cell transplantation of PNH patients.

No conflict of interest to declare
P085
Post Hematopoietic Stem Cell Transplantation Thrombocytopenia is Related to the Low CD34 Cell Count.

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Background
Post hematopoietic stem cell transplantation thrombocytopenia (PTT) is one of the most common problems in hematopoietic stem cell transplantation (HSCT) and known to be related to survival in allogeneic stem cell transplantation.

Method
We analyzed patients who survived 3 months after SCT to evaluate the risk factors for PTT. PTT was defined as sustained thrombocytopenia less than 100k/ul until 3 months after transplantation despite successful granulocyte engraftment.

Results
Between 2005 and 2010, 134 patients underwent HSCT at Ajou University Hospital. One hundred twenty-two patients survived longer than 3 months. Of them, 77 patients received allo-HSCT and 45 received auto-HSCT. Eighty-three cases were PBSCT and 39 cases were BMT. Thirty patients (25%) showed PTT and median post-transplant platelet count was 37k. The incidence of PTT was no different in allogeneic or auto transplantation (16 allo-HSCT vs 14 auto-HSCT, p=0.31). There was no difference in age, sex, primary diagnosis between the patients with PTT and without PTT. In patients with PTT, the infused CD34 cell count was significantly lower (3.59 x 10^6/kg vs 6.03 x 10^6/kg, p=0.021) regardless of stem cell source. Granulocyte engraftment day was also delayed (12.5 days vs 11 days, p=0.04) in PTT patients. Though the pre-transplant platelet count was significantly lower in PTT patients (104k/ul vs 141k/ul, p=0.04), the number of pre-transplant platelet transfusion was no different between two groups.

In patients who received allo-HSCT, there were no differences in donor type (related or unrelated), HLA and ABO matching status between two groups. GVHD and CMV infection incidence were also not different. Survival analysis showed poor survival tendency in patients with PTT (7.5 months vs 26 months, p=0.15).

Conclusions
In HSCT, the infused CD34 cell count is an important risk factor for development of PTT. Solid megakaryocytic engraftment seems to need more CD34 cells than granulocyte engraftment.

No conflict of interest to disclose
P086

Professional Oral Health Care Reduces Oral Mucositis and Febrile Neutropenia in Patients Treated with Allogeneic Hematopoietic Stem Cell Transplantation

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Aim

Little is known about the effects of professional oral health care (POHC) on the outcome of hematopoietic stem cell transplantation (HSCT). We evaluated the effects of POHC given by dentists and dental hygienists on the development of oral mucositis and febrile neutropenia (FN) after allogeneic bone marrow transplantation (BMT).

Methods

We retrospectively studied 140 adult patients who had received allogeneic BMT, with or without POHC, in our hospital consecutively between February 2002 and December 2009. Oral mucositis was evaluated according to the World Health Organisation (WHO) scale.

Results

The incidence of oral mucositis was 66.7% (52/78) in the patients who had received POHC, compared to 93.5% (58/62) in the non-POHC group (P<0.001). The incidence of FN and the maximal level of CRP were also significantly lower in the POHC group. Multivariate analysis revealed that the POHC was significantly associated with the incidence of oral mucositis (odds ratio, 7.58; 95%CI, 2.45-23.34; P<0.001).

Conclusions

We concluded that POHC reduced the incidences of oral mucositis and FN by upgrading the overall oral hygiene during HSCT.

No conflict of interest to disclose
P087
BEAM vs LACE Conditioning Regimen in Lymphoma Transplants: Comparison of Toxicity, Efficacy and Outcome.

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Aim
BEAM is a commonly used conditioning regimen in lymphoma transplants. LACE (lomustine-200 mg/ m^2 x 1d, etoposide 1000mg/ m^2 x1d, ara-c 2000 mg/m^2 x 2d and cyclophosphamide 1800mg/ m^2 for 3d) is well tolerated and effective. We retrospectively compared the two conditioning regimens for toxicity, efficacy and outcome.

Method
Hodgkin’s disease (HD) and Non Hodgkin’s lymphoma (NHL) patients with primary refractory or relapsed disease from August 1994 to May 2011 were included. BEAM was used between August 1994 and March 2007 while from April 2007 onward, all but three received LACE. Thirty five patients received BEAM and 52 LACE. Fifty-nine patients had HD (BEAM-24, LACE-35) while 28 had NHL (BEAM-11, LACE-17). Oral mucositis was graded by WHO criteria and all other toxicities by CTCV3. Categorical data was analyzed using chi-square and continuous data with the Mann Whitney Test. OS and PFS were analyzed using Kaplan Meier survival analysis.

Result
Grade 3 and 4 oral mucositis was higher in BEAM (54% vs 3.8%; P=0.01). More patients in BEAM required parenteral nutrition (66% vs 29%; P=0.001). No patient in LACE developed VOD compared to 4 in BEAM. Transplant related mortality was higher in BEAM (31.4% vs 6%; P=0.002). The median days to neutrophil engraftment (11 vs 9; P=0.0000004) and platelet engraftment (18 vs 12; P=0.000015) were shorter in LACE. Two patients in BEAM and one in LACE developed pulmonary toxicity. Median duration of hospitalization was less in LACE (3 vs 4 weeks P=0.016). At 2.5 years, OS (BEAM -54.5%, LACE -66.2%) and PFS for all patients (BEAM-47%, LACE-34%) were comparable, though in HD subgroup, OS was superior with LACE (BEAM-45.7%, LACE-80.6%; P=0.014)

Conclusion
In our retrospective audit LACE is a better regimen with lesser toxicity, earlier engraftment and comparable survival rates. Randomised trials comparing the 2 regimens are needed.

No conflict of interest to disclose
**P087**

**BEAM vs LACE Conditioning Regimen in Lymphoma Transplants: Comparison of Toxicity, Efficacy and Outcome**

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**Aim**

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**Method**

Hodgkin's disease (HD) and Non Hodgkin's lymphoma (NHL) patients with primary refractory or relapsed disease from August 1994 to May 2011 were included. BEAM was used between August 1994 and March 2007 while from April 2007 onward, all but three received LACE. Thirty five patients received BEAM and 52 LACE. Fifty-nine patients had HD (BEAM - 24, LACE - 35) while 28 had NHL (BEAM - 11, LACE - 17).

Oral mucositis was graded by WHO criteria and all other toxicities by CTCV3. Categorical data was analyzed using chi-square and continuous data with the Mann Whitney Test. OS and PFS were analyzed using Kaplan Meier survival analysis.

**Result**

Grade 3 and 4 oral mucositis was higher in BEAM (54% vs 3.8%; P=0.01). More patients in BEAM required parenteral nutrition (66% vs 29%; P=0.001). No patient in LACE developed VOD compared to 4 in BEAM. Transplant related mortality was higher in BEAM (31.4% vs 6%; P=0.002). The median days to neutrophil engraftment (11 vs 9; P=0.0000004) and platelet engraftment (18 vs 12; P=0.000015) were shorter in LACE. Two patients in BEAM and one in LACE developed pulmonary toxicity. Median duration of hospitalization was less in LACE (3 vs 4 weeks P=0.016). At 2.5 years, OS (BEAM - 54.5%, LACE - 66.2%) and PFS for all patients (BEAM - 47%, LACE - 34%) were comparable, though in HD subgroup, OS was superior with LACE (BEAM - 45.7%, LACE - 80.6%; P=0.014)

**Conclusion**

In our retrospective audit LACE is a better regimen with lesser toxicity, earlier engraftment and comparable survival rates. Randomised trials comparing the 2 regimens are needed.

There is no conflict of interest to disclose.

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**P088**

**Transplantation Outcomes of Patients Who Did Not Receive Methotrexate on Day 11 Posttransplant Because of Severe Mucositis and Neutropenic Fever**

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**Aim**

The disadvantages of a short course of methotrexate for GVHD prevention in allogeneic stem cell transplantation are mucositis and delayed hematologic recovery. We have omitted methotrexate infusion on day 11 post transplant in patients that had severe mucositis or life-threatening infection. The aim of this study is to know if this modification may increase the risk of GVHD and lower the survival rate of those patients.

**Method**

We analyzed the transplant outcomes comparatively between patients who received the full schedule of methotrexate (group A) and patients who did not receive methotrexate on day 11 post transplant (group B). A total of 51 patients were enrolled. Twenty-four patients were in group A and twenty-seven were in group B.

**Results**

Absolute neutrophil count recovery and platelet recovery are not different between the two groups (P=0.165 and 0.304, respectively). The number of patients that required anti-CMV treatment (P=0.773) and the number of patients that had CMV disease (P=0.289) did not differ significantly. The cumulative incidence of acute GVHD (grade ≥2) was 25.6% in group A and 29.13% in group B (P=0.581). That of chronic GVHD at 2 years was 76.4% in group A vs. 54.7% in group B (P=0.096). Non-relapse mortality (36.7% in group A vs. 36.7% in group B; P=0.877) and relapse incidence (P=0.892) at 3 years were no different between the two groups. Disease-free survival and overall survival at 3 years did not differ between the two groups.

**Conclusion**

The transplantation outcomes of patients that did not receive methotrexate on day 11 post transplant due to severe mucositis or life-threatening infection were no different from those of patients that received the full schedule of methotrexate. This study demonstrates methotrexate infusion on day 11 posttransplant can be omitted without increase of risk of GVHD and non-relapse mortality in those patients.

There is no conflict of interest to disclose.
Donor Chimerism after Reduced-Intensity Allogeneic Stem Cell Transplantation: Kinetics and Predictors

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Aim
We conducted a retrospective review of patients with plasma cell myeloma (PCM) and acute myeloid leukaemia (AML) undergoing reduced-intensity conditioning (RIC) allogeneic stem cell transplantation (allo-SCT), in order to assess the kinetics and predictors of donor chimerism (DC), in an attempt to better identify patients at risk for graft rejection and relapse.

Method
Thirty-five patients transplanted between May 2008 and June 2011 were included: 19 patients with PCM (after planned autologous stem cell transplant [ASCT]) and 16 with AML. Sixteen had a matched related donor (MRD) and 19 a matched unrelated donor (MUD). Conditioning consisted of fludarabine and low-dose TBI (2 Gy on day 0). Peripheral blood DC (CD3+ and CD33+) was assessed by short tandem repeat PCR at days 30, 60, 90, 180 and 365 after transplant and then yearly.

Results
AML patients, who were older (mean age 60 versus 50 years; p < 0.0001), had lower mean CD3+ DC at days 60 (70% versus 94%; p = 0.0015) and 90 (72% versus 96%; p = 0.0053) than PCM patients. Similar significant differences at 2, 3 and 6 months were seen between older (>60 years) and younger (<60 years) patients. PCM patients undergoing allo-SCT within 4 months of ASCT had higher mean CD3+ DC at day 60 (97% versus 89%; p = 0.0357). Similarly, patients with a MUD had higher mean CD3+ DC at day 60 (93% versus 74%; p = 0.0136) than those with a MRD. AML patients who relapsed had lower CD3+ chimerism at day 60 (49% versus 87%; p = 0.0008) and progression-free survival was superior (100% versus 33% at 1 year; p = 0.0420) in patients whose day 60 CD3+ DC was ≥80%.

Conclusion
PCM patients (who were significantly younger) had better CD3+ DC than AML patients between 2 and 6 months after transplant. This may reflect absent thymic recovery in patients older than 60 years. Proximity of ASCT in PCM patients also appeared to improve early CD3+ DC. In this cohort, transplantation from a MUD resulted in improved early CD3+ DC. Day 60 CD3+ DC may be an important predictor of outcome in RIC transplantation for AML.

No conflict of interest to disclose.
P090

Impact of Iron Overload in Patients After Allogeneic Hematopoietic Stem Cell Transplantation

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Aim
Iron overload is a significant risk factor of nonrelapse mortality in hematopoietic stem cell transplantation (HSCT). We analyzed patients who underwent allogeneic HSCT, using pretransplantation serum ferritin as a surrogate marker of iron overload.

Methods
We retrospectively evaluated the prognostic effect of serum ferritin level on the post-transplantation outcome of 27 patient with myeloid malignancy who underwent allogeneic HSCT.

Results
Patients with high serum ferritin level group (>=2000ng/ml) had a significantly lower overall survival rate than those with low serum ferritin level group (<2000mg.) (P=0.00355)

Conclusion
We reconfirm the significance of pretransplant iron overload which has adverse effect on post transplantation survival rate. Early intervention on iron overload may improve the outcome of allogeneic HSCT.

No conflict of interest to disclose
P091
Allogeneic Hematopoietic Stem Cell Transplantation With TBI and G-CSF-combined High-dose Cytarabine for Elderly Patients With Myeloid Malignancies

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Aim
We have previously reported a favorable outcome of allogeneic hematopoietic stem cell transplantation (HSCT) with myeloablative conditioning using TBI and G-CSF-combined high-dose cytarabine (CA). However, the safety and efficacy of the conditioning are not evaluated in elderly patients. Therefore, we retrospectively analyzed the outcome of allogeneic HSCT using this conditioning in elderly patients with myeloid malignancies.

Patients and methods
Twenty patients aged 50 or greater with myeloid malignancies including AML (n=12), advanced MDS (n=5), CMML (n=2), and MPN (n=1) could be evaluated. Median age at transplant was 52 years (range, 50-58). In 12 AML patients, 5 were in CR1, 4 were in CR2, and 3 were not in CR at transplant. The conditioning consisted of 12 Gy of TBI and high-dose CA (3g/m²) every 12 hours for 4 days in combination with continuous infusion of G-CSF. Stem cell sources were bone marrow (BM) or PBSC from related donor (n=11) and BM from unrelated donor (n=9). Cyclosporine A or tacrolimus with short-term methotrexate was given for the prophylaxis against GVHD.

Results
The 5-year overall survival, disease-free survival, and transplant-related mortality rates were 62.8% (95% CI, 40.7%-84.9%), 63.6% (95% CI, 41.8%-85.4%), and 28.9% (95% CI, 7.3%-50.46%), respectively. The causes of death were disease recurrence (n=2), infection (n=2), GVHD (n=2), and graft failure (n=1).

Conclusion
These results suggest that TBI plus G-CSF-combined high-dose CA could be a feasible and promising conditioning regimen of allogeneic HSCT for elderly patients with myeloid malignancies.

No conflict of interest to disclose
P092
Treatment of Hepatic Sinusoidal Obstruction Syndrome (SOS) with Defibrotide +/- Ursodeoxycholic Acid Prophylaxis - A Single Centre Experience of 31 Patients

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Background
Sinusoidal Obstruction Syndrome (SOS) is a potentially fatal complication of hematopoietic stem cell transplantation (HSCT). We performed a retrospective case analysis of 31 patients who developed hepatic SOS after allogeneic HSCT in our hospital to assess the response to defibrotide treatment with or without ursodeoxycholic acid prophylaxis.

Method
Patients were allocated in to two groups where one group received prior ursodeoxycholic acid prophylaxis (N-14) and the other group with no ursodeoxycholic acid prophylaxis (N -17). Defibrotide was given when SOS was diagnosed. Univariate analysis of different patient factors was performed to assess impact on survival in Kaplan Meir survival analysis.

Results
21 out of 31 patients (67%) had complete resolution of SOS. In this group the day 100 survival was 60%. Mortality in non-remission patients was 100%. Onset occurred at a median of 10 days post transplant, and the median duration was 15.5 days. The median dose of defibrotide used was 19.2 mg/kg; median duration of use was 14.5 days. The factors that were significant for survival were patient age, resolution of SOS, type of donor (related or unrelated donors), duration of SOS and presence of multi organ failure. Use of total body irradiation, high dose busulphan, high dose cyclophosphamide, intensity of conditioning, prior hepatitis, and use of ursodeoxycholic acid prophylaxis did not impact survival.

Conclusion
A randomized dose finding study published by Richardson et al. demonstrated no difference between 25 mg/kg/day and 40 mg/kg/day, and showed overall remission and day 100 survivals of 42 % and 46 % respectively. Our retrospective observation showed better response and survival rates (67% and 60% respectively) but was likely confounded by the retrospective nature of this study. Mortality from SOS can be reduced significantly by early intervention. These results support initiation of a multicentre study using defibrotide in SOS in HSCT.

No conflict of interest to disclose
P093
Early Engraftment of G-CSF Primed Allogeneic Bone Marrow Transplantation in Pediatric Patients regardless of Donor-Recipient Weight Differences

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Aim
Harvesting sufficient progenitor cells from bone marrow (BM) for pediatric patients is a challenging process especially from smaller donors. Growth factor administration to donors prior to harvest results in an enrichment of the graft and leads to early engraftment. This study is to find out whether G-BM can reach to the sufficient cell dose and lead to a proper engraftment without increasing GVHD in pediatric allogeneic bone marrow transplantation even with smaller donors than recipients.

Method
A total of 41 patients received an HLA-identical sibling transplantation using granulocyte colony-stimulating factor (G-CSF)-primed BM. All donors received G-CSF 10 µg/kg/day for 2 days prior to harvest.

Result
The median weight difference between donor and recipient was 3.9 kg (range, -29.8–32 kg), and the median CD34+ cells harvested were $4.16 \times 10^6$/kg (range, 1.17–31.9×10^6/kg). The median time to neutrophil engraftment was 12 days (range, 10–27) and the time for platelet was 20 days (range, 12–64). The cumulative incidence of acute grade 2-3 graft-versus-host disease (GVHD) and chronic GVHD was 4.9% and 5.1%, respectively. An analysis according to the weight difference of donor and recipient showed there was no significant difference in harvested CD34+ cell dose and in time required for engraftment between recipients with smaller and heavier donors.

Conclusion
G-CSF primed BM allows successful engraftment and provides a valuable alternative to unstimulated BM and peripheral blood stem cells with good engraftment, and tolerable GVHD even in patients with smaller donors.

No conflict of interest to disclose
**P094**

**Survival Benefit of Infant Leukemia with More Intensive and Early Intensification Chemotherapy Followed by Hematopoietic Stem Cell Transplantation**

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**Aims**

Compared with older children, acute leukemia in infants has a dismal outcome, despite introduction of intensive multiagent chemotherapy. Patients aged under one year and with mixed-lineage leukemia (MLL) gene rearrangements are the most high risk group. In this study, we investigate the outcome of more intensive and early intensification chemotherapy and the role of hematopoietic stem cell transplantation (HSCT) in infant leukemia.

**Methods**

This study analyzed 21 infants with acute leukemia who were diagnosed and treated between 1987 and 2010 at Severance hospital. Among 21 patients, 11 patients were under 6 months old (median age = 6 months). We compared the event free survival (EFS) rate between group A (from 1987 to 2001, no HSCT) and group B (from 2002 to 2010, who received more intensive and early intensification chemotherapy and HSCT). The indication of HSCT in group B was age at diagnosis is under 6 months or white blood cell at diagnosis are over 50,000 /uL or presence of MLL gene rearrangements. The event was defined as relapse or death. We analyzed medical records of whole patients with retrospective manner.

**Results**

The EFS rate of group B (76.2±0.1%) was significantly higher than that of group A (36.4±0.1%) (p=0.041). The EFS rate of group B patients (100.0%) older than 6 months old was significantly higher than that of group A children (25.0±15.3%), statistically (p=0.02). In group B, the EFS rate for infants younger than 6 months (33.3±27.2%) was lower than those of older than 6 months (100.0%, p=0.038). However, in group A, the EFS rate for infants according to age (6 months old) was 66.7±27.2% (<6 months) and 25.0±15.3% (≥6 months), respectively (p>0.05). In this study, 12 (57.1%) out of 21 patients were confirmed gene rearrangement. Ten (83.3%) out of 12 gene rearranged patients presented MLL-gene rearrangement. In MLL-gene rearrangement group (N=10), the EFS rate was 64.8±16.5%.

**Conclusions**

Outcome of infant leukemia is very poor, because rearrangement of the MLL-gene is found for about 80% of these patients. The results of this study suggest that early Intensification of chemotherapy followed by HSCT shows more promising results. Moreover, this newer treatment protocol for infant leukemia presents feasible results for patients with MLL-gene rearrangement. *No conflict of interest to disclose*
P095

HLA-Matching Degree and Graft Source-Based Analyses of Long-Term Outcomes of Unrelated Donor Transplants in Adult High-Risk Acute Lymphoblastic Leukemia

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Aim
To assess the effect of HLA-matching degree and graft source, we analyzed long-term outcomes of 109 consecutive unrelated donor (URD) transplants with adult high-risk ALL (2000-2008).

Method
Using allele-level typing, graft sources were classified as 8/8-matched BM (n=31), 8/8-matched PBPC (n=19), 7/8-matched BM (n=35), and 7/8-matched PBPC (n=24). Patients received full-intensity (n=95) or reduced-intensity (n=14) conditioning regimens.

Result
After a median follow-up of 57 months, the 5-year cumulative incidence of relapse was 19% for 8/8-matched BM, 16% for 8/8-matched PBPC, 37% for 7/8-matched BM, and 48% for 7/8-matched PBPC (P=0.013). Conversely, no significant difference in the 5-year cumulative incidence of non-relapse mortality was observed between each group of patients. Consequently, disease-free survival (DFS) at 5 years was inferior using 7/8-matched BM (46%) or 7/8-matched PBPC (42%), compared with 8/8-matched grafts (65% for BM and 58% for PBPC) (P=0.044). However, when considering pre-transplant disease status and conditioning intensity, no influence of HLA-matching degree and graft source on outcomes was found in transplants receiving full-intensity conditioning in CR1. Notably, outcomes were similar between BM and PBPC transplants in each group of patients showing the same HLA-matching degree. Regardless of HLA-matching degree and graft source, pre-transplant disease status (CR1 versus >CR1) was an independent predictive factor affecting relapse (RR 3.09, P=0.005) and DFS (RR 2.69, P=0.003). The absence of chronic GVHD was also associated with higher relapse (RR 5.60, P<0.001) and poorer DFS (RR 2.49, P=0.005).

Conclusion
Our data suggest that outcomes are similar for transplantation using BM or PBPC in the setting of 8/8- or 7/8-matched URD transplantation in adult high-risk ALL.

No conflict of interest to disclose
P096
Unrelated Double Umbilical Cord Blood Transplantation for Adults with High-Risk Acute Lymphoblastic Leukemia in Korea

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Aim
Graft cell dose is an important determinant of outcomes following umbilical cord blood transplantation (UCBT), limiting the use of this strategy for low body weight patients. To overcome this barrier, infusion of two partially HLA-matched UCB units was adopted as a new strategy (double UCBT). Here, we report the results of double UCBT for 14 consecutive adults with high-risk acute lymphoblastic leukemia (ALL) in Korea (median age, 31 years [range, 16-52 years]; median weight, 68 kg [range, 47-84 kg]).

Method
All patients had one or more high-risk features, including 7 Philadelphia-positive ALL. Eleven patients (78.6%) were transplanted in CR1. All patients received the same conditioning (TBI [12 Gy] + fludarabine [150 mg/m²] + cytarabine [9 g/m²]) and GVHD prophylaxis (tacrolimus + mycophenolate mofetil). UCB unit was selected according to the number of nucleated cells and HLA compatibility (HLA-A and -B by serotyping and HLA-DRB1 by genotyping).

Result
The median cell doses infused were 3.60x10⁷ nucleated cells/kg (range, 2.62-7.88), 1.80x10⁸ CD34/kg (range, 0.65-5.85), and 8.04x10⁷ CD3/kg (range, 6.24-13.49). All patients achieved a successful engraftment with full donor chimerism. Neutrophil recovery occurred at a median of 25 days (range, 17-109 days), and platelet recovery occurred at a median of 39 days (range, 20-185 days). Acute GVHD occurred in 10 of 14 patients (7 grade II, 2 grade III, 1 grade IV) and chronic GVHD occurred in 4 of 13 evaluable patients (2 mild, 2 moderate). After a median follow-up of 18 months (range, 6-63 months), 9 patients are alive with a leukemia-free status. Cumulative incidence of relapse and transplant-related mortality at 2 years were 21.4% and 9.1%, respectively, and disease-free survival at 2 years was 70.7%.

Conclusion
Our data suggest that adult high-risk ALL patients without suitable related or unrelated donors should be considered as candidates for double UCBT.

No conflict of interest to disclose
G-CSF-treated Donor CD4\(^+\) T-cells Develop Less Acute GVHD Lethality via Decreasing Polarization Toward Th17 Cells

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Aim
Immunoregulatory effects of granulocyte colony-stimulating factor (G-CSF) have been demonstrated on donor T-cells to reduce acute graft-versus-host disease (GVHD). However, the underlying mechanism is still not fully understood. Here, we investigated whether decreasing polarization of donor CD4\(^+\) T-cells towards IL-17 producing CD4\(^+\) T-cells (Th17 cells) is related to the regulatory mechanism of G-CSF.

Methods
B6 (H-2\(^{b}\)) donor mice were subcutaneous injected with recombinant human G-CSF (10 \(\mu\)g) daily for 5 days. Lethal irradiated B6D2F1 (H-2\(^{b/d}\)) recipient mice were transplanted with vehicle (PBS)- or G-CSF-treated CD4\(^+\) T-cells (2\(\times\)10\(^6\)) and T-cell-depleted bone marrow cells (5\(\times\)10\(^6\)) via tail vein injection. Survival and clinical scores were monitored daily. The effect of G-CSF on Th17 polarization was determined by both cytokine assay using cytokine bead array system and flow cytometric intracellular staining and ROR\(\gamma\)t expression using real-time PCR and flow cytometry. SOCS3 expression was determined by real-time PCR and immunoblots. The Kaplan-Meier product-limit method was used to obtain survival curves. Survival data were analyzed by the log-rank test.

Results
Recipients that transplanted with allogeneic CD4\(^+\) T-cells from G-CSF pretreated donors showed a significantly improvement of survival compared with the recipients transplanted with control donor CD4\(^+\) T-cells. We found that G-CSF-treated donor CD4\(^+\) T-cells significantly reduced the number of IL-17 producing and ROR\(\gamma\)t expressing cells in the secondary lymphoid organs of allogeneic recipients after transplantation compared with control. Finally, we found that SOCS3 expression was strongly induced in G-CSF-treated CD4\(^+\) T-cells, but not normal cells.

Conclusion
Our results demonstrate that inhibition of TH17 polarization by SOCS3 induction is associated with immunoregulatory functions of G-CSF on CD4\(^+\) T-cell-mediated acute GVHD.

No conflict of interest to disclose
P098
Influence of Stem Cell Mobilization After Cyclophosphamide, Thalidomide and Dexamethasone (CTD) Regimen in Patients With Newly Diagnosed Multiple Myeloma

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Backgrounds
CTD regimen has been known as an effective induction therapy in patients with newly diagnosed MM. But there were inconsistent results for the autologous stem cell yield for transplantation. The aim of the present study was to identify the influence of CTD therapy on outcomes of peripheral blood stem cell (PBSC) collection.

Methods
Forty-eight patients received 4 cycles of CTD therapy. Stem cells were mobilized with cyclophosphamide (3.0 g/m²) and G-CSF (10 mg/kg, daily) or G-CSF alone. Patients failing to collect ≤ 4.0 x 10⁶ CD34⁺ cells /kg received a second mobilization course.

Results
The median age at diagnosis was 56 years (range, 39-69). Median duration from start of CTD therapy to first collection was 4.6 months (range, 3.3-8.7). Forty-four patients were mobilized with cyclophosphamide following with G-CSF and 4 patients G-CSF alone. The median day of apheresis was 3 days (range, 2-7). The response rate for CTD regimen at mobilization was 10% (5/48) of CR, 25% (12/48) of VGPR and 63% (30/48) of PR. A median number of harvested CD34⁺ cells was 8.6 x 10⁶ cells/kg. At the first mobilization 83% (40/48) of patients had reached the minimal PBSC collection target of ≥ 2.0 x 10⁶ CD34⁺ cells/kg, and 71% (34/48) of patients achieved the collection ≥ 4.0 x 10⁶ CD34⁺ cells/kg. At the end of the second mobilization, 90% (43/48) of patients had yields of at least ≥ 2.0 x 10⁶ CD34⁺ cells/kg and 77% (37/48) of patients had yields of ≥ 4.0 x 10⁶ CD34⁺ cells/kg. During the mobilization period, three patients developed grade 3/4 non-hematologic adverse events.

Conclusion
CTD regimen is an effective induction therapy in patients with newly diagnosed MM showing high response rates and acceptable rates of autologous stem cell yield without any detrimental effect for the following stem cell collection.

No conflict of interest to declare
P099
The Clinical Comparison of Different Non-myeloablative Conditioning Regimen Based on Antithymocyteglobulin or Fludarabine for Haematologic Diseases

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Aim
To investigate the influence of nonmyeloablative HLA-matched sibling donor hematopoietic stem cell transplantation, with anti-thymocyte globulin(ATG) or fludarabine (Flu) based conditioning regimen, on hematopoietic reconstitution and graft –versus-host disease for malignant hematologic diseases.

Methods
The clinical data of 28 patients who underwent non-myeloablative transplants were analysed. Patients were divided into two groups of ATG (n=12) and Flu (n=16). Conditioning regimens consisted of ATG 8mg·kg⁻¹·d⁻¹×3d or Flu 30~35mg·m⁻²·d⁻¹×5d combined BU(injection) 1.6mg·kg⁻¹·d⁻¹×4d or BU/tablet) 2 mg·kg⁻¹·d⁻¹×4d and CTX 60 mg·kg⁻¹·d⁻¹×2d, or combined melphalan 120mg/m²×1d. We compared myelosuppression, peripheral blood and bone marrow recovery, blood group and cherimeria status, platelets and red blood cell requirements post-transplant Clinical characters of GVHD and relationship of GVHD and survival, infection, relaps of primary disease between the two groups were compared.

Results
All patients achieved hematopoietic stem cell engraftment. The minimum number of WBC (×10⁹/L) of Flu was significantly lower than the ATG group (0.095 ± 0.085 VS 0.15 ± 0.15, p <0.01). The time(d) to reachBPC ≥50×10⁹/L, Flu was significantly faster in the ATG group (14.6 ± 6.6 VS 18.5 ± 5.6, p <0.05). The time(d) at transformation of blood group (40.5±15.5 VS 85.5±20.5) and full donor chimerism (45 VS 135±105)( p <0.01) of Flu was significantly faster in the ATG group. The numbers of transfused red cells (Unit) (4.2 ± 2.5 VS 6.3 ± 4.5) and platelets (Unit) (6.4 ± 2.5 VS 9.2 ± 3.5) of Flu group was lower than the ATG group (p <0.05). The time (d) of hematological remission of Flu group was significantly faster than the ATG group (60 ± 15 VS 135 ± 45, p <0.05). The incidence and severity of aGVHD between Group of Flu and ATG were different significantly (I/II aGVHD: 43.75% vs16.67%; III/IV aGVHD: 6.25% vs 0%) (p<0.05). The incidence of overlap syndrome in Flu group was higher than in the ATG group (25.00% vs 16.67%, p <0.05). The incidence of classic cGVHD in Flu group was higher than in ATG group (25% vs 16.67%, p <0.05); cGVHD in Flu group was higher than in the ATG group (50.00% vs 33.33%, p <0.05). The overall disease-free survival of 3 years were 72.2% [95%CI:60.9~82.2] of Flu group and 50.00% [95% CI:45.5~65.6] of ATG group respectively, and there was not different significantly (p>0.05).

Conclusion
Hematopoietic reconstitution after non-myeloablative transplant based on Flu was faster than ATG group. The incidences and severity of aGVHD in Flu group were higher. After 100 days, there was no difference in late aGVHD. The incidences of overlap syndrome, classic cGVHD and GVHD with cGVHD characteristics in Flu group were higher than in the ATG group.
P100
Clinical Outcome of Once-daily intravenous Busulfan and Fludarabine Conditioning Regimen for Allogeneic Hematopoietic Stem Cell Transplantation in Pediatric AML: Single Center Experience in Korea

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Aim
The combination of intravenous busulfan with fludarabine, rather than a second alkylating agent, is introduced as a myeloablative, reduced-toxicity conditioning regimen for patients with acute myeloblastic leukemia (AML). However, until recently there has been little research and limited data available on the safety and efficacy of once-daily intravenous busulfan and fludarabine in pediatric allogeneic HSCT. We report the outcomes for allogeneic hematopoietic stem cell recipients, evaluating engraftment status, regimen related toxicities (RRT), and relapse after use of once-daily intravenous busulfan and fludarabine conditioning regimen for allogeneic HSCT in children with AML in a single pediatric center of Korea.

Method
From March 2003 to December 2010, 16 AML children who received once daily iv busulfan/fludarabine based conditioning regimen for allogeneic HSCT were reviewed, with median age of 10 years (range, 2-17 years). 12 boys and 4 girls were enrolled. The median period from diagnosis to transplantation was 9 months (range, 5-29 months). Bu/Flu±ATG consists of intravenous fludarabine (40 mg/m²) and busulfan (120~130 mg/m², once daily iv) on days -6 to -3, and antithymocyte globulin (ATG) (3 mg/kg) on days -3 to -1. All patients received tacrolimus and minidose MTX for GVHD prophylaxis.

Result
As a stem cell source, peripheral blood stem cells (PBSC) were 12 cases (75%), bone marrow and cord blood were 2 cases in each. The median follow-up for patients was 35 months. All patients except one who had an unrelated cord blood transplant were successfully engrafted. The median time to ANC recovery (ANC> 500 x 10⁶/L) and platelet recovery (platelet> 20,000 x 10⁶/L) were 13 days, 16 days in each. The incidence of acute GVHD was 25.0%, while severe grade III/IV GVHD was observed in only 1 patient (6.3%). There was only one case of limited type chronic GVHD in this study. Hepatic toxicity was relatively common but transient. There were three clinically diagnosed cases of veno-occlusive disease (VOD), but most recovered by fluid restriction and diuretics. Grade II stomatitis occurred in 6 patients (37.5%). Four patients (25%) showed positive CMV antigen/PCR but only one patient developed CMV colitis. Five patients died: 4 due to relapse/disease progression, 1 due to severe acute GVHD. The actual relapse rate at 1 year was 18.8%. Day 100 survival was 87.5%, and the overall survival was 68.8%.

Conclusion
Overall, once-daily intravenous busulfan and fludarabine was less toxic and effective as conditioning regimen in pediatric AML patients undergoing allogeneic transplantation.

No conflict of interest to disclose
Allogeneic Hematopoietic Stem Cell Transplantation in Pediatric Acute Lymphoblastic Leukemia: National Taiwan University Hospital Experience from 1989 to 2009

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Aim
In Taiwan, modern chemotherapy for childhood acute lymphoblastic leukemia (ALL) has achieved a five-year event-free survival rate of 77%, but some patients still suffer from relapses or refractory disease with a dismal outcome. A number of these patients may benefit from allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Method
Allo-HSCT for childhood ALL has been performed at National Taiwan University Hospital since 1989. We retrospectively searched our Pediatric HSCT Registry for patients with ALL between January 1988 and December 2009. Patients' demographic data, disease characteristics, clinical course, HSCT treatment, and outcome were analyzed.

Result
Total 61 (19.4%) of 315 pediatric patients undergoing allo-HSCT during this period have the diagnosis of ALL. There are 41 males and 20 females, with a median age of 9.9 years (range 0.2-26.7). The disease status before transplantation includes CR1 in 19 (31.1%), CR2 in 25 (41.0%), PR in 8 (13.1%), and refractory in 9 (14.8%). The conditioning regimen is based on fractionated total body irradiation (n=44; 72.1%) or busulfan (n=17; 27.9%). Mean CD34+ cell dosage is 7.6 (0.13–34.73) ×10^6/kg, with engraftment rate 86.4%. Kaplan-Meier analysis reveals that the 18-year probability of overall survival is 31.1% (19.5%–43.4%). Cox proportional hazards modeling in 54 patients (88.5%) with complete data identifies that pre-transplant disease status confers the strongest influence to survival (hazard ratio for remission vs. non-remission, 0.34 [0.16–0.76], p=0.008); CD34+ cell dosage and stem cell source also show borderline significance; while age, gender, cytogenetics, conditioning regimen, and era of HSCT have no significant effect. The top three major causes of death are disease progression (44%), infections (24%), and graft-versus-host disease (12%).

Conclusion
Approximately one-third of pediatric patients with poor-risk, refractory, or relapsed ALL have survived after allo-HSCT. A risk stratification system for decision-making is warranted.

No conflict of interest to disclose
P102
A Clinical Study on Double-Unit Umbilical Cord Blood Transplantation in Adults with Hematologic Disease

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Objective
In this study, we explored the efficiency and toxicity of 46 cases of double-unit CBT in adults with hematologic disease.

Methods
The tolerance; transplant related complications; survival rate and disease free survival rate were observed and analyzed. A non-myeloablative conditioning regimen was used. Cyclosporine combined mycophenolate mofetil and ATG were used to prevent graft versus host disease (GVHD).

Results
All these 46 patients tolerated the therapy well while four patients had graft failure. Severe acute GVHD was presented in 6 patients. Chronic GVHD was occurred in 18 patients. Fatal infection complications occurred in 7 patients and 5 patients relapsed after transplantation. In the follow-up duration of 29 months on median, the expected 3-year relapse mortality was 16.7%; non-relapse mortality was 26.1%; overall survival was 57.7%, and disease free survival was 48.2%.

Conclusion
The use of double-unit CBT after reduced intensive conditioning therapy in adults with hematologic disease is an effective and safe treatment.

No conflict of interest to disclose
P103
Comparison of High-Dose Cyclophosphamide Plus G-CSF With G-CSF Alone for Peripheral Blood Stem Cell Mobilization in patients With Multiple Myeloma

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Aim
Autologous peripheral blood stem cell transplantation for multiple myeloma (MM) offers higher response rates and improved survival compared with conventional chemotherapy. However, the best method for peripheral blood stem cell mobilization for MM remains controversial. We analyzed the results of two different methods of stem cell mobilization for autologous transplantation in 34 patients with MM.

Method
In group I (n = 15), High dose-Cyclophosphamide (CY), 4 g/m² i.v., was administered followed by G-CSF, 10 µg/kg/day s.c., until the end of collection, starting the leukapheresis after hematological recovery (WBC >5,000 x 10⁶/L). In group II (n = 19), G-CSF, 10 µg/kg/day s.c., was used alone until the last day of collection, starting consecutive apheresis on the 5th day.

Result
Both groups were comparable for age, sex and prognostic features as well as previous therapies. Patients receiving CY had higher median total yield of CD34+ cells. However significant differences were not observed between two groups (9.0 x 10⁶ cell/kg vs. 6.44 x 10⁶ cell/kg, P=0.26). Successful collection more than 5.0 x 10⁶ CD34+ cells/kg and not failed collection of at least 2.0 x 10⁶ CD34+ cells/kg were achieved in similar proportions in two groups (60.0% vs. 57.9%, P>0.999 and 73.3% vs. 89.5%, P=0.37). Hospitalization for mobilization was longer in group I (18days vs. 9days, p<0.001) and treatment-related toxicity was greater in this group: 7 patients (47%) developed fever requiring antibiotics during the neutropenic period after HD-CY and seven (47%) patients required transfusion support.

Conclusion
These data suggest that although High dose-CY plus G-CSF is superior to G-CSF alone based on median CD34+ cell yield, adequate CD34+ cell collections can be achieved with G-CSF alone in most MM patients with less toxicity and with simplification of the procedure.

No conflict of interest to disclose
**P104**

**Successful Hematopoietic Stem Cell Transplantation for Severe Aplastic Anemia from an Unrelated Donor Following Living-related Liver Transplantation for Fulminant Hepatitis**

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**Aim**

Aplastic anemia is a rare complication of liver transplantation for fulminant hepatitis. We report a case of successful hematopoietic stem cell transplantation (HSCT) from an unrelated volunteer donor in a patient with severe aplastic anemia (SAA) who had undergone a living-related liver transplantation (LRLT) from an HLA-mismatched sister for fulminant non-A, non-B, non-C hepatitis.

**Method**

Retrospective review of medical records was performed.

**Results**

A 15–year-old male received an LRLT from an ABO-compatible, HLA-mismatched sister for acute liver failure following non-A, non-B, non-C hepatitis. After 3 months of LRLT, the patient developed thrombocytopenia and neutropenia, and subsequently progressed to pancytopenia. The patient was diagnosed with SAA based on bone marrow biopsy. The patient showed no response to immunosuppressive therapy consisting of cyclosporine (CSA), rabbit-antithymocyte globulin (r-ATG), and corticosteroids. Eleven months following liver transplantation, the patient received an allogeneic HSCT from an HLA-mismatched (at 1 allelic locus), ABO-compatible unrelated donor. The conditioning regimen consisted of fludarabine 200 mg/m² cyclophosphamide 120mg/kg and rabbit-antithymocyte globulin 7.5 mg/kg. The dose of fludarabine was reduced to 160 mg/m² due to a septicemia during conditioning period. GVHD prophylaxis was done with CSA and a short course of methotrexate. WBC and platelet engraftments were achieved at 15 days and 27 days posttransplant, respectively. The septicemia, which occurred during the conditioning period, was resolved with broad-spectrum antibiotics after WBC engraftment. The patient had a CMV reactivation, which was treated with ganciclovir. The patient developed grade 2 skin GVHD, which was successfully treated with corticosteroids. At 47 days posttransplant, the patient developed a myelopathy of unspecified cause, which resolved spontaneously. He is transfusion-independent and free of any signs of chronic GVHD or liver dysfunction 2 years post transplant.

**Conclusion**

Allogeneic HSCT from an unrelated donor may be a feasible treatment option for SAA following an LRLT for fulminant hepatitis.

*No conflict of interest to disclose*
Unrelated Hematopoietic Stem Cell Transplantation from Foreign Donors: Current Status in Japan

Since the Japan Marrow Donor Program (JMDP) started international cooperation activities in 1997, unrelated donors from overseas have become an important stem cell source for the patients who need hematopoietic stem-cell transplantation (HSCT) but cannot find a suitable donor in the JMDP or the Japanese Cord Blood Bank Network (JCBBN). To collect and analyze data on the current condition and results of HSCT specializing in foreign donors, the JSHCT established the working group named “HSCT from foreign donors in Japan” in 2010. From 1997 to 2009, there were 140 (133 BMTs and 7 PBSCTs) unrelated HSCTs from foreign donors in Japan. The median age at transplantation was 32 (range from 0 to 60) years old and they were 91 (65%) males and 49 (35%) females. Their diseases included AML (n=37, 26%), ALL (n=36, 26%), CML (n=33, 24%), MDS (n=11, 8%), other hematological malignancies (n=11, 8%), and non-malignant diseases (n=11, 8%). The ethnicity of the donors were categorized into Asian-Pacific Islanders (APIs) including Japanese (n=105, 75%), other than APIs (n=17, 12%), and unknown (n=18, 13%). A total of 83 transplants (59%) were performed between 1997 and 2000, 39 (28%) between 2001 and 2004, and only 18 (13%) after 2005. Among 125 patients who succeeded to engraft, 60 developed grades II-IV acute GVHD; II was 33, III was 22, and IV was 5. The three-year survival rate was 53% among the all cases. By multivariable analysis, HSCT for high-risk malignant diseases compared with non-malignant or standard-risk malignant diseases and the donor ethnicity other than API compared with API were significantly associated with inferior survival. As we have accelerated global cooperation in HSCT recently, this survey will help us research the most appropriate donors from foreign countries by comparing the results of traditional HSCT with the use of domestic donors.

No conflict of interest to disclose.
Patients Receiving HLA-haploidentical allo-HSCT Can Gain Desirable HRQoL Which Comparable to Even Better Than Patients Receiving HLA-identical Allo-HSCT

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Aim
To investigate the health-related quality of life (HRQoL) of patients receiving HLA-haploidentical allo-HSCT (allogeneic hematopoietic stem cell transplantation) and to compare with that of patients receiving HLA-identical allo-HSCT.

Methods
A total of 275 patients receiving allo-HSCT at the Institute of Hematology, Peking University from 2004 to 2008 were enrolled (132 with HLA-identical allo-HSCT and 143 with HLA-haploidentical allo-HSCT). HRQoL post-transplantation was evaluated by the Mos 36-item short-form health survey. The effects of various factors on the HRQoL were analyzed, including age, gender, diagnosis, disease status at transplantation, gender of donors, follow-up time after transplantation, acute graft-versus-host disease (aGVHD), chronic graft-versus-host disease (cGVHD), and donor lymphocyte transfusion (DLI).

Results
A large proportion of patients receiving HLA-haploidentical allo-HSCT achieved good recovery in HRQoL. Patients receiving HLA-haploidentical allo-HSCT had a higher chance of good recovery in physical functioning, role-physical functioning, general health, bodily pain, and vitality. In terms of social functioning, role-emotional functioning, and mental health, similar rates of good recovery were observed in both groups. HRQoL of survivors more than three years after transplantation was significantly superior to that of patients surviving less than three years after transplantation. Multivariate analysis showed that cGVHD was the most adverse factor affecting HRQoL and histocompatibility of the donor had no effect on HRQoL.

Conclusion
The HRQoL of patients receiving HLA-haploidentical is comparable to even better than the patients receiving HLA-identical allo-HSCT. Follow-up time and cGVHD are the most important factors affecting HRQoL. Patients receiving HLA-haploidentical allo-HSCT can gain desirable HRQoL.

No conflict of interest to disclose
PI07
Allogeneic Stem Cell Transplantation for Acute Myeloid Leukaemia: A Single Centre Experience from 1998-2010

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Aim
To evaluate the outcome of allogeneic stem cell transplant (Allo HSCT) in 121 consecutive patients with Acute Myeloid Leukaemia (AML) in a single institution from 1998-2010. To assess factors associated with improved survival in this group particularly in the era of both myeloblative and reduced intensity transplants.

Method
This was a retrospective analysis with data acquired from the Haematology Department Stem Cell Transplant database. All analyses was performed using Stata software, P values less than p 0.05 were considered significant.

Results
The median age was 43 years (15-67), with 55.4% male. 45.5% of donors were siblings and 40.5% of patients were in CR1, 27.3% in CR2. The majority of patients received CyTBI (n=42), BuCy (n=42) or Fludarabine Melphalan (n=30) conditioning with Cyclosporine/methotrexate GVHD prophylaxis and no T-cell depletion. Transplant related mortality was 14.9% at D100. The cumulative incidence of Acute GVHD was 54.5%, and Chronic GVHD was 60.3%. Overall Survival at 5 years for all patients post HSCT was 52.1%. Multivariate analysis revealed presence of Chronic GVHD (HR 0.31, p = 0.006) and BuCy conditioning (HR 0.55, p = 0.04) to be associated with improved survival. Chronic GVHD appeared to exert a particularly strong anti-leukaemic effect (as shown below). Patients not in remission had a poor disease-free survival (HR 3.47, P<0.001). There was no effect of donor type and in particular no inferior survival with Flu Mel conditioning.

Conclusion
These data confirm that Allo HSCT can provide a strong Graft Versus Leukaemia effect when Chronic GVHD is present. Transplanting patients in remission with either myeloblative or reduced intensity conditioning provides excellent leukaemia-free survival in this cohort.

No conflict of interest to disclose
P108
Quality of Life Before and After Allogeneic Stem Cell Transplant – A Prospective Study of 34 Patients

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Aim
To prospectively evaluate the Quality of Life (QOL) of patients undergoing allogeneic stem cell transplant (HSCT) in a single centre with one and two year follow up.

Method
This was a prospective study performed in 34 patients who provided written informed consent prior to HSCT from May 2008 until November 2009. All patients completed the Functional Assessment of Cancer Therapy – Bone Marrow Transplant (FACT-BMT) scale at baseline (D-7 of HSCT) and 1 and 2 years post HSCT. Scores were collated using the FACT-G (general well being) scale and the total FACT-BMT scale at these 3 time points. A higher score suggests a better QOL.

Results
There were 34 patients who consented to the trial during this time period. The median age was 47 years (19-69) with 14 undergoing sibling allografts, 18 unrelated allografts and 2 cord blood transplants. Eleven patients died or relapsed during follow-up. All patients underwent CyTBI, BuCY or FluMEI conditioning. Median baseline FACT-G score was 81.5 (51-105, n=34)) increasing to 85 (55-104, n=18) at 1 year and 90 (54-106, n=14) at 2 years. Median baseline FACT-BMT score was 112 (68-139, n=34). This figure remained relatively constant over the follow up period with values of 113.5 (79-135, n=18) at 1 year and 115 (72-143, n=14) at 2 years.

Conclusion
The preliminary results from this prospective QOL study for HSCT patients suggest that overall, patients can maintain a stable QOL despite undergoing the hardships of a HSCT. The general well-being component of the FACT questionnaire appeared to show a good QOL for these patients as they attempted to return to a normal lifestyle post HSCT.

No conflict of interest to disclose
PI09
Safety and Benefit of Autologous Stem Cell Transplant for Auto-immune Diseases: 15 Years Experience from a Single Institution

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Aim
To describe a single centre experience of autologous stem cell transplant (HSCT) in 32 consecutive patients with severe, refractory auto-immune diseases (AID).

Method
This study was a retrospective analysis of consecutive patients transplanted between 1997 and 2011. All patients provided written informed consent and fulfilled the eligibility criteria for the trials conducted. All patients were assessed for suitability by their referring physicians and in many cases by independent external assessors.

Results
32 heavily pre-treated and refractory patients were transplanted within the study period with a median age of 38 years (23-61). 82% of the patients were female. Diseases transplanted include: Rheumatoid arthritis (RA-16, all in the pre anti-TNF era), Systemic sclerosis (SSc-13), Vasculitis (V-2), SLE (1) and Multiple Sclerosis (MS-1). The conditioning regimen was cyclophosphamide 100-200mg/kg for RA, cyclophosphamide 200mg/kg and ATG 40mg/kg for SSc, SLE and Vasculitis and BEAM/ATG for MS. Transplant related mortality was 0% at 100 days and 1 year post HSCT. The response rate and duration varied between diseases groups: RA: 81% with only 7.5 months duration, SSc: 100% response rate with 33 months duration. All patients in the other disease categories responded however one of the vasculitic patients had only an 8 week response. Overall survival is 89.2% of evaluable patients (5 lost to follow up) with 3 patients (2SSc and 1RA) dying of their diseases. There have been no secondary malignancies.

Conclusion
These data demonstrate that HSCT can be safely performed in heavily pre-treated patients with severe AID. Response rates vary between diseases and careful patient selection in the context of clinical trials is likely to provide benefit to AID patients with severe disease.

No conflict of interest to disclose
P110
Allogeneic Hematopoietic Stem Cell Transplantation with Reduced-intensity Conditioning for Refractory or Relapsed Follicular Lymphoma

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Background
Although the treatment outcome of follicular lymphoma has been improved, allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative remedy of refractory or relapsed disease. However, transplant-related mortality (TRM) and disease relapse after HSCT are the most important factors interfering with the success of transplantation. We present the outcome of allogeneic HSCT with RIC consisting of fludarabine and melphalan for refractory or relapsed follicular lymphoma.

Patients and Methods
Twenty-one patients with relapsed and refractory follicular lymphoma underwent allogeneic HSCT after conditioned with RIC at Keio University Hospital. The median age of the patients at transplant was 48 years (range: 34-62). Fludarabine (125 mg/m²) and high-dose melphalan (140 mg/m²) were given as a conditioning. Additional low-dose total body irradiation was delivered in two patients undergoing cord blood transplantation. Stem cell sources and donor types were bone marrow or peripheral blood stem cells from human leukocyte antigen (HLA)-identical sibling (n=6), bone marrow from HLA-serologically matched unrelated donor (n=13), and cord blood from unrelated donor (n=2). For the prophylaxis of graft-versus-host disease (GVHD), cyclosporin A or tacrolimus with short-term MTX was given.

Results
All patients achieved neutrophil engraftment, and there was no case of early death before engraftment. The median follow-up period of the 18 surviving patients was 70.5 months (range: 23.5-102 months). Causes of deaths (n=3) were GVHD in 2 patients, and bacterial infection in 1 patient. The 5-year overall survival was 85.7% (95% CI: 70.8%-100%), and progression free survival was 79.4% (95% CI: 58.0%-94.4%). Although disease relapse was observed in 2 patients undergoing CBT, complete remission was achieved solely by the discontinuation of immunosuppressive therapy in both patients.

Conclusion
These results strongly suggest that allogeneic HSCT with RIC using fludarabine and melphalan could be a safe and curative remedy for refractory or relapsed follicular lymphoma.

No conflict of interest to disclose.
Reduced-Intensity Conditioning Allogeneic Stem Cell Transplantation in the Patients with Acute Leukemia

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Aim

Allogeneic hematopoietic stem-cell transplantation (HSCT) following myeloablative regimens has been used to treat patients with acute leukemia. Reduced-intensity conditioning (RIC) has been developed for older patients which are associated with a high risk of transplant-related mortality (TRM). We investigated the outcomes of allogeneic HSCT with RIC in the patients of acute leukemia.

Method

Among the patients with acute leukemia who have received allogeneic HSCT in Ewha Womans University Hospital between 1998 and 2010, outcomes of the patients who received HSCT with RIC were analyzed retrospectively.

Result

Twenty-two patients were enrolled in this study including 8 patients of acute lymphoblastic leukemia in first CR, 14 patients of acute myeloid leukemia in first CR (n=9), second CR (n=2), and not in CR (n =3). Seventeen patients with hematopoietic cell transplant comorbidity index (HCT-CI) score 0 were included in this analysis. Twelve patients received fludarabine (150mg/m²) with melphalan (140mg/m²), and 10 patients received fludarabine (150mg/m²) with busulfan (6.4mg/m²) as conditioning regimen, and followed by transplantation from matched sibling (n=16), unrelated donors (n=5), or unrelated cord blood (n=1). Incidence of grade 2-4 acute and chronic GVHD were 18.2% and 45.5%. Donor chimerism in bone marrow at 30 day and in blood at 100 day was median 99.0% and 97.0% after HSCT. The rate of relapse was 22.7% after median 7.5 months of follow-up. TRM at 100 day and 1 year were 13.6% and 27.3%. Disease-free survival (DFS) and overall survival (OS) were median 33.0 month and 42.7 month by Kaplan-Meier analysis.

Conclusion

These results suggest that allogeneic HSCT using RIC for treatment of acute leukemia may be a potential therapeutic approach considering less toxic and acceptable treatment outcomes even in young adult ALL without comorbidity which has not been considered as candidate of allogeneic HSCT using RIC.

No conflict of interest to disclose
A Population-Based Analysis of the Effect of Autologous Haematopoietic Cell Transplantation in the Treatment of Multiple Myeloma

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Aim
Persons diagnosed with multiple myeloma (MM) may proceed to a number of treatments including autologous haematopoietic cell transplant (HCT). The aim of this study was to assess whether outcome was superior for MM patients who proceeded to autologous HCT compared to those who did not.

Method
Data for New South Wales (NSW) residents in the age group 35 to 70 years diagnosed with MM during the years 2002 to 2005 were retrieved from the NSW Central Cancer Registry (CCR). From this list, patients who proceeded to autologous HCT during 2002-2006 were identified via a data linkage with the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR).

Results
The final analysis dataset comprised 716 patients, of whom 274 (38%) had received an autologous HCT. The results of a multivariate logistic regression indicated that males, persons younger than 60 years and residents of rural areas were more likely to proceed to HCT. The post-diagnosis unadjusted 2-year survival of HCT recipients was significantly better than those who did not proceed to HCT (83% vs 54%, P<0.001). In multivariate survival analysis the one significant favourable risk factor for overall survival was an autologous HCT (hazard ratio 0.39, 95% confidence interval 0.27 – 0.59, P<0.001).

Conclusion
This study confirms the results of international clinical trials and shows for the first time at a population level that autologous HCT provides a survival benefit in MM and should be considered as an early treatment option.

No conflict of interest to disclose
P113
Incidence, Etiology, Risk Factors and Treatment Outcomes of Patients Developing Hemorrhagic Cystitis During Hematopoietic Stem Cell Transplantation - Experience from a Tertiary Cancer Center in India

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Aim
Hemorrhagic cystitis (HC) is an important complication post stem cell transplantation (SCT). There are no Indian data on its epidemiology, and therefore we undertook this retrospective study.

Method
Between November 2007-April 2011, 159 patients underwent SCT. Patients developing HC underwent quantitative PCR for BK and adenovirus in blood and urine. The severity was graded by Droller’s grading. Patients ≤ grade 3 received hydration. Those with ≥ grade 3 HC also received cold saline irrigation. Intravesical G-CSF was used in patients with ≥ 25 days of HC. Patients with adenoviremia ≥ 10^5 copies/ml received cidofovir.

Result
Twenty (12%) patients developed HC (6 - BK viral, 3 - adenoviral, 8 - both, 1 - cyclophosphamide induced and 2 - uncertain etiology) with median grade of 3. Median day of onset and duration of HC was 31 and 40 respectively. Median day to adenoviral and BK viral reactivation was 35 and 40 respectively. The median peak titer of adenovirus in blood and urine were 1.3 x 10^3 copies/ml and 1.1 x 10^3 copies/ml respectively. The median peak titer of BK viruria was 1.8 x 10^4 copies/ml. Nine patients developed ≥ grade 3 HC. The median time to resolution of grade 2 and grade 3 was 8 and 38 days respectively. Four patients who received intravesical G-CSF had resolution within 10 days of first dose. Three patients received cidofovir. HC was seen in allogeneic setting (65%), those receiving steroids (55%) for acute and chronic GVHD or engraftment fever and those with concomitant CMV reactivation (40%). No patient died due to HC related complications.

Conclusion
HC was mostly viral induced. Risk factors included allogeneic setting, steroid use and CMV reactivation. Patients with prolonged duration of HC and adenoviremia may benefit from intravesical G-CSF and cidofovir respectively.

No conflict of interest to disclose
P114
Donor Lymphocyte Infusion for Relapsed Hematologic Malignancies after Allogeneic Stem Cell Transplantation

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Aim
Relapse, occurring in nearly 40% of all hematologic malignancy patients, remains the most common cause of death after allogeneic hematopoietic cell transplantation (HCT). Management of relapse after allogeneic HCT relied on augmentation of the graft-versus-tumor (GVT) effect either by reduction or by elimination of immune suppression or administration of donor lymphocyte infusion (DLI). We retrospectively assessed the efficacy of DLI in patients who relapsed in the post transplant setting.

Methods
We retrospectively reviewed the medical records of patients received DLI to treat relapsed hematologic malignancies after allogeneic HCT. Between 2002 and 2009 clinical data were collected retrospectively from Soonchunhyang university hospital.

Results
Fourteen patients were included with following diagnoses : AML (n=8), ALL (n=1), ABL (n=1), CML (n=1), lymphoma (n=1), and others (n=2). The median age was 45 (range 16-22). The donors for HSCT were HLA-identical sibling 8 (47%) and unrelated 5 (49%). With median 32.3 months of follow up (range, 1.4 to 93.3 months), 6 patients died. Nine patients (64%) developed GVHD after DLI and DLI related mortality was 14%. The 1 year overall survival rate was 45%, respectively. In univariate analysis, DLIs givens within 6 months after transplantation were associated with longer overall survival than DLIs given >6 months of SCT.

Conclusion
DLI is a viable option for patients who relapse after allogeneic HCT. Patients should be considered for DLI early after relapse but collaborative multicenter prospective trials are needed.

No conflict of interest to disclose
**P115**
The Effect of Cyclophosphamide and Prednisone as a First-line Treatment for Non-transplant Candidates Multiple Myeloma

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**Aim**
For many years, conventional treatment for elderly myeloma patients, or young myeloma patients who are not ineligible for high dose therapy due to poor performance states, has been the combination of oral melphalan and prednisone (MP). However, melphalan-based regimens are associated with numerous complications, and another alkylating agent, cyclophosphamide has similar effect for multiple myeloma and fewer reports of complications. Therefore, cyclophosphamide-based regimens usually have been used as a salvage therapy in patients with refractory or relapsed multiple myeloma despite newly-developed drugs. The purpose of this report was to evaluate the efficacy and tolerability of cyclophosphamide and prednisone (CP) as a first-line therapy for multiple myeloma (MM).

**Method**
From February 2005 to April 2011, we are retrospectively analysis of 29 patients with newly non-transplant candidates multiple myeloma who received a treatment regimen that consisted of intravenous cyclophosphamide 1000mg/kg for 1 day and prednisone 100mg for 4 days.

**Result**
The rate of response to this regimen was 46 percent among response evaluable 26 patients. The median progression-free survival (PFS) was 54 weeks. The median overall survival (OS) was 145 weeks. Cyclophosphamide-based combination was well tolerated. Above the grade III adverse effect; reduced absolute neutrophil count (ANC) was 26.3%, reduced hemoglobin (Hb) was 36.8%, and reduced AST was 5.3%. These adverse effects were easily adjustable. No one developed MDS, hemorrhagic cystitis. And no one increased ALT, bilirubin, creatine.

**Conclusion**
Although PFS was less than Melphalan-Predisone regimen (76 weeks), median OS was better than Melphalan-based regimen(110 weeks). Furthermore, adverse effect was well-tolerable for poor performance state patients. This Cyclophosphamide-based combination may represent effective and well-tolerated first therapy for poor condition patients with MM.

*No conflict of interest to disclose*
P116
High-Dose Chemotherapy and Autologous Hematopoietic Stem Cell Transplantation for Breast Cancer: Data of Long-term Follow-up

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Background
We tried to figure out the long-term follow-up data of high-dose chemotherapy and autologous hematopoietic stem cell transplantation (HDC-HSCT) which is not a suitable treatment modality for metastatic breast cancer any longer.

Method
Medical records of patients who have received HDC-HSCT at Ajou University Hospital between Aug 1995 and Mar 2001 for metastatic or high risk breast cancer were reviewed retrospectively. Patients with 10 or more lymph node metastasis at diagnosis were considered as a high risk group.

Results
46 female patients were analyzed. The median follow-up duration was 158.75 months and the median age was 43 (27 – 59). Ten patients were heavily treated before HSCT (3 or 4 lines of chemotherapy) and 27 patients (58.7%) received radiation therapy before HSCT. Median count of the infused CD34 positive cell was 8.1 x 10^6/kg. All patients engrafted successfully and the rate of transplantation-related mortality was 10.9% (5/46, 1 of hepatic VOD, 4 of sepsis). High-risk group showed 53.9% of overall survival rate at 175 months after HSCT and the median duration of relapse-free survival was 114 months whereas the survival rates of primary metastatic, sensitive relapse, and refractory relapse group were 33.3%, 15.4%, and 0%, respectively. HDC-HSCT as a salvage therapy had a hazard ratio of 2.73 to live short (vs. as an adjuvant therapy, p = 0.05) and previous radiation therapy showed a hazard ratio of 1.55 to higher mortality, but both were not significant. Three or more lines of previous chemotherapy significantly decrease life expectancy with a hazard ratio of 8.2 (p = 0.025).

Conclusion
HDC-HSCT might be useful as an adjuvant therapy for high risk patients with breast cancer such as 10 or more lymph node metastasis at diagnosis especially when the patient is not heavily treated before transplant.

No conflict of interest to disclosure
PI17
CD34 Specific Viability Assay But Not Total Cell Viability Assay Correlate With Actual HPC Viability Following Cryopreservation

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Background and Aim
Hemopoietic stem cells (HPC) are being frequently used for the treatment of hematologic malignancies. The autologous HPC products are routinely processed and cryopreserved. Three important parameters such as enumeration of CD34+cells, viability and cultures are essential quality indicators. It is hypothesized that the viability of the HPC products may be compromised due to increased number of granulocytes, autolytic enzymes, proinflammatory cytokines, free radicals, and consumption of nutrients in the HPC product. The present study is undertaken to see whether the increased number of granulocyte has any effect on the viability of HPC product.

Study Design and Method
The cryopreservation charts of 140 patients were retrospectively reviewed. The viability was usually measured by flowcytometry within 24 hours after cryopreservation and thawing. Total WBC, CD34 mab, CD45 mab and 7AAD were used for viability. The standard cut off value for viability was set at 70%. Total cell viability in CD45 window was performed initially and then CD34 specific viability was determined.

Result
Of 140 HPC products, total cell viability was decreased below 70% in 39 HPC products. The percent decrease in viability ranged from 19.2% to 69.2%. 5 HPC products were discarded. All 34 HPC products have shown CD34 specific viability of >70%. All 34 patients engrafted. The increased number of granulocytes apparently did not have any effect on the viability of HPC.

Conclusion
CD34 specific viability should be integrated in the viability assay system in place of total cell viability. Apparently the number of granulocytes content in the HPC product does not have any effect on the overall HPC viability. However lysis of granulocytes, their released enzymes and cytokines effects are not determined. At present, a prospective study using enzymes and cytokines is underway that may have a detrimental effect on the overall viability.

No conflict of interest to disclose
Monitoring of Cytomegalovirus-Specific CD8+ T-cell response using QuantiFERON-CMV Assay in Adult Allogeneic Hematopoietic Stem Cell Transplant Recipients

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Aim
Cytomegalovirus (CMV) infection in hematopoietic stem cell transplant (HSCT) recipients is a major cause of morbidity and mortality. CMV infection causes a strong virus-specific cytotoxic T-cell response thus the evaluation of T-cell response will be a useful tool in monitoring and predicting CMV infection in HSCT recipients. In this study, we monitored CMV-specific immune response in adult HSCT recipients with QuantiFERON-CMV assay in correlation with CMV antigenemia.

Methods
35 adult patients who received allogeneic HSCT from October 2008 to May 2011 were recruited. Monitoring schedule was on week 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 after HSCT. CMV-specific CD8+ T-cell response was measured using QuantiFERON-CMV assay. CMV antigenenemia assay was performed with immunofluorescence staining method.

Results
Among the study patients, 71% (25/35) experienced positive-CMV antigenemia at least for one time during the study period and 96% (24/25) of CMV infection developed during the first 12 weeks. Notably, patients with positive QuantiFERON-CMV test showed less CMV infection on week 4, 6, 8 and 10 than those with negative QuantiFERON-CMV test. The recipients from QuantiFERON-CMV positive sibling donors showed faster recovery of CMV immunity compared with the recipients from QuantiFERON-CMV negative sibling donors (median 3 weeks vs 12 weeks, respectively).

Conclusions
This study shows that CMV-specific CD8+ T-cell response is important in protecting HSCT recipients from CMV infection, especially within 12 weeks post-HSCT. QuantiFERON-CMV assay may be a useful tool in monitoring the HSCT recipients for the development of protective immunity against CMV infection.

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Itraconazole for Secondary Prophylaxis of Invasive Fungal Infection in Haematological Disease: Results from China

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Aim
Invasive fungal infection (IFI) is a major cause of treatment-related morbidity and mortality in patients with haematological disease. The risk of recurrence of fungal infection following chemotherapy or HSCT is high. To further evaluate itraconazole for secondary prophylaxis of previous proven or probable IFI, our study was conducted in patients undergoing chemotherapy or allogeneic HSCT.

Method
A prospective, open-label, multicenter trial was conducted evaluating Itraconazole (200 mg q12h intravenously d1-2, 200 mg /d) as secondary antifungal prophylaxis in chemotherapy patients or allogeneic stem cell transplant recipients with previous proven or probable invasive fungal infection. Itraconazole was started when patients’ neutrophils <0.5*10⁹/L, and stopped when chemotherapy patients’ neutrophils >0.5*10⁹/L; allogeneic stem cell transplant recipients’ neutrophils >1.0*10⁹/L. The primary end-point of the study was the incidence of proven or probable invasive fungal infection.

Results
One hundred fifty patients were enrolled, 123 completed the trial. The median duration of itraconazole prophylaxis was 14 days. No patients died during the trial. Five invasive fungal infections during the trial, all of them were received chemotherapy. The cumulative incidence of invasive fungal disease was 4.1%. Two patients were withdrawn from the study due to treatment-related adverse events (i.e. liver toxicity and phlebitis).

Conclusions
Itraconazole appears to be safe and effective for secondary prophylaxis of systemic fungal infection after chemotherapy and allogeneic stem cell transplantation. The observed incidence of 4.1% is considerably lower than the relapse rate reported in historical controls, thus suggesting that itraconazole is a promising prophylactic agent in this population.

No conflict of interest to disclose  (ClinicalTrials.gov identifier: NCT01198236).
A Case of Evans Syndrome Occurred after Allogeneic PBSCT in a Patient with Idiopathic Myelofibrosis

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Post-transplantation hemolytic anemia usually occurred in ABO mismatched setting. Evans syndrome is a rare disease characterized by autoimmune hemolytic anemia and immune thrombocytopenia or immune neutropenia. We report a case of successfully treated Evans syndrome which occurred after ABO-matched, related allogeneic peripheral blood stem cell transplantation (PBSCT) in patient with idiopathic myelofibrosis.

A 37-year-old female was admitted with dizziness with marked splenomegaly. Initial CBC was WBC 32,340/uL, hemoglobin (Hb) 3.8 gm/dL, platelet 9,000/uL. She was diagnosed with JAK2 V617F wild type chronic idiopathic myelofibrosis. She was heavily dependent on platelet transfusion. Therefore she treated with a non-myeloablative preparative regimen that included fludarabine, busulfan, and antithymocyte globulin followed by allogeneic PBSCT from her HLA-matched sister. Neutrophil engraftment was on D+14, but platelet engraftment was delayed. Bone marrow (BM) study showed fibrosis was markedly regressed, and full donor chimerism was achieved by STR on D+28. The spleen was not palpable. Spontaneous intracerebral hemorrhage occurred on D+38. Two weeks after the cyclosporin tapered off at 5 months after transplant, severe pancytopenia, WBC 1,720/uL, Hb 4.7 gm/dL, platelet 1,000/uL, recurred. Follow-up BM biopsy showed findings of graft failure and unilaterally worsened myelofibrosis. STR also showed mixed chimerism status. We prepared retransplantation. But irregular antibody screening test for PRC transfusion converted to positive. Direct anti-IgG and indirect Coombs tests were strong positive. The diagnosis of Evans syndrome was made. Response to per oral prednisone 1 mg/kg/day was rapid, and she was on platelet transfusion independent after 14 days. 5 months after steroid treatment, WBC and Hb rose to normal range, and platelet increased to 86,000/uL.

No conflict of interest
**P121**
The Incidence and Risk Factors of Invasive Fungal Infection after Haploidentical Hematopoietic Stem Cell Transplantation Without *in vitro* T-cell Depletion

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**Aim**
In recent years, we have successfully established a novel method of haploidentical hematopoietic stem cell transplantation (HSCT) without *in vitro* T-cell depletion, which can achieve comparable outcomes with HLA-matched transplantation or unrelated transplantation. Data related to invasive fungal infection (IFI) under this new model are scarce. This study aimed to analyze the incidence and the risk factors of IFI under this transplantation method.

**Method**
291 patients who received haploidentical HSCT from January 1st, 2007 to December 31st, 2008 were enrolled. Diagnosis of IFI was documented according to the EORTC/MSG criteria, and only proven or probable cases of IFI were regarded as true cases.

**Results**
Forty-one patients were documented as IFI, including 4 proven cases and 37 probable cases. The median time of diagnosis was 29 days (6-405 days) after transplantation. The cumulative incidence of IFI at 1, 2 and 3 years after transplantation was 13.7%, 14.1% and 14.1%, respectively. Multivariate analysis identified platelet engraftment time (>17 days) (p=0.019; HR 2.460, 95% confidence interval 1.161-5.263), high-risk disease (p=0.002; HR 2.794: 95% confidence interval 1.471-5.305), and grade III-IV acute GVHD (p=0.027; HR 2.265: 95% confidence interval 1.095-4.684) as risk factors for IFI. The incidence of IFI in patients with 0, 1, 2 or 3 risk factors during 3 years was 4.47%, 8.57%, 30.98% and 30.77%, respectively.

**Conclusion**
IFI is an important complication following haploidentical HSCT without *in vitro* T-cell depletion. Patients with high-risk underlying disease, slow platelet engraftment and grade III-IV acute GVHD are at high risk of IFI.

No conflicts of interest

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**P122**

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

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**Aim**

We compared the clinical outcomes of unrelated cord blood transplantation (UCBT) with related bone marrow transplantation (BMT) and/or peripheral blood stem cells transplantation (PBSCT) in adults with hematologic malignancies.

**Method**

Between Oct. 2001 and June 2011, 152 adults with hematologic malignancies received unrelated cord blood transplantation (CBT, n=51) and related bone marrow transplantation (BMT) and/or peripheral blood stem cells transplantation (PBSCT)(n=101). All patients received myeloablative transplantations from single center.

**Result**

Median follow-up was 17 months (range, 0.8-109.6 months) for BMT/PBSCT and 6.2 months (range, 1.0-96.7 months) for CBT recipients. Recipients of unrelated CBT were younger (median, 22 vs. 30 years of age; P<0.05) and had more advanced disease at the time of transplantation (78.4% vs. 34.7%, P<0.05) than recipients of related BMT/PBSCT. All related transplants were HLA matched, whereas 21.6 percent of CBT grafts were HLA mismatched (P<0.05). The median number of nucleated cells infused was 0.37×10^6 per kilogram of the recipient's body weight for CBT and 5.65×10^6 per kilogram for BMT/PBSCT recipients (P<0.05). Neutrophil recovery was significantly delayed after CBT (median, 19 vs. 12 days post transplantation, P<0.05). In multivariate analysis, the overall engraftment rates showed lower trend in CBT recipients(94% in CBT and 100% in BMT/PBSCT recipients, P=0.062). There was no significant differences in transplantation-related mortality (TRM) (31.4% in CBT and 21.8% in BMT/PBSCT recipients), relapse rate (9.8% in CBT and 14.9% in BMT/PBSCT recipients), the incidence of grades III to IV acute graft-versus-host disease (aGVHD) (6.3% in CBT and 5.0% in BMT/PBSCT recipients), 1 year leukemia-free survival (DFS)(57.2% in CBT vs. 71.5% BMT/PBSCT recipients) and 1 year over survival (OS) (57.2% in CBT vs. 71.5% BMT/PBSCT recipients) between both groups, but extensive chronic GVHD was lower in CBT recipients (0% vs. 15.8%).

**Conclusion**

HLA-mismatched cord blood should be considered an acceptable source of hematopoietic stem-cell grafts for adults in the absence of an HLA-matched adult donor.

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**II:123**
P123
The Quality of Life (QOL) of Family Members as Caregivers for Recipients of Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

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Aim
Little is known about the QOL of their caregivers for HSCT recipients late after the transplants. The purpose of this study was to explore the long-term effects of HSCT on QOL and health problems of family members who are the primary caregivers for their HSCT recipients.

Methods
Family members who were nominated as caregivers by their recipients of allogeneic HSCT between 2003 and 2009 at Keio University Hospital were enrolled in this cross-sectional observation study. Their QOL was measured at a mean of 50.9 months after HSCT by using Profiles of Mood States (POMS), SF-36, and Caregiver Reaction Assessment (CRA-J).

Results
The median age of family members was 54.5 (range 21-87 years). The relationship with their recipients included father (5), mother (9), husband (29), wife (17), child (2), sister (1), and unknown (3). Forty-nine (74.2%) family members had at least one subscale of POMS where the score was lower than the population norm. SF-36 also showed that 27 (40.1%) had at least one dimension where the score was lower than the population norm. Frequently impaired dimensions of SF36 were role functioning—physical, role functioning—emotional and social functioning. CRA-J, a measure of burden of recipients' care, was lower in 3-21% of family members, and the score of CRA-J correlated with those of POMS and SF-36. Wife-as-caregiver also associated with impaired QOL measured by the above scales, but husband-as-caregiver and time from transplants did not.

Conclusions
The QOL of family members for transplant recipients was often impaired throughout the HSCT trajectory. Studies for screening and counseling/supports based upon the identification of factors affecting their QOL after SCT is clearly warranted.

No conflict of interest to disclose
P124
Haemorrhagic Cystitis in Paediatric Haematopoietic Stem Cell Transplant: Report from a Paediatric Transplant Center in Singapore

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Background
Haemorrhagic cystitis is a known morbidity in the haematopoietic stem cell transplant (HSCT) setting. We describe the cases in our institute over the last 12 years.

Methods
This is a retrospective chart review of HSCT patients in our institute from 1999 to 2011 (12 years). Data on patient characteristics, type of HSCT as well as description of the hemorrhagic cystitis episodes, their management and outcomes were collected.

Results
There were 9 patients with haemorrhagic cystitis over the 12 years. During this period 105 cases of HSCT were performed in our center. This gave an incidence of 8 % in our study. The age ranged from 3 to 13 years. There were 5 girls and 4 boys. The majority (8/9 88%) had allogeneic HSCT. The indications for the HSCT in these patients were: chronic myeloid leukemia (3 patients), myelodysplastic syndrome (2 patients), acute lymphoblastic leukaemia (2 patients), acute myeloid leukaemia (1 patient) and high-risk neuroblastoma (1 patient, autologous HSCT). Conditioning for all the allogeneic HSCT included cyclophosphamide – BuCy (5 patients) or CyTBI (3 patients). The onset of hemorrhagic cystitis ranged from day +5 to day +68. Duration was 3 to 153 days. Urine BK virus was positive in 6 patients (urine BK virus studies were not performed for the first 3 cases). Three of these received intravenous (IV) with/without intravesical cidofovir, with unsatisfactory results. All patients received blood product support for thrombocytopenia. Six (66 %) patients, 3 of whom had BK viruria, required urine catherization and bladder washout, and eventually underwent cystoscopy and diathermy. One of these 6 patients needed a suprapubic catheter. Another underwent intravesical prostaglandin (PGF2α) with disappointing results. Two patients, both BK positive, had spontaneous resolution.

Conclusion
From our series, haemorrhagic cystitis was a significant morbidity in HSCT patients. Most of these patients had to undergo invasive interventions such as cystoscopy and diathermy, and intravesical drugs. BK viruria is a common cause of haemorrhagic cystitis in our study. Optimal management of this problem is still unknown.

No conflict of interest to disclose
P125
Haematopoietic Stem Transplantation for Primary Immunodeficiency Disease: A South-East Asian Perspective

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Haematopoietic stem cell transplants (HSCT) are the only cure for primary immunodeficiency disease (PID). If left untreated, PID is associated with high mortality from serious infections in childhood. We described our experiences on HSCT for children with PID in our population.

Method
We performed HSCT for 14 children with PID over a period of 12 years from Dec 1996 to Feb 2009. Of these, 10/14 (71%) received unrelated cord blood transplants (CBT), 3/14 had MSD and only one had MUD marrow transplant. The underlying diseases included SCID (5), HIgM (5), CGD (2), WAS (1), LAD (1). The median age at transplant was 35 months (range 3 to 204 months). Conditioning regimes consisted of myeloablative Busulphan (BU)/ Cyclophosphamide (CPA) +/-ATG regimes for all except for the 5 SCID who had reduced intensity conditioning (RIC). The RIC regime consisted of Fludarabine +/-Melphalan +/-CPA. GVHD prophylaxis consisted of cyclosporine A +/- or a combination of methotrexate, methylprednisolone or mycophenolate mofetil.

Results
Fourteen percent (2/14) had graft rejection. TRM was 14% (2/14), both had unrelated CBT, one died from pneumonia and the other from chronic GVHD in liver and lung. Overall survival remained good at 79% (11/14). Of those with unrelated CBT, 7/10 (70%) are alive. Pre and post transplant infective complications were significant in our patients; they ranged from disseminated BCGitis seen in SCID cases to systemic fungal infections.

Conclusions
PID is highly curable with HSCT. MSD transplants give 80 to 90% while MUD gives 50 to 60% cure reported in literature. The unrelated CBT in our study showed an intermediate range of cure 70%. Unrelated cord blood is an important source of stem cells for our diverse racial local population.

No conflict of interest to disclose
P126
Dynamic Detection of Minimal Residual Disease for Patients with High-Risk Acute Leukemia Post Allogeneic Hematopoietic Stem Cell Transplantation

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Aim
To find the relationship between minimal residual disease (MRD) and the outcome of patients with high risk acute leukemia (AL) who underwent allogeneic hematopoietic stem cell transplantation (HSCT).

Method
Using four or five-color multi-parameter flow cytometry (MFC, CD45/SSC gating) to detect MRD pre-(day-30) and post-transplant (day+30, +60, +100, 6 months, 9 months and 12 months, we retrospectively analyzed the MRD levels and prognosis of 90 high risk AL patients (AML: 31 cases, ALL: 59 cases). According to the MRD cutoff value of 0.1%, low level and high level groups were defined. In the high level group, patients were divided into two sub groups according to the subsequent treatment (receiving intervention therapy group and without intervention therapy group).

Result
The median follow-up was 24 (1-102) months. Patients with high levels of MRD post-transplant (+60d and +100d) showed higher relapse rates than that of the low level group. Multivariate Cox regression analysis revealed that the MRD level on day +100 was independent parameter to predictive for OS, differences were significant among three groups (MRD high level with intervention group, MRD low level group and MRD high level without intervention group) including 1 year relapse-free survival (RFS) (100% vs 91.3% vs 60.87%). 1 year overall survival (OS) (100% vs 91.67% vs 60.87%) and 3 years RFS (85.71% vs 68.48% vs 44.72%, all P<0.05).

Conclusion
MFC-based quantification of MRD post transplant reveals important prognostic information in patients with high risk AL. MRD check point at day +100 (cutoff: 0.1%) allows to discriminate different risk populations, those patients with MRD levels ≥0.1% should receive early intervention at an early stage when the tumour burden is still low to reduce the relapse rate and increase survival.

No conflict of interest to disclose
P127
Life-threatening Complications Suspected Due to Clinical Pharmacokinetic Interaction Between Cyclosporine A and Triazole Antifungal Agents in Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) Recipients

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Aim
It is well documented that the co-medication with the triazole antifungal agents is associated with a flattening of the CSA blood concentration profile via the cytochrome P450 3A4 dependent metabolic pathway in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Our aim is to evaluate the tolerability, toxicity and clinical outcome of the co-administration of CSA and triazole antifungal agents in allo-HSCT recipients.

Method
A retrospective review of the medical records of 104 consecutive patients undergoing allo-HSCT for hematologic malignancies at our transplant center over past 5 years was conducted. The causality of administration of CSA in combination with triazole in 12 cases (11.54%) with graft rejection or life-threatening complications experiencing supratherapeutic trough levels of CSA was identified and analyzed.

Result
Out of 12 patients, 5 patients received itraconazole prophylaxis, 3 voriconazole treatment, 4 itraconazole prophylaxis sequenced with voriconazole treatment. 2 cases developed acute graft rejection but are still alive, in which CSA dosage and trough levels were not influeneced by the presence of itraconazole prophylaxis. That indicates the trough plasma concentration might inadequately reflect CSA absorption profile. In other 10 patients, the CSA trough levels remained higher than therapeutic range even after gradual tapering of CSA dosage. Shortly after the engraftment, these 10 patients eventually died of non-infectious pulmonary complication or multi-focal leukoencephalopathy (PML), which might be ascribed to CSA-related adverse effects. The median survival time was 46.5 days.

Conclusion
Although preemptive CSA dosage reduction and the close monitoring of its whole blood trough levels may minimize the drug-toxicity when co-administration with triazole antifungal agents, the different clinical spectrum and different disease evolution post transplant in this group warns that in the setting of hematopoietic cell transplantation with CSA as immunosuppression agent the individual variables influence the drug-exposure and drug-toxicity.

No conflict of interest to disclose
PI28
Survival After HSCT: Overall Survival Estimates from the Cancer after Stem Cell Transplantation (CAST) Study

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Aim
To evaluate overall survival following haematopoietic stem cell transplantation (HSCT) in patients treated for cancer.

Method
Over 13,000 HSCT recipients treated from 1992-2007 in Australia were assembled for the CAST study. Deaths following HSCT were identified from the Australasian Bone Marrow Transplant Recipient Registry and through data linkage with the National Death Index. Overall survival estimates for acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), myelodysplasia (MDS), non-Hodgkin’s lymphoma (NHL), Hodgkin’s disease (HD) and multiple myeloma (MM) were calculated for 7,006 patients who survived at least 2 years post-HSCT. The influence of age and sex on survival for each diagnosis was examined using Cox proportional hazards models.

Results
Among recipients of allogeneic HSCT, overall survival was 77.6% for ALL, 76.1% for AML, 86.7% for CML, 77.4% for MDS and 75.6% for NHL 10 years post-HSCT. For those with AML, an increased risk of death was found for patients in age groups 15-29, 30-44 and ≥45 years compared to patients 0-14 years (p≤0.01). There was no significant difference in survival between males and females diagnosed with AML, CML, MDS or NHL (p>0.05). However, males with ALL were almost twice as likely to die as females with ALL (HR=1.79, 95%CI=1.07-3.00). Among recipients of autologous HSCT, overall survival was 80.2% for AML, 75.2% for HD, 34.9% for MM and 66.4% for NHL 10 years post-HSCT. AML patients aged ≥45 years were almost 5 times more likely to die than AML patients aged 0-14 years (HR=4.61, 95%CI=2.17-9.82). However, no significant difference in survival was observed between AML patients aged 0-14 years and 15-29 or 30-44 years (p>0.05).

Conclusion
Survival following HSCT can vary by age and sex for some cancer diagnoses. Transplant-related data currently being collected for the CAST study will further define risk factors for death following HSCT. No conflict of interest to disclose.
P129
Oral Mycophenolate and Intravenous Tacrolimus as GVHD Prophylaxis in Umbilical Cord Blood Transplantation for Elderly Patients with Hematological Diseases

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Aim
The optimal drug combination for GVHD prophylaxis following reduced-intensity umbilical cord blood transplantation (RI-UCBT) has not been established. Since December 2005, elderly patients, 55 years and older, received oral MMF and intravenous tacrolimus (Tac) as a GVHD prophylaxis in our institute, and we conducted a single center retrospective study to investigate safety and efficacy of Tac+MMF combination.

Method
RI-UCBT recipients, 55 years and older, in 0-3 ECOG performance status, free from active infection at the date of transplant, and who received RI-UCBT as their first transplant were retrospectively reviewed. Tac was administered intravenously from day -1 of transplant aiming for serum concentration of 10-15 ng/mL. Oral MMF was started on day 0 at 15-30 mg/kg/day and was discontinued or started to taper down on the day of neutrophil engraftment in the absence of active GVHD.

Result
One hundred and five patients were included. 66 (63%) were male, and the diagnoses were AML/MDS (n=84), CML (n=4), ALL (n=2), ML (n=12), and SAA (n=3). 87 (83%) were in a high risk disease status (i.e. MDS RAEB and beyond, or AL, ML not in remission). Median age was 62 (range, 55-82). Median total nucleated cells and CD34⁺ cells infused were 2.5 (1.8-4.3) x 10⁷/kg and 0.84 (0.3-1.85) x 10⁵/kg, respectively. Cumulative incidence of neutrophil recovery (>500/ul) up to 50 days post-transplant was 79% (median 20 days post-transplant, range, 12-48). Median observation period of survivors post-transplant was 356 (17-1674) days. Cumulative incidences of NRM at 30 days, 100 days, and 1 year post-transplant were 5.8%, 24.2% and 30.2%, respectively. Cumulative incidence of relapse at 1 year post-transplant was 34.5%. Overall survival and event free survival at 1 year were 45.8% and 31.8%, respectively.

Conclusion
For patients 55 years and older, GVHD prophylaxis using Tac+MMF showed acceptable neutrophil recovery, low early mortality, and survival. (299 words)

No conflict of interest to disclose
P130
Pre-engraftment Syndrome after Unrelated Donor Umbilical Cord Blood Transplantation in Patients with Hematologic Malignancies

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Aim
Pre-engraftment syndrome (PES) after umbilical cord blood transplantation (CBT) remains poorly characterized, and the prognosis and appropriate management are unclear. Therefore, we retrospectively analyzed the incidence, risk factors, manifestations, and clinical outcomes of PES in CBT recipients who had been treated for hematological malignancies at our transplantation center.

Method
Eighty-one patients (median 18 years, range 3-48) with hematologic malignancies received unrelated CBT between April 2000 and November 2010 at the Department of Hematology of Anhui Provincial Hospital, China. PES was defined as unexplained fever higher than 38.3°C that is not associated with documented infection and unresponsive to antimicrobial manipulations; and/or unexplained erythematous skin rash occurring prior to neutrophil engraftment. A total of 81 patients received either myeloablative (n=72) or nonmyeloablative (n=9) conditioning.

Result
Neutrophil engraftment was achieved in 69 of the 81 cases (86.2%, 95% confidence interval [CI] = 78.9%-94.1%), and the median time to more than 0.5×10^9/L ANC was 19 days (range, 12–39). Fifty-one patients (63.0%) developed PES at a median of 7 days (range 3-15) post-transplant: 46 patients had both rash and unexplained fever; one patient had unexplained fever alone and 4 patients had rash only. 47 patients (92.2%) received IV methylprednisolone (MP) at a median dose of 1 mg/kg (range 0.4-3). All patients treated with MP responded as evidenced by fever resolution combined with resolution of rash. Univariate analysis identified myeloablative conditioning and younger age as significant risk factors for developing PES. Cumulative incidence of grade II-IV acute graft-versus-host disease (aGVHD) in the PES+ and PES- groups was 51.5 % (95% CI = 38.0%-70.0%) and 17.0% (95% CI = 6.9%-41.7%), respectively, and we found significantly increased risk of grade II-IV aGVHD among PES patients (P=0.035). However, PES was not associated with sustained donor engraftment, the day to neutrophil recovery, chronic GVHD, transplant-related mortality (TRM) at day 180 and overall survival.

Conclusion
We conclude that PES, which is common after CBT, is a strong predictor of aGVHD, and patients with PES respond promptly to corticosteroids.

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Allogeneic-Hematopoietic Stem Cell Transplantation (Allo-HSCT) For Chronic Active EBV Infection (CAEBV): Report of Three Cases

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Background
CAEBV is defined as a systemic EBV-associated NK/T-cell lymphoproliferative disorder characterized by recurrent infectious mononucleosis-like symptoms and high mortality. We report three cases of CAEBV treated with Allo-HSCT.

Case 1: A 26-year-old female with CAEBV had been suffering from recurrent fever and liver dysfunction for 5 years. She received prior chemotherapy and umbilical cord blood transplantation (UCBT) after reduced-intensity conditioning (RIC) consisting of 4 Gy of total body irradiation (TBI), 150mg/m² of fludarabine (Flu), and 120mg/kg of cyclophosphamide (CY). CMV colitis developed on day 30, and then improved. T-cell chimerism at day 14, 28, 56 was 81.2%, 87.6%, 100% respectively. The EBV-DNA load has been kept at undetectable levels for 20 months after UCBT. Moreover, she got pregnant and had a delivery at 32 weeks of gestation.

Case 2: A 26-year-old female with CAEBV had been suffering from severe mosquito bite allergy for more than 15 years. She received Allo-BMT from HLA 7/8 loci-matched unrelated donor after RIC (Flu+CY+TBI4Gy). Although grade2 acute GVHD and mild bronchiolitis obturans developed, the symptoms were controlled with immunosuppressive therapy. Mix T-cell chimerism was observed (90.4%) at day 550 after BMT, but EBV-DNA load has fallen to undetectable levels.

Case 3: A 26-year-old male was diagnosed with NK/T-cell lymphoma derived from CAEBV. He had been suffering from recurrent fever and lymphadenopathy for 6 month. Although the first diagnosis was CAEBV, the reassessment of lymphnode and bone marrow biopsy revealed the progression to NK/T-cell lymphoma. He received CHOP therapy, but gained insufficient response. He was treated successfully with intensive regimen, SMILE therapy, and we are preparing to perform UCBT.

Conclusion
Allo-HSCT with RIC regimen is an effective approach for the treatment of CAEBV. It is important to assess the disease status, because patients in progressive disease states might need more intensive chemotherapy and/or Allo-HSCT with conventional myeloablative regimen.

No conflict of interest to disclose
PI32
Autologous Stem Cell Transplantation as First Line Therapy in Aggressive Non-Hodgkin’s Lymphoma

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Aim
The role of high dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) as front-line therapy in high-risk aggressive non-Hodgkin’s lymphoma (NHL) patients is still a matter of debate, but several studies demonstrated the efficacy of HDT in pre-Rituximab era. Since Rituximab added to CHOP chemotherapy, OS and PFS were significantly improved. Therefore, we analysed to compare conventional chemotherapy with HDT followed by ASCT in aggressive NHL in the post-Rituximab era.

Methods
We retrospectively reviewed the medical records of 367 patients with primary diagnosed aggressive NHL from January 2002 to December 2009. Among them, we selected patients who achieved complete or partial remission after first induction chemotherapy or had ≥3 International Prognostic Index (IPI) scores or stage III / IV. Among 367 patients, 43 patients younger than 65 years were enrolled and categorized as two groups: conventional chemotherapy group (n=33, 77%) and HDT followed by ASTC group (n=10, 23%).

Results
The median age at the time of diagnosis was 46 years (range, 15-64). Diffuse large B-Cell lymphoma (DLBLC, 47%), T-cell lymphoma (32%) and lymphoblastic lymphoma (7%) were included. The proportion of Rituximab including regimen for induction chemotherapy were 49%. The five-year survival rate (±SD) was not significantly different between two groups (00±0 percent in chemotherapy group vs. 00±0 percent in HDT group, P=). And the estimated progression free survival at five years was not significantly different between two groups (00±0 percent in chemotherapy group vs. 00±0 percent in HDT group, P=).

Conclusion
The efficacy of HDT followed by ASTC during first-line treatment in patients with aggressive NHL does not improve the outcome in post-Rituximab era and should be evaluated in randomized trials.

No conflict of interest to disclose
P133
KIR Genotypes of Donor-Recipient Pairs Influence the Rate of CMV Infection Following Hematopoietic Cell Transplantation

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Cytomegalovirus (CMV) is one of the common sources of opportunistic infections after hematopoietic stem cell transplantation (HSCT). The mortality of CMV disease is over 80% early in HSCT. Prevention and early diagnosis were the key measures to reduce the occurrence of CMV disease. Besides T-cells, natural killer (NK) cells are the primary effector populations that suppress CMV replication. NK cell functions are regulated by a complex repertoire of cell surface molecules including the killer-cell immunoglobulin-like receptors (KIR). The KIR genotype was determined by Sequence-specific primer polymerase chain reaction (PCR-SSP) in 138 pairs of donors and recipients before HSCT. Posttransplant monitoring for CMV infection was performed by indirect immune histochemically assays. The allele frequency of 2DS2 and 2DS003-007 of donors was decreased in the CMV positive group. The CMV infection rate was higher in patients who received KIR haplotype AA donor than in the others received KIR haplotype BB donor (P<0.05). With multivariate analysis, 2DS003-007 and KIR haplotype BB of donor reduce CMV infection adjusting for confounding variables. The results may guide the selection of donors as well as identify patients who may benefit from closer CMV monitoring and additional strategies to prevent CMV reactivation post transplant.
PI34
Unrelated Hematopoietic Cell Transplantation as Good as Identical Sibling Transplant for Chronic Myelogenous Leukemia in First Chronic Phase

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Allogeneic hematopoietic cell transplantation (HCT) is still the only curative therapy for chronic myelogenous leukemia (CML) although the therapeutic strategy for CML has been changed in the era of tyrosine kinase inhibitor (TKI). To learn the outcomes of HCT from identical sibling (SIB) or unrelated donor (URD) for CML in first chronic phase (CP1), 82 patients (SIB, n=47; URD, n =35) in our hospital during April 2004 to December 2010 were analyzed. HCT in SIB cohort was grafted with bone marrow (BM) plus peripheral blood stem cell (PBSC) (n=32) or BM (n=8) or PBSC (n=7). HCT in URD cohort was received PBSC only. In URD transplant, HLA 10/10 or 6/6 matched HCT was 12, 1 respectively, 9/10 matched in 10, 8/10 matched in 11, and 7/10 matched in 1. BuCy2 regimen was used in SIB HCT and BuCy2 plus ATG in URD HCT as conditioning. The median age of recipients and donors in SIB HCT arm was older than that in URT HCT arm. SIB HCT patients had shorter course from diagnosis to transplant (5.5 versus 6.5 months, p=0.03). All patients achieved sustained engraftment. The incidences of grades II to IV acute graft-versus-host disease in SIB and URT transplant cohorts were 17.2%, 17.1% (p=.92), respectively. The incidence of chronic GVHD did not differ significantly between two cohorts (67.0% vs. 79.6%, p =0.367). The median follow up time was 33, 34 months, respectively (p=0.86). Three-year leukemia-free survival and overall survival were 90.4% vs. 85.6% (p=0.40) and 90.4% vs. 82.8% (p=0.25) in SIB and URD HCT respectively. In conclusion, URD HCT has achieved clinical outcomes as good as SIB HCT in CML-CP1. Therefore, HCT from SIB and URD could be one of the choices for the patients with CML-CP1 who could not afford or tolerate TKI or has poor response to TKI.

No conflict of interest to disclose
Differentiation Induction of Human Placenta-derived Stem Cells into Hematopoietic Stem Cells

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Background/Aims
The placenta is a specialized organ which has both fetal and adult tissues. The existence of mesenchymal stem cells and hematopoietic stem cells in fetal side of placenta has been reported. However the so called “mesenchymal stem cells” might have a more broad differentiation potential beyond mesenchyme because these stem cells are more closely associated with embryonic stem cells than adult stem cells. Based on this concept, we hypothesized that hematopoietic stem cells in placenta originate from other placental stem cells which have been called “mesenchymal stem cells”. To prove this hypothesis, this study is designed to perform in vitro differentiation of placental stem cells directly into the hematopoietic stem cells.

Methods
Placental stem cells were obtained from surgically isolated placental chorionic plates from healthy women who had undergone abortion at 6-8 weeks of gestation. After mesenchymal differentiation potential was identified in these cells by adipogenic and osteogenic differentiation, they were induced to be differentiated into hematopoietic stem cells by cytokine cocktail which was known to induce in vitro differentiation of embryonic stem cells into hematopoietic lineage (erythropoietin, Flt-3/Flk-2 ligand, G-CSF, GM-CSF, IL-6/7/15, stem cell factor, and thrombopoietin). In vitro differentiation was performed in conventional cell culture dish and low attachment dish for 3 weeks, respectively. After 3 weeks in vitro differentiation, expression of hematopoietic stem cell marker was analyzed using flowcytometry and RT-PCR, and colony forming unit (CFU) assay was performed.

Results
In the group cultured in conventional cell culture dish, no hematopoietic differentiation was identified regardless of the concentration of cytokine cocktail. However, in the group cultured in low attachment dish, expression of hematopoietic markers, CD 235a and GATA-2, was observed. Especially there was a trend that the expression of hematopoietic markers was increased dependent on the concentration of cytokine cocktail. CFU-E, CFU-G, and CFU-M colony was identified by 3 weeks in CFU assay.

Conclusion
The placental stem cells might be differentiated into the hematopoietic stem cells in vitro. The differentiation efficacy was dependent on the physical feature of culture dish and the concentration of cytokines in the medium. However, because we could not observe all kinds of CFU colonies, further refinement in culture condition should be required.

No conflict of interest to disclose
PI36
Retrospective Survey of Bleeding and Thrombotic Complications on the Early Phase of Hematopoietic Stem-Cell Transplantation (HSCT)

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Thrombotic and bleeding events are common and potentially fatal complications in patients receiving hematopoietic stem-cell transplantation (HSCT). In order to determine the clinical significance of hemostatic factors in thrombotic and bleeding events after HSCT, we retrospectively investigated the outcomes and the risk factors of thrombotic and bleeding complications in 478 HSCT recipients (133 auto-HSCT and 345 allo-HSCT recipients). The overall incidence of bleeding disorders was 65.9% (315 cases), in which 243 cases (77.1%) are allo-HSCT recipients. Minor bleeding was seen in 74.3% (234 cases), moderate bleeding was seen in 22.5% (71 cases), and severe bleeding was seen in 3.2% (10 cases) of all bleeding patients. The organs of hemorrhage involve skin or mucosa (36% in all HSCT recipients), gastrointestinal tract (36%), lung (1.2%), brain (0.4%), and urinary (39%). In regards to thrombotic complications, 16 recipients (3.3% in all HSCT recipients) developed thrombotic events, including 11 veno-occlusive diseases (VOD), 2 transplantation related thrombotic microangiopathy (TA-TMA), 1 pulmonary embolism (PE) and 2 deep vein thrombosis (DVT). The overall mortality after thrombotic events was 62.5% (10 cases) in all HSCT recipients with thrombotic complications. Both thrombotic and bleeding disorders were significantly correlated with age, disease category and pretreatment regimen (P<0.05), but not associated with gender, transplantation types, routine hemostatic parameters, and biochemical indicators (P>0.05). No difference was found in the reconstruction time required for haematogenesis between recipients with or without bleeding disorders (P>0.05), but the survival rate was correlated with the site and intensity of bleeding disorders (P<0.01). In addition, polyoma viruria and II-IV grade aGVHD were the independent risk factors for hemorrhagic cystitis. PAI-1 level in the HSCT recipients with thrombotic complications are significantly higher than those with other complications and toxicities (graft-versus-host disease, infections, and preparative regimen toxicity) (P<0.01). Our study showed that the HSCT patients with bleeding disorders presented high morbidity while the HSCT recipients with the thrombotic complications had high mortality. Some of the risk factors and hemostatic parameters were correlated with thrombotic or bleeding complications. All the finding suggested the necessity to diagnose and treat hemostatic complications early to improve the prognosis of HSCT recipients.

No conflict of interest to disclose
Allogeneic Stem Cell Transplantation as a Potential Treatment for a Patient with a Combined Disorder of Hereditary Spherocytosis and Chronic Myelogenous Leukemia

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Objective
Human severe hereditary spherocytosis (sHS) is life-threatening and transfusion-dependent. Allogeneic stem cell transplantation is a potential treatment for a patient with a combined disorder of hereditary spherocytosis and chronic myelogenous leukemia (CML).

Materials and Methods
Allogeneic stem cell transplantation is an excellent therapeutic intervention for sHS and CML. The patient was rescued by transplantation. Donor cell implantation and blood parameters were monitored periodically and bcr/abl was determined post-HSCT.

Results
sHS recipients with 100% donor erythroid cells have significantly improved red blood cell counts throughout life when compared with pre-HSCT. Total bilirubin levels are corrected in recipients. Donor-implanted HS patient at 1 to 18 months, have normal mean cell hemoglobin concentration. Reticulocyte counts normalize, bcr/abl was cleared away.

Conclusion
Our case is a rare example with a combined disorder of hereditary spherocytosis and CML following allogeneic bone marrow transplantation. Hereditary spherocytosis in itself was not a contraindication for patient in the matched sibling transplant setting.

No conflict of interest to disclose
PI38
Prolonged Thrombocytopenia Following Allogeneic Hematopoietic Stem Cell Transplantation and Its Association with a Reduction in Ploidy and an Immaturity of Megakaryocytes

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Prolonged thrombocytopenia is a frequent complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT); however, its pathogenesis has remained obscure. In the present study, we used flow cytometry to determine the frequency of bone marrow megakaryocytes (MKs) and MK ploidy distributions in allo-HSCT recipients with or without prolonged thrombocytopenia (n=32 and 27, respectively) and healthy volunteers (n = 13). In addition, the expression of c-Mpl in MKs was measured. The results indicate that the proportions of MKs in marrow mononuclear cells or the percentages of CD110+MKs in total MKs did not significantly differ between the 3 groups; however, in a comparison of nonthrombocytopenic allo-HSCT recipients to healthy volunteers, the allo-HSCT patients who had prolonged thrombocytopenia exhibited significant shifts toward low ploidy cells (left shift), which were accompanied by a marked increase in ≤8N cells (P<0.036 and P<0.001, respectively) and significant decreases in 16N cells (P=.001 and P<.001, respectively) and ≥32N cells (P =.01 and P<.001, respectively). These results indicate that there were more immature MKs in allo-HSCT recipients who had prolonged thrombocytopenia, in comparison with nonthrombocytopenic allo-HSCT recipients and healthy volunteers. We conclude that prolonged thrombocytopenia and slow platelet engraftment after allo-HSCT may be related to a reduction in ploidy and an immaturity of MKs.
Wilms’ Tumor Gene 1 Expression: An Independent Acute Leukemia Prognostic Indicator Following Allogeneic Hematopoietic Stem Cell Transplantation

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To evaluate the prognostic significance of Wilms’ tumor gene 1 (WT1) expression for monitoring minimal residual disease (MRD) and predicting relapse in patients with acute leukemia (AL) following allogeneic hematopoietic stem cell transplant (allo-HSCT), the WT1 expression levels of 138 AL patients were measured using real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) at designed time points after allo-HSCT. All patients were divided into four groups based on the HSCT outcomes and intervention application. A low level of WT1 expression following HSCT indicated a low risk of relapse, whereas WT1 expression higher than 1.05% was indicative of a higher probability of relapse. Only the advanced stage of disease (HR=2.73; 95% CI=1.337-5.573, p=0.006) and a WT1 expression ≥0.60% (HR=4.774; 95% CI=2.410-9.459, p=0.000) were associated with lower disease-free survival. Relapse (HR=0.119; 95% CI=0.056-0.250, p=0.000) and a WT1 expression ≥0.60% (HR=2.771; 95% CI=1.316-5.834, p=0.007) were associated with lower overall survival. In conclusion, the WT1 expression level is an independent prognostic factor that can predict clinical outcomes for AL patients after HSCT and provide a guide for suitable interventions.

No conflict of interest to disclose
**P179**

**Efficacy of Intermittent Ganciclovir in Preventing Cytomegalovirus (CMV) Reactivation Following Alemtuzumab-Based Reduced Intensity Conditioned (RIC) Allogeneic Haematopoietic Stem Cell Transplantation (HSCT)**

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**Background**

Intermittent ganciclovir, pioneered by Atkinson, for CMV prophylaxis following an allo-HSCT is not assessed in patients receiving allogeneic HSCT conditioned with alemtuzumab, a monoclonal anti-CD52 antibody associated with high incidence of CMV reactivation.

**Aim**

In this retrospective analysis we aim to determine the efficacy of intermittent use of ganciclovir in prevention of CMV disease following alemtuzumab based RIC allogeneic HSCT.

**Methods**

Patients undergoing alemtuzumab-based RIC allogeneic HSCT and with pre-HSCT CMV sero-positivity either in the donor or the recipient (or both) were included. Following allo-HSCT, all patients were planned to receive ganciclovir at 5 mg/kg/day three times a week starting with adequate haemopoietic recovery till day +84 maximum. Patients received valaciclovir if ganciclovir not continued till day +84. The conditioning regimen consisted of alemtuzumab 10 mg/m² day -8 to -4, fludarabine 25 mg/m² days -7 to -3 and melphalan 140 mg/m² day -1. Plasma CMV was monitored weekly.

**Results**

Eighteen patients fulfilled the criteria. The pre-transplant CMV serologies were: D+/R+ in 7, D+/R- in 5 and D-/R+ in 6. Total 17 patients (94.4%) reactivated CMV within 33 months following HSCT; 10 (55.5%) within day 84 at a median of 31.5 days (20-81). Six patients reactivated while still receiving ganciclovir, 4 shortly after cessation but within 84 days. Reactivation of CMV in 2/6 occurred during intensification of immunosuppression to treat graft versus host disease. By day +84, one patient had viral load >1000 copies/ml but no CMV disease was seen. Fourteen patients did not receive the complete course of ganciclovir (duration 7-70 days); either due to toxicity, reactivation of other viruses or logistical reasons; two receiving valganciclovir afterwards.

**Conclusion**

Use of intermittent ganciclovir in the context of alemtuzumab-based transplant conditioning is effective in preventing CMV reactivation in majority of patients; and high viral titres and CMV disease are uncommon.

*No conflict of interest to disclose*
P140
The Effects of Vanilloid-Like Agents on Platelet Aggregation

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Aim
It has been proposed that consumption of vanilloid-like agents, including capsaicinoids, the ‘hot’ principles found in chilli inhibit platelet aggregation and may protect against the development of cardiovascular disease. The aim of this study was to investigate the effects of a range of vanilloid-like agents on in vitro platelet aggregation.

Method
Venous blood was collected from healthy subjects who avoided antiplatelet medications and dietary chilli for at least 10 and 2 days, respectively. ADP-induced (10 and 5 µmol/L) platelet aggregation was determined using platelet rich plasma (PRP; 250x10^9/L) in the absence and presence the capsaicinoids [capsaicin and dihydrocapsaicin] and the endocannabinoid/endovanilloid agents [N-oleoyldopamine (OLDA) and N-arachidonoyl-dopamine (NADA)]. %Maximum aggregation (%Max), % area under curve (%AUC) and slope of platelet aggregation were determined. Platelet LDH release was also investigated to determine the direct toxic effects of these agents on platelets.

Result
ADP-induced (5 µmol/L) platelet aggregation was inhibited in a concentration-dependent manner by capsaicin (%Max, mean±SEM; 0 vs 100 µmol/L, 83.8±0.9% vs 45.2±2.4%, n=6, p<0.001); dihydrocapsaicin (0 vs 50 µmol/L, 47±0.8% vs 39±3%, n=6, p<0.001); OLDA (0 vs 100 µmol/L, 71.6±8.2% vs 9.4±1.4%, n=4, p<0.001); and NADA (0 vs 100 µmol/L, 71.5±5.9% vs 38.2±1.4%, n=4, p<0.008). Similar results were observed using 10 µmol/L ADP. The inhibition of platelet aggregation by all agents was not due to direct toxic effects as LDH release from platelets was unaffected.

Conclusion
Capsaicin, dihydrocapsaicin, OLDA and NADA inhibit ADP-induced aggregation of platelets from healthy subjects, a mechanism that is not due to direct toxic effects of these agents. Further studies are warranted to determine interaction(s) of these vanilloid-like agents with platelet ADP receptors, platelet granules, and potentially vanilloid receptors e.g., TRPV1 and signaling pathways.

No conflict of interest to disclose.
Food and Postprandial Platelet Aggregation in Healthy Individuals

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Aim
An association between plasma glucose, insulin and hyper activation of platelets is thought to be one of the possible links for development of cardiovascular disease in diabetes. However it is not known whether acute changes in plasma glucose and insulin concentrations in response to food intake affect postprandial platelet aggregation in healthy individuals.

Method
A randomised cross-over design study investigated the effects of three different meals on postprandial platelet aggregation in 32 participants from Northern Tasmania (mean ± SD; age 59.9 ± 11.7 years, BMI 27.1 ± 3.7 kg/m²). The isocaloric meals were: a low glycaemic index high carbohydrate (LGI-HC); a high glycaemic index high carbohydrate (HGI-HC) meal; and a low glycaemic index moderate protein/fat meal (LGI-MPF). Blood samples collected at fasting and at 60 and 120min postprandially were analysed for ADP induced platelet aggregation, plasma glucose, serum insulin and triglyceride responses, using mixed-methods regression corrected for repeated measures.

Result
Each meal led to significant reductions in postprandial platelet aggregation (all p<0.04). However, there was no difference between the meals at fasting or any of the postprandial times. Glucose was similar at baseline and 120min for all meals, but was significantly elevated at 60min after the HGI-HC compared to both LGI-HC and LGI-MPF meals (all p<0.03) as expected. Insulin was significantly higher after the HGI-HC meal compared to both LGI-HC and LGI-MPF (all p<0.01) meals at both 60 and 120min, and significantly lower after the LGI-MPF meal than the LGI-HC meal at 60min (p=0.04). Serum triglyceride concentrations were not significantly different between the three meals at any time. No significant associations were observed between platelet aggregation and measured metabolic variables.

Conclusion
In healthy individuals, a mechanism independent of glucose and insulin response may be responsible for positively reducing postprandial platelet aggregation.

No conflict of interest to disclose
PI42
Impaired Control of the Tissue Factor Pathway in SLE

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Aim
Thrombosis is a frequent manifestation in patients with systemic lupus erythematosus (SLE) although the precise mechanisms remain unclear. We investigated the tissue factor (TF) pathway in SLE patients and determined whether there were associations with other abnormalities present in SLE e.g., antiphospholipid antibodies (APA), inflammation and endothelial cell damage.

Method
101 subjects [40 SLE patients and 61 age- and sex-matched controls] were recruited from Tasmania, Australia. Markers of the TF pathway [TF antigen/activity, free and total tissue factor pathway inhibitor (TFPI) antigen, and TFPI activity], hypercoagulability [thrombin-antithrombin (TAT) complexes and prothrombin fragment 1+2 (F1+2)], inflammation [IL-1, IL-6, IFN-γ, TNF-α], and endothelial cell damage [soluble E-Selectin (sE-Selectin)] were determined in plasma. Serum levels of APA (aCL IgG and IgM isotypes, LAC, anti-β2GP1 and anti-prothrombin antibodies) were also determined.

Results
Patients had higher levels of LAC (p=0.0102), anti-β2GP1 (p=0.0139), anti-prothrombin (p=0.0139) and IFN-γ (p=0.0002) compared to controls. Furthermore, patients had higher levels of TFPI free antigen (patients vs controls; mean±S.E.M) (11.64±0.89 ng/mL vs 6.43±0.42 ng/mL; p<0.0001), but lower TFPI activity (0.66±0.07 U/mL vs 1.22±0.03 U/mL; p<0.0001). Patients had elevated TAT (18.18±6.27 μg/L vs 4.79±0.96 μg/L; p=0.01) and F1+2 (472.8±51.6 nmol/L vs 355.9±22.2 nmol/L; p=0.0212) compared to controls. No TF pathway marker was significantly associated with APA, inflammation or endothelial cell damage in SLE patients.

Conclusion
A significant increase in the ‘bioactive’ free form of TFPI was demonstrated in SLE patients, but was not matched by a corresponding increase in TFPI activity. Changes to the TF pathway were not associated with abnormalities in SLE, suggesting that hypercoagulability in SLE may (in part) be due to reduced TFPI activity, a mechanism that appears to be independent of other abnormalities of SLE.

No conflict of interest to disclose
P143
Variable Effects of Antiphospholipid Antibodies on in vitro Platelet Aggregation

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Aim
Antiphospholipid antibodies contribute to the development of thrombosis, although precise mechanisms remain to be elucidated. We determined the effects of affinity purified anti-β2GP1 and anti-PT antibodies on in vitro platelet aggregation.

Method
Platelet rich plasma (PRP) was collected from normal donors and standardised to 250x10^9/L for all experiments. ADP (5, 2.5 µmol/L) and collagen (5, 2.5 µg/mL) induced platelet aggregation was performed using an AggRAM platelet aggregometer (Helena Laboratories). PRP (200µL) was incubated with anti-β2GP1 or anti-PT antibodies (25µL) to final concentrations of 1.25-10 µg/mL for 10 minutes at 37°C. Results for parameters including %Maximum aggregation (%Max) and Slope were determined as the difference between test and a baseline control.

Result
Anti-β2GP1 antibodies significantly reduced platelet aggregation (%Max) in a concentration dependent manner using both 5 µmol/L (p=0.0007) and 2.5 µmol/L (p=0.038) ADP, but did not significantly affect the primary slope (rate) of aggregation. In contrast, the same antibodies significantly enhanced platelet aggregation (% Max) in a concentration dependent manner using 2.5 µg/mL (p=0.0018), but not 5 µg/mL collagen. Anti-PT antibodies significantly enhanced 5 µg/mL collagen-induced platelet aggregation (% Max) in a concentration dependent manner (p=0.034), but did not affect ADP-induced platelet aggregation.

Conclusion
Purified anti-β2GP1 antibodies reduced in vitro ADP-induced aggregation, but not the slope/rate, suggesting these antibodies interfere with secondary platelet aggregation. Potential mechanisms may include interference to the G-protein coupled membrane ADP receptors, P2Y1 and P2TAC, or by binding to anionic phospholipids associated with platelet membranes to reduce the release of arachadonic acid and subsequent thromboxane A2 production. The results suggest interactions between anti-β2GP1 and/or anti-PT antibodies and platelets are complex, but potentially have a significant effect on the overall balance of haemostasis in patients with SLE.

No conflict of interest to disclose
PI44
In vitro Modeling of Microparticle Production Occurring in Diabetic Nephropathy

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Aim
High levels of Advanced-Glycosylation-End-products (AGEs) and free fatty acids occur in diabetes and can cause degeneration of kidney epithelium leading to diabetic nephropathy (DN). CD36 has been shown to be a cellular receptor for these pathological ligands where signaling through CD36 can directly induce the apoptosis of renal tubular epithelial cells. We have recently established that a cell free form of plasma CD36 that is considered to be a diabetes biomarker is entirely associated with microparticles (MPs). We hypothesize that some of the elevated MPs levels in diabetes can be attributed to DN and here we aim to develop an in vitro model of this process using the HK-2 human tubular epithelial cell line.

Method
AGEs were prepared by incubating D-glucose with BSA. HK-2 cells grown either in low-glucose or high-glucose media to regulate the levels of CD36 were treated with AGEs and palmitic acid (PA) to induce apoptosis. MPs were analysed using Western blotting after either ultracentrifugation or immunoprecipitation and also using multi-parameter flow cytometry.

Results
Flow cytometry and immunoblotting showed increased CD36 expression on HK-2 cells and increased CD36+MPs production upon apoptosis in high glucose conditions. Results from three experiments showed that AGEs were able to generate 2.5 times more CD36+MPs and 1.8 times less phosphatidylserine (PS)+MPs in comparison with PA that was able to generate higher total MPs numbers.

Conclusion
Treatment of HK-2 cells with AGEs and PA induced apoptosis resulting in the production of MPs and this correlated with CD36 expression levels. Quantitative and qualitative differences in MP production were observed with PA inducing MPs most similar to those reported for diabetic subjects. Further investigations are needed to establish the origins of plasma MPs in diabetes but we envisage that our in vitro model of MP production in DN will help direct this investigation.

No conflicts of interest to disclose
The Current Status of Australian Veterans Taking Warfarin

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Aim
Warfarin remains a high-risk drug for adverse events, particularly in the elderly. We aimed to determine the impact of prescriber-pharmacist collaborative medication reviews conducted in primary care on the rate of hospitalisation due to bleeding and thrombosis and to determine the influence of these reviews on INR control in veterans taking warfarin.

Methods
We undertook a retrospective cohort study using administrative claims data for Australian veterans and war-widows who were regularly dispensed warfarin during the study period, resided at home and provided their consent to be involved. The Department of Veterans Affairs (DVA) identified a cohort of veterans, half of whom had received a Home Medicines Review (HMR) and half of whom had not, and invited them to contact the research team. Pathology providers were subsequently contacted to provide INR results.

Results
818 veterans participated in the study; 281 veterans had received an HMR and 537 veterans had not. 64.4% were male and the median age was 83 years. HMRs were not associated with a reduction in hospital admissions for combined bleeding and thrombotic events (1.4% vs. 1.1%, p = 0.74). The time in therapeutic range (TTR) for veterans whose INR results were available was also not influenced by HMRs (63.0% vs. 67.0%, p = 0.27). The overall TTR for the veteran cohort was 64.0%, and the median testing interval was 15.6 days. Veterans living in remote and outer regional areas had a significantly lower TTR than veterans living in inner regional and metropolitan areas (49.9% versus 65.3%, p < 0.01).

Conclusion
HMRs were not associated with a reduction in hospitalisations due to bleeding or thrombotic events; nor were they associated with improved INR control. The level of INR control was high, and comparable to that achieved in recent randomised trials involving warfarin.

No conflict of interest to disclose. This research was supported by the Australian Government Department of Veterans’ Affairs. The Department of Veterans’ Affairs reviewed the abstract.
P146
Interim Australian Results From an International, Multi-center, Single-arm Study Evaluating the Safety and Efficacy of Romiplostim in Adults With Primary Immune Thrombocytopenia (ITP)

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

Methods
Eligibility criteria were broad: ≥18 years-old; prior ITP therapies (≥1 or ≥3, dependent on protocol amendment); low platelet counts (≤10, 20 or 30 x 10⁹/L) or uncontrolled bleeding; no history of myeloproliferative neoplasms, MDS or bone marrow stem cell disorder. Romiplostim was initiated at 1 µg/kg/week, and adjusted to maintain platelets ≥50 x 10⁹/L. Rescue medications were allowed at any time; concurrent ITP therapies were reduced when platelets were > 50 x 10⁹/L. Primary endpoint was incidence of adverse events (AEs) and antibody formation. Secondary endpoint was platelet response, defined as (1) doubling of baseline count and ≥ 50 x 10⁹/L or (2) ≥20 x 10⁹/L increase from baseline.

Results
As of April 2009, 19 (86%) of 22 Australian patients enrolled remained on study and 3 (14%) had withdrawn. Median (Q1, Q3) time from ITP diagnosis was 4.2 (1.0, 15.2) years; 15 (68%) patients were splenectomised, 14 (64%) were receiving concurrent ITP therapies. Median (range) baseline platelet count was 14.0 (2.0–27.0) x 10⁹/L. Median (Q1, Q3) treatment duration and average weekly romiplostim dose were 13.57 (10, 28) weeks (maximum 60 weeks) and 3.48 (1.99, 4.91) µg/kg, respectively. Incidence and type of AEs were consistent with the overall study population. No antibodies to romiplostim, or thrombotic/thromboembolic events were reported. 19 (86%) patients achieved each platelet response definition; median (Q1, Q3) time to response was 1 [(1): (1.0, 3.0); (2): (1.0, 2.0)] week for both.

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

<table>
<thead>
<tr>
<th>AEs – Subject Incidence</th>
<th>N=22</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>All events</td>
<td>18 (82)</td>
<td></td>
</tr>
<tr>
<td>Serious</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>Treatment-related</td>
<td>10 (46)</td>
<td></td>
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<tr>
<td>Serious, treatment-related</td>
<td>0</td>
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This research was supported by Amgen. The company funded the study, performed the data analysis and provided medical writing support.
PI47
Massive Splanchnic Venous Thrombosis as a Presentation of Congenital Hypodysfibrinogenemia

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Background
Congenital dysfibrinogenemia is a rare disorder, associated with thrombosis and bleeding. We describe a 20 year-old man with extensive spontaneous thrombosis, a strong family history and concordant laboratory studies.

Case report
A previously well non-smoker with no prior history of thromboembolism, presented with nausea, vomiting and epigastric pain. CT abdomen demonstrated renal and splenic infarcts, without any underlying anatomical cause. History revealed a paternal uncle who had succumbed to unprovoked PE at age 42.

FBC, film and thrombophilia testing, including antiphospholipid antibodies, JAK-2(V617), PNH, FV Leiden and prothrombin gene mutation, were unremarkable, other than free protein S (59%) – possibly related to acute thrombosis - and fibrinogen (0.9g/L). Clinical symptoms resolved, but he represented after 3 weeks with severe abdominal pain. Triple phase CT confirmed extensive thrombus from the splenic hilum to the porta hepatitis, with almost complete obliteration of the portal venous system. Transoesophageal echocardiogram excluded a cardio-embolic source. Fibrinogen (Clauss method) was again reduced (1.0g/L), PT 15 secs, APTT 29 secs, TCT and reptilase both 20 secs. Suspicious of a congenital dysfibrinogenemia, his family was tested, with father demonstrating reduced fibrinogen – 0.8g/L. Genetic studies and testing of other family members is currently being performed.

The patient commenced indefinite anticoagulation with a therapeutic challenge to titrate the INR in the setting of low fibrinogen. Factor assays were performed with an INR of 2.5 to demonstrate efficacy.

Discussion
The primary function of fibrinogen is clot formation, with an ancillary role in platelet aggregation, fibrinolysis and thrombin binding. Hereditary hypodysfibrinogenemia may give rise to potentially fatal thrombosis or bleeding, although 50% of affected individuals are asymptomatic. Transmission is commonly autosomal dominant, so prompt recognition with family screening is appropriate to reduce disease mortality and morbidity.

Ref range
Fibrinogen 2.0 – 4.0 (g/L) Protein C 70 – 140 (%) Protein S 50 – 134 (%) PT 10-14 (s) APTT 23-35(s)
Propositus 0.9 73 59 15 29
Father 0.8 177 93 12 25
Mother 3.4 112 70 11 27

No conflicts of interest to declare.
P148
Splenectomy and Treatment Failure in Australian Patients with Primary Immune Thrombocytopenia (ITP) Receiving Romiplostim or Standard of Care (SOC) in an International Multi-Center Study

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Aim
Compare splenectomy and treatment failure in Australian ITP patients receiving romiplostim or SOC.

Methods
Non-splenectomised patients with platelet counts < 50 x 10⁹/L were randomized (2:1) to open-label romiplostim or SOC for 52-weeks. SOC was administered per institutional practices or therapeutic guidelines. Co-primary endpoints were splenectomy and treatment failure (platelet count ≤ 20 x 10⁹/L for 4 consecutive weeks at highest recommended dose, or major bleed, or change in treatment due to intolerable side-effect or bleeding symptoms). Patients discontinuing from the study were considered as having achieved either endpoint. Following the treatment period, patients entered another romiplostim study or 6-month off-treatment follow-up.

Results
27 Australian patients were randomized (18 romiplostim, 9 SOC); 18 completed the study (14 [78%] romiplostim, 4 [44%] SOC), 11 entered the follow-up period (5 [28%] romiplostim, 6 [67%] SOC). Median (range) time from ITP diagnosis was 3.33 (0.0–33.22) years; 16/27 (59%) patients had received ≥2 prior ITP therapies. Patient characteristics were similar between treatment groups. Incidences of splenectomy and treatment failure were lower in the romiplostim group, with statistical significance reached for splenectomy. During the treatment period, adverse events occurred in 17/18 (94%) romiplostim- and 9/9 (100%) SOC-treated patients. Serious adverse events occurred in 7/18 (39%) romiplostim- and 5/9 (56%) SOC-treated patients, these were considered treatment-related in 3/18 (17%) romiplostim-treated patients. One patient (SOC) died on-study from hepatic failure, which was considered unrelated to treatment.

Conclusions
Romiplostim significantly lowered the incidence of splenectomy in adult ITP patients compared to SOC. The safety profile of romiplostim was similar to that of SOC, and to that reported previously.

<table>
<thead>
<tr>
<th></th>
<th>SOC (N=9)</th>
<th>Romiplostim (N=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of splenectomy – n (%)</td>
<td>4 (44)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Odds Ratio (95% CI)</td>
<td>0.074 (0.007, 0.817); p=0.0161</td>
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</tr>
<tr>
<td>Incidence of treatment failure – n (%)</td>
<td>2 (22)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Odds Ratio (95% CI)</td>
<td>0.206 (0.016, 2.655), p=0.2024</td>
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This research was supported by Amgen. The company funded the study, performed the data analysis and provided medical writing support.
PI49
Flow Cytometry Identifies Platelet Dysfunction in Patients With Thrombocytopenia and Thrombocytosis.

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Aim
Traditional methods for assessment of platelet function rely upon a minimum number of platelets. In this study, we aimed to detect changes in platelet function/reactivity using flow cytometry in cases of thrombocytopenia and thrombocytosis secondary to known haematological disorders.

Methods
Blood samples were obtained from 10 patients with essential thrombocythaemia (ET) and 16 patients with thrombocytopenia (9 immune thrombocytopenic purpura (ITP)/7 myelodysplasia (MDS)). Normal ranges were established using samples obtained from 9 controls. Platelet activation (PAC-1, CD62p, CD63) and platelet-derived microparticles (CD41+/Ann V+) were assessed using flow cytometry in resting and agonist stimulated samples. Reticulated platelets and platelet dense granules were assessed by thiazole orange and mepacrine staining respectively.

Results
7 thrombocytopenic and 3 ET subjects had decreased PAC-1 binding, 6 thrombocytopenic and 3 ET subjects had decreased CD62p exposure and 3 thrombocytopenic and 2 ET subjects demonstrated decreased CD63 exposure. Absolute platelet-derived microparticle counts were significantly higher in patients with ET (2,828 ± 805 MP/µL) when compared to normal controls (794 ± 148 MP/µL, p=0.031), and were similarly increased in collagen (35,675 ± 6,484 vs 14,074 ± 2,414 MP/µL, p=0.009) and TRAP (7,637 ± 1,966 vs 1,906 ± 332 MP/µL, p=0.017) stimulated samples. When microparticle counts were expressed as a percentage of platelets, there was no significant difference between ET patients (0.351% MP/Plt) and normal controls (0.377% MP/Plt, p=0.780). There was no significant difference between the percentage of mepacrine labelled or reticulated platelets between patients and controls.

Conclusions
Decreased platelet activation could be demonstrated in subjects with thrombocytosis and thrombocytopenia. Elevated microparticle counts in ET patients reflect the increased platelet count and not an increase in platelet reactivity.

No conflict of interest to disclose
P150
Effect of In-Vitro Platelet Transfusion on Platelet Function Testing in subjects taking Aspirin

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Aim
It is routine practice in many institutes to give platelet transfusions to patients with normal platelet counts, who are on anti-Platelet agents prior to surgery or in the trauma setting. There is limited evidence for the effectiveness of this practice. We have performed PFA-100 and Light Transmission Aggregometry (LTA) on subjects taking aspirin before and after spiking samples with both Normal platelets and Platelet concentrate.

Method
3 normal subjects each took 320mg Aspirin prior to testing. PFA-100 using Collagen-Epinephrine and Collagen-ADP cartridges and LTA using ADP, Collagen, Epinephrine and Arachidonic Acid as agonists were performed at baseline. Samples were then mixed 50:50 with Platelet Rich Plasma (PRP) from a normal donor and also resuspended Platelet concentrates from a Pooled Bag obtained from the Blood bank, to raise Platelet counts by 50%. PFA-100 and LTA were re-performed post mixing.

Results
Initially all subjects had a prolonged PFA-100 with no aperture closure using the Collagen-Epinephrine cartridge. This abnormality improved significantly after addition of Normal Platelets but showed no change after addition of Platelet concentrate. LTA in all three subjects at baseline showed only primary aggregation with ADP, reduced or absent aggregation with Epinephrine, Arachidonic Acid (AA) and Collagen. There was complete or almost complete correction of defects with addition of normal platelets, however on addition of Platelet concentrate there was improvement only with AA, but little or no change with other agonists.

Conclusion
Addition of Blood Bank Platelets shows little effect on Platelet function in-vitro. Further in-vivo studies are warranted to better guide practice.

No conflict of interest to disclose
P151
Update on Procoagulant Phospholipid as a Marker for Hypercoagulability and Late Platelet Activation

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Procoagulant phospholipid (PPL) is an essential cofactor for clotting which is not considered in conventional testing panels although significantly increased levels have been reported in conditions such as acute coronary syndromes, stroke, malignancy and DVT.

Aim
To investigate factors which may have adversely affected some clinical evaluations of PPL and to suggest improvements to blood collection practices.

Method
Factor Xa activated clotting tests (XACT) for PPL were carried out on plasmas obtained after various storage times from normal citrated bloods containing various additives. Double centrifugation was carried out using 3 protocols and plasmas were tested fresh as well as after freezing.

Results
Fresh plasmas from 40 healthy donors showed that the mean PPL(SD, ng/ml) was highest for citrate, 602(98) and slightly lower for EDTA and CTAD. After freeze-thawing the apparent PPL levels became 1213(75) for citrate but did not change significantly for EDTA and CTAD plasmas. Normal bloods collected into regular citrate tubes displayed increasing expression of PPL with time. This occurred also with citrated platelet rich plasma (PRP) but could be prevented by Abciximab, EDTA and reduced pH. PPL was increased by collagen and sample agitation. However the highest levels of PPL (approximately 100x those in fresh plasmas) occurred after freeze-thawing PRP samples. PPL from residual platelets within frozen plasmas can sometimes overcome the low baseline levels of PPL apparent before freezing.

Conclusion
The XACT is useful test for PPL whether on activated platelets or on microparticles. Some evaluations of XACT have been compromised by the use of inappropriate samples. Tests for assessing PPL in vivo should be carried out either on fresh plasmas (where resting platelets display minimal PPL) or on frozen samples which should be platelet free or doubly centrifuged CTAD or EDTA plasmas. Reduced pH and various platelet stabilizers are also helpful.

This project was partly supported by Haematex which developed the XACT test but this did not influence result interpretation.
P152
When is a Phospholipid Dependent Coagulation Inhibitor not a Lupus Anticoagulant? Considerations on a False LA

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1 Haematex Research, Hornsby, NSW. 2 Haematology Dept, Liverpool Hospital, Liverpool, NSW. 3 Phlebology Research Lab, Bondi Junction, Sydney, NSW, Australia.

The majority of acquired anticoagulants detected by coagulation labs are now classified as lupus anticoagulants (LA) and often this is used to support a diagnosis of Anti-Phospholipid Syndrome (APS). However several subtypes of LA are not diagnostic for APS.

Aim
To assess the value of a new reagent for detecting some false LA. Also to tighten up the definition of LA for the diagnosis of APS.

Method
LA-sensitive APTT and dRVT clotting tests were used. Plasma from a patient (F76) with B-cell lymphoma (?SMZL) was found to display strong LA-like activity. The patient did not show clinical features of APS despite also being strongly positive for anticardiolipin antibody (IgM). The inhibitor and IgM paraprotein disappeared after splenectomy. This patient’s pheresis plasma has been used in Australasian QAP exercises assessing methods for LA detection.

Results
Serendipitously, this LA-like coagulation inhibitor was included in a study investigating the potential thrombogenic effects of sclerosants on known anticoagulants. We found that this strong LA-like activity could be almost fully overcome by adding the sclerosant sodium tetradecyl sulphate. A related compound, sodium lauryl ether sulphate was used to develop a reagent (FLAN=False LA Neutralizer) which could be used routinely to assess the incidence of similar anticoagulants. A total of 55 random LA from several coagulation labs were tested for false LA using FLAN. None of these LA reacted similarly to the index case wherein the attenuated LA was able to be neutralized by FLAN.

Conclusion
We have identified agents which can specifically neutralize the “lupus anticoagulant” occurring in a single patient with SMZL. The incidence of such “false” LA among cases currently classed as LA positive appears to be very low. However, this finding supports the contention that not all phospholipid dependent clotting inhibitors should be classified as LA.

No conflict of interest to disclose
P153
Prospective Study of Venous Thromboembolism Incidence in Community Based Heart Failure Patients

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Background
Heart failure is a known risk factor for venous thromboembolism (VTE) with many studies showing the increased risk of VTE in hospitalised heart failure patients. However, an increasing number of heart failure patients are being managed within the community and no studies have been done to assess whether these patients remain at risk of developing VTE.

Aims
The proposed study aims to compare the incidence of VTE in community-based heart failure patients to similar aged community-based control subjects without heart failure.

Method
The study will recruit approximately 95 heart failure patients (NYHA III and IV) and 95 controls. Study subjects will be screened for asymptomatic deep vein thrombosis using bilateral lower limb compression ultrasound. Other VTE risk factors including immobility will be recorded and accounted for using multiple regression analysis.

Results
Screening so far has found detected 4 asymptomatic deep vein clots in 37 heart failure patients and no clots in 15 similar aged control subjects. Final results will be presented at the conference.

Conclusion
Although medically ill patients have an established VTE risk in a hospital setting, this is the first study of VTE incidence in community based medically ill patients. If the incidence rate shows a clinically relevant difference between heart failure VTE incidence and control VTE incidence, there will be evidence to suggest at least heart failure patients should be considered for thromboprophylaxis even outside a hospital setting.

This project has been partly funded by a VTE scholarship awarded by Covidien Pty Ltd.
Apixaban versus Enoxaparin After Knee or Hip Surgery: Efficacy and Safety in Key Clinical Subgroups

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Aim
Apixaban was more effective than enoxaparin 40 mg, without increased bleeding, for thromboprophylaxis after knee or hip replacement in ADVANCE-2 and -3. The aim was to assess the consistency of the efficacy and safety of apixaban compared to enoxaparin in clinically relevant subgroups.

Method
Two randomized, double-blind, phase 3 trials compared apixaban 2.5 mg twice daily with enoxaparin 40 mg once daily. Study drugs were continued for 10-14 days after knee, and 32-38 days after hip replacement. The statistical analysis plan prespecified evaluation of a limited number of key clinical subgroups, including age, body mass index (BMI) and renal function. Relative risks and absolute risk differences with 95% confidence intervals were calculated for the outcomes of any venous thromboembolism (VTE)/all-cause death, major VTE, and bleeding for apixaban versus enoxaparin.

Result
Heterogeneity was observed for bleeding by degree of renal impairment (p=0.03 for interaction). Among other subgroups evaluated, no significant treatment by subgroup interactions were identified.

Conclusion
The efficacy and safety of apixaban versus enoxaparin was generally consistent across subgroups of age and BMI. More precise estimates of bleeding risk are needed for patients with moderate-severe renal impairment (CrCL less than or equal to 50).

This research was supported by Bristol-Myers Squibb and Pfizer. The study was designed and supervised by the steering committee members. Data were collected and analysed by the study sponsors, Bristol-Myers Squibb and Pfizer. The statistical analysis plan was approved by the steering committee before the database was locked and unblinded.
P155
ACL antibodies and TFPI extend CAT Lag Phase

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Aim
We examined the rate and characteristics of thrombin generation in patients with first ever ischaemic stroke and examined whether there were differences in parameters between an age and sex matched control group and if the changes persisted 6 months after stroke.

Methods
164 consecutive patients with first acute ischaemic stroke along with age and sex matched controls were studied. Blood was collected within 7 days of stroke prior to heparin therapy and at 6 months follow-up. Thrombin generation was measured using the Calibrated Automated Thrombinoscope (CAT, Diagnostica Stago) in platelet poor plasma using 5pM tissue factor. Parameters studied were lag time, peak height, endogenous thrombin potential (ETP) and start tail that describes the cessation of thrombin generation. Samples were also tested for IgG and IgM anticardiolipin (Medical Innovations, Australia) and for TFPI function (Asserachrom Free TFPI, Diagnostica Stago) and activity (chromogenic in house method).

Results
Stroke patients had significantly longer lag time mean±SD 5.5±3.1min (control 3.4±1.5 min, p=<0.001), ETP 1634±571 nmole (control 1559±450 nmole, p=0.04) and start tail 31.8±9.6 min (control 25.4±6.2min, p<0.001). At follow-up at 6 months, all thrombin generation parameters returned to baseline and were not different from control.
There is a significant correlation with IgG ACL in both cases and controls. There is a significant correlation with TFPIa and TFPIf in cases only.

Conclusion:
CAT lag phase is prolonged and correlates with increased ACL antibodies and TFPI levels. However thrombin generation is increased overall in ischaemic stroke patients with a pattern of slower initiation but prolonged production which disappears at six months after the acute event.

No conflict of interest to disclose
P156
An Audit of Inherited Thrombophilia Testing at the Royal Hobart Hospital

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Aim
This audit was created to examine the indications and circumstances of testing for all inherited thrombophilia tests performed at the Royal Hobart Hospital (RHH) over a one-year period.

Methods
A retrospective medical record of all inherited thrombophilia requests at the RHH from March 2010 to March 2011 was performed. Data was collected on test indications, test circumstances (time of testing, anticoagulation and pregnancy status), results and management outcomes. The appropriateness of indication for testing was assessed according to existing local guidelines.

Results
200 testing episodes were identified representing the results of 194 individual patients. Approximately 80% of total requests were for the indications of either VTE, arterial disease (predominantly stroke and TIA) or pregnancy loss. 53 out of the total 200 episodes (27%), returned an abnormal inherited thrombophilia result. 39 episodes (20%) returned either a low antithrombin, protein C or protein S result. When likely acquired deficiency and confounders were considered, 90% of these phenotypic tests were deemed to be probable false positives for an inherited abnormality.

The majority of indications did not comply with local guidelines. In only 15% of patients was it clear that an appropriate thrombophilia screen had been performed. A large number of screens were missing one or more tests (63%). In terms of outcomes, there was no demonstrable change in patient management as a result of inherited thrombophilia testing over this timeframe.

Conclusion
Much of the thrombophilia testing at the RHH is inappropriate according to local standards. No inherited thrombophilia tests in the last year demonstrably changed management. This is likely to be partly due to low detection rates in poorly selected patients. There are however, emerging doubts within the literature of the clinical utility of inherited thrombophilia testing in the management of venous thrombosis, pregnancy morbidity and arterial disease.

No conflict of interest to disclose
P157
A Solution of 4% Albumin Can Replace FVIII Deficient Plasma as the Diluent in the Nijmegen Bethesda Assay for FVIII Inhibitors

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Aim
We compared FVIII inhibitor titres obtained using Nijmegen Bethesda Assay (NBA) to those obtained using a modified assay where 4% albumin replaced FVIII deficient plasma as both sample and control tube diluent. The aim of the study was to implement a substantially more cost efficient Bethesda assay without sacrificing test specificity or accuracy.

Method
A total of 59 FVIII inhibitor-containing citrated plasma samples from 35 patients were assayed, comprising 45 samples from 23 patients with congenital Haemophilia A (CHA) (15 severe, 1 moderate, 7 mild) and 14 samples from 12 patients with acquired Haemophilia A. A further 49 samples from 35 inhibitor-free CHA patients undergoing routine surveillance for inhibitors were assayed prospectively. Eighteen samples (13 positive, 5 negative) issued by the External Quality Control for Assays and Tests proficiency program and the Royal College of Pathologists of Australasia proficiency program were tested prospectively to compare results of the albumin-modified NBA assay with consensus results. Imidazole buffered pooled normal plasma was prepared in-house. The FVIII deficient plasma for the NBA and one-stage assays was congenital Haemophiliac plasma. Automated factor VIII assays were performed on the STA-R analyser.

Result
Compared to results from our existing Nijmegen Bethesda Assay, overall median titre increased from 6.55 BU/mL to 7.50 BU/mL when 4% albumin replaced FVIII deficient plasma in the test setup. No sample was found where a difference in measured titre between methods would have altered clinical management. Agreement was very close in samples with titres less than 2 BU/mL and in negative samples. The albumin-modified assay yielded results closer to the NBA sub-group mean in EQA samples.

Conclusion
The use of 4% albumin as the diluent in place of FVIII deficient plasma is a simpler and more cost-efficient way of performing Bethesda assays for FVIII inhibitors while maintaining the improved specificity of the Nijmegen Bethesda Assay.

No conflict of interest to disclose
P158
An Unusual Case of Bleeding with Inhibition of the Intrinsic Coagulation Pathway

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Background
Acquired coagulation factor inhibitors are usually antibodies that inhibit the function of the missing coagulation factors that cause serious bleeding diathesis. Although reduced levels of each coagulation factor VIII, XI and XI are believed to be as result of development of autoantibodies against these coagulation factors, it is very rare to demonstrate a pan-inhibition effect to the entire intrinsic coagulation pathway including factor XII. It is worth noting that there is no clinical relevance between FXII-deficiency and bleeding. However, the incidence for some acquired single coagulation factor inhibitor is probably 1 per million per year with high mortality rate ranging between 12.5% and 22%, usually because of fatal haemorrhage.

Case report
We report on a 67 year old Caucasian female who presented to the Launceston General Hospital with a significant history of bleeding. Apart from osteoarthritis she has no significant health issues. Her APTT was moderately elevated (50 s) with normal INR and Fibrongen. Mixing studies with normal plasma suggested inhibition to intrinsic coagulation factor(s). Repeated factors studies confirmed low factors VIII; 26 IU/dL, FIX; 17 IU/dL, FXI; 2 IU/dL and FXII; 17 IU/dL. There was no evidence for lupus anticoagulant or anticardiolipin antibodies or immune disorder. Protein electrophoreses showed constant small para-protein levels of IgA kappa ranging between 3 and 4 g/L. Further radiological imaging with MRI and sestamibi bone marrow scans showed no evidence for myeloma. The patient had several episodes of serious spontaneous bleeding that responded well to FVIIa. Also numerous surgical procedures were performed safely with FVIIa cover. Since diagnosis she is doing well with continuous monitoring. Although recombinant activated factor VII molecule has been evaluated clinically for some years with promising results in case of acquired coagulation factor inhibitors, it is worthwhile to demonstrate similar efficacy in patients with non-specific intrinsic factors inhibitor in association with bleeding, and also in whom a surgical procedure was indicated.

No conflict of interest to disclose
Rotational Thromboelastometry (ROTEM®): A Rapid Way to Assess Snake Bite Coagulopathy and its Treatment

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Background
Coagulopathy, a potentially life threatening sequelae of snake bite envenomation, has traditionally been assessed by clinical features and the standard laboratory coagulation measurements (INR, APTT, fibrinogen). Rotational Thromboelastometry (ROTEM®) is a newer coagulation assay and measures viscoelastic properties during whole blood clot formation and lysis. ROTEM® assesses EXTEM (tissue factor triggered extrinsic pathway), INTEM (ellagic acid activated intrinsic pathway) and FIBTEM (cytochalasin D evaluating the contribution of fibrinogen to clot formation). We describe two envenomated patients and their ROTEM® results.

Cases
Two male patients, aged 70 and 53, presented to Fremantle Hospital Emergency Department post snake bite and envenomation was confirmed by clinical assessment plus positive wound swab and urine assay. Neither patient was taking anticoagulants.

ROTEM® on the first patient showed markedly prolonged INTEM clotting time (CT) of 3607s and EXTEM CT of 3339s at baseline which, after antivenom neutralisation, had improved by 1.5 hours and normalised by 3.9 hours post antivenom. INR and APTT took more than 23 hours to normalise, and fibrinogen was still low at 38.5 hours post antivenom.

ROTEM® on the second patient also showed markedly prolonged INTEM CT of 3605s and EXTEM CT of 3606s at baseline which, after antivenom neutralisation, had improved at 8.5 hours and nearly normalised by 14.5 hours post antivenom. INR and APTT had normalised by 15 hours post antivenom, but fibrinogen was still low at 26 hours post antivenom. Both patients made a full recovery without haemorrhagic complications and were discharged home.

Discussion
In these two cases, ROTEM® reliably detected coagulopathy and response to antivenom more rapidly than conventional coagulation parameters. Other advantages of ROTEM® include faster processing speed, using whole blood rather than a centrifuged sample, and good sensitivity as suggested by normal ROTEM® in four non-envenomated control patients.

No conflict of interest to declare
**PI60**

**Factor V Leiden and Slovak Population**

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**Aim**
The aim of the study was to evaluate the clinical manifestation of inherited resistance to activated protein C (APC-R) caused by factor (F) V Leiden mutation in the Slovak population.

**Methods**
The study involved 1078 subjects (486 men and 592 women, mean age: 36.81 years). There were 559 symptomatic patients (mean age:41.06 years; 241 men and 318 women), 371 asymptomatic carriers (mean age: 29.09 years; 160 men and 211 women) and 148 healthy controls (mean age:40.11 years; 85 men and 63 women). ProC Global and PCR were used to detect the mutation of factor V Leiden.

**Results**
The total number of FV Leiden mutation carriers was 930, symptomatic patients made up 60.1% (n = 559). Deep vein thrombosis (DVT) of low extremities was the most common clinical consequence (in 75% cases; n = 350); in 40 patients (11%) DVT was complicated by pulmonary embolism. Superficial thrombophlebitis was detected in 11% of patients (n = 53). In 65% of patients thrombophilia was manifested until 40 years of age. Half of the patients (n = 232; 49.9%) had one episode of venous thromboembolism (VTE), in a quarter (n = 108; 23.2%) VTE was recurrent once and in a last quarter of patients (n = 125; 26.9%) it was recurrent two or more times. FV Leiden was the only thrombophilia in majority of patients with VTE (n = 332; 71.4%). 47 patients (4.4% of total group; 8.4% of symptomatic patients) suffered from arterial thrombosis. FV Leiden mutation was detected in 47 women (4.4% of total group; 8.4% of symptomatic patients) with recurrent spontaneous fetal loss (n = 40; 85.1%) or another complications during pregnancy (n = 7; 14.9%).

**Conclusions**
Resistance to activated protein C caused by FV Leiden mutation is the most common hereditary thrombophilia. Clinically it results mainly in spontaneous and recurrent venous thrombosis in young age.  
*No conflict of interest to disclose.*

Acknowledgement: This work was supported by grant Vega 1/0018/10, Vega 1/0029/11 and by the “Center of Excellence for Perinatology Research II” project (CEPV II), which is co-financed from EC sources.
P161
Risk of Arterial Cardiovascular Events in Chinese Patients after Acute Pulmonary embolism

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Aim
Recent epidemiological studies showed an association between venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), and arterial cardiovascular events. The risk of arterial cardiovascular events is higher in unprovoked VTE than provoked VTE. However, these results are inconsistent among studies. It is still unknown whether this association is present in Chinese patients or not. This study was conducted to investigate the relationship between arterial cardiovascular events and acute pulmonary embolism in Chinese patients.

Method
This is a retrospective cohort study. Ninety-five patients with acute PE were recruited. Among them, 35 (36.8%) patients had unprovoked PE and 60 (63.2%) patients had provoked PE. They were compared with a control group of 279 patients in whom a clinically suspected PE was ruled out by CT pulmonary angiography or ventilation-perfusion scintigraphy. The primary endpoint was occurrence of arterial cardiovascular events.

Results
Median follow-up time was 2.7 years. The baseline arterial cardiovascular risk profiles (age, hypertension, diabetes mellitus, history of arterial cardiovascular diseases and tobacco smoking) were comparable among the PE and control groups. Fifty-nine arterial cardiovascular events were recorded (incidence rate 5.4 per 100 patient-years). Adjusted hazard ratio for arterial cardiovascular events was higher in patients with unprovoked PE than control patients (2.7; 95% CI 1.4-5.5; p=0.0005). However, patients with provoked PE and control patients did not differ in arterial cardiovascular risk (1.7; 95% CI 0.83-3.37; p=0.15).

Conclusion
This study demonstrated unprovoked PE is associated with a higher arterial cardiovascular risk in Chinese patients.

No conflict of interest to disclose
P163
Importance of Recovery Tests in Hemophilia A Patients

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Aim
Because there are refractory bleedings in some hemophilia A patients despite sufficient dose of factor VIII (FVIII) concentrate, we decided to check individual recovery of plasma FVIII level after medication.

Method
The baseline plasma FVIII levels were checked after cessation of using FVIII concentrate over 3 days. And respective FVIII levels were examined at 15, 30, 60 minutes after administration of 25 IU/kg FVIII concentrates in hemophilia A patients.

Result
Six hemophilia A patients (all males) agreed to take recovery tests. Their mean age was 30 years (ranging from 9 to 67). And their mean baseline FVIII level was 1.5% (ranging from 0.2 to 5.2%). The mean plasma FVIII level after injection of 25 IU/kg FVIII concentrate was respectively 42.9% (29.8 ~ 60.4%), 41.8% (28.4 ~ 60.9%), and 39.5% (28.9 ~ 54.8%) at 15, 30, 60 minutes. Among 6 patients, only 2 showed expected an increase of FVIII level. The other four patients revealed lower FVIII level than expected.

Conclusion
There are wide variations in the plasma FVIII levels after infusion of the same dose (25 IU/kg) of FVIII concentrates to hemophilia A patients. However, most hemophilia A patients are managed with a standardized dose of FVIII concentrate for bleeding control or prophylaxis of hemorrhage during surgery. Considering these results, individualized dose determination is very important in hemophilia A patients. We propose routine recovery tests in all hemophilia A patients before use of FVIII concentrate.

No conflict of interest to disclose
Thrombophilia Testing is Over-utilised - An Audit of 500 Consecutive Screens

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Aims
To conduct an audit of thrombophilia testing (TPT) in a laboratory servicing two medium sized hospitals, specialists and general practitioners.

Methods
We audited 500 consecutive screens for TPT noting the history of venous thromboembolism (VTE), the defects found and in a subset of patients, the timing of testing.

Results
There were 228 females and 272 males (median age 49 years, average number of tests 4). Overall, 17% of patients had thrombophilia defects. Deficiencies of PC, PS, AT3 were infrequent, being 1, 1.5 and 1.3% respectively (excluding those on warfarin or heparin). Lupus anticoagulant positivity was 1.8%. FV Leiden was found in 12.2% and prothrombin G20210A in 3.3%. A history of VTE was specified for 59% of patients. Specialist vascular physicians requested 43% of the screens, were more likely to specify VTE (82%) and less likely to test anticoagulated patients but with a defect rate of 16% did not select patients for TPT any better than average (17%). In a group of 72 hospital in-patients, 18% did not appear to have any indication for TPT, 32% VTE was not proven before testing and 50% were tested at the acute stage of VTE. Australian health insurance rules restrict rebates to those with a history of VTE. 26% of patients lacked this history and could not be billed.

Conclusions
The TP defect rate in our patient population was low, indicating that patient selection by our clinicians was poor. We conclude that TPT is vastly over-utilised in our patient population, especially in view of recent guidelines that testing is not indicated in unselected patients and that the decision to test should be based on whether results are likely to influence treatment decisions. We intend to disseminate these guidelines in our institution as part of an education programme to reduce the rate of inappropriate TPT and to conduct a further audit later.

No conflict of interest to disclose.
Evaluation of the HemosIL HIT-Ab Latex Enhanced Immunoassay in the Diagnosis of Heparin-induced Thrombocytopenia (HIT) as Compared to Established Methods

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Aim
The clinico-pathological syndrome of HIT is a serious complication and poses an ongoing diagnostic challenge for both clinicians and laboratories. A new automated latex immunoassay (HemosIL HIT-Ab™) has been evaluated against established functional (Serotonin Release Assay) and antigenic assays (ELISA) in conjunction with the 4T pretest probability scores. The HemosIL HIT-Ab™ offers a quick turnaround time on the routine automated platform.

Method
31 samples were assayed using the HemosIL HIT-Ab™(PF4-H) on the ACL TOP coagulation instrument as well as the Diagnostica Stago Asserachrom HPIA-IgG and IgG,A,M ELISA. These were compared to the Serotonin Release Assay (SRA) results. The 4T pretest probability scores had been determined by clinicians independently prior to testing.

Result

<table>
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<th></th>
<th>Low n (%)</th>
<th>Intermediate n (%)</th>
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<td>5 (45)</td>
<td>4 (27)</td>
<td>1 (20)</td>
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<td>6 (55)</td>
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<tr>
<td>Monospecific IgG ELISA</td>
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<td>8 (53)</td>
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<tr>
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<td>6 (55)</td>
<td>7 (47)</td>
<td>5 (100)</td>
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<td>Pos</td>
<td></td>
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<tr>
<td>Polyspecific IgG,A,M ELISA</td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Neg</td>
<td>11 (100)</td>
<td>15 (100)</td>
<td>5 (100)</td>
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Conclusion
Based on the small number of samples tested and ignoring the low 4T score group, who should not proceed to laboratory testing, the HemosIL HITAb™ immunoassay performed better than the polyspecific ELISA but not as well as the monospecific IgG ELISA when compared to the gold standard SRA method. Further evaluation is required to fully assess the utility of the latex immunoassay.

This research was supported by Beckman Coulter. The company had no role in analysing the data or preparing the abstract.
**P166**

**Improved Rates of Retrieval of Temporary IVC Filters with the G2X Filter and Multidisciplinary Follow-up: The Auckland Hospital Experience**

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**Aims**

Inferior vena cava (IVC) filters are frequently inserted to prevent pulmonary embolus on a temporary basis. While studies focused on specific devices have reported high rates of retrieval, published audits have noted lower rates of success due to a combination of filter thrombus, filter tilt (preventing removal) and patients being lost to follow up without attempted retrieval. We report audit results of two time periods at our institution comparing retrieval success.

**Methods**

Following ethics approval, the notes of all patients who underwent IVC filter insertion at Auckland City Hospital between 1/1/2007 and 31/12/2010 were reviewed. An initial audit of all filters inserted during 2007 was undertaken during 2008. On this basis of the results, the type of filter inserted was changed from the Celect (Cook, IN USA) to the G2X (Bard, AZ USA) and greater multidisciplinary supervision of filter removal was undertaken for the second time period 2009-10. Statistical analysis was undertaken with GraphPad software using Fisher’s exact test.

**Results**

IVC filters were inserted in 139 patients; 51% were female with an average age of 62 years (range 14-87). During 2007-08, 80% of insertions were consistent with ACCP recommendations and during 2009-10 90.2%. In the first two years, 40/67 (59.7%) filters were inserted for temporary indications. Of these, 5 patients died, and of the remainder the filter was successfully retrieved in 15 patients (42.9%). In 2009-10 78.9% (56/71 filters) were placed on a temporary basis; 8 patients died and the filter was successfully retrieved from 32 patients (66.7%). Overall there was a significant improvement in filter retrieval comparing 2009-10 to 2007-08(p=0.043). This improvement was partly due to a reduction in patients lost to follow-up, and partly due to significant improvement in rates of retrieval of the G2X filter. Of these, 91.6% of retrieval attempts were successful, compared to 55.8% of attempts with the Celect (p=0.0024). No G2X filters were left in situ because of filter tilt compared to 16/56 (28.6%) of temporary Celect filters.

**Conclusions**

Retrieval of temporary IVC filters has significantly improved following a change in filter type and greater multidisciplinary follow up. Since 2011, a prospective database of all IVC filter patients has been established to monitor retrieval and ensure all filters are removed where possible.

*No conflict of interest to disclose*
The Flavonols Quercetin and 3’, 4’-Dihydroxyflavonol Selectively Inhibit Dense Granule Release in vitro, and Delay Thrombus Formation in a Model of Arterial Thrombosis in vivo

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Aim
We investigated the effects of the flavonols quercetin (Que) and 3’, 4’-dihydroxyflavonol (DiOHF) on human platelet aggregation in vitro, and arterial blood flow in an in vivo model of acute arterial thrombosis.

Methods
Platelet aggregation stimulated by collagen (5 µg/ml), ADP (10 µm) or arachidonic acid (AA, 0.5 mM) was determined using platelet rich plasma. Dense granule release was quantitatively assessed by quinacrine uptake and thrombin (0.5 U/mL) induced release using flow cytometry and laser confocal microscopy. Alpha granule release (demonstrated by CD62P expression) and GPIIb/IIIa receptor activation (demonstrated by PAC-1 binding) induced by ADP, AA or thrombin receptor activating peptide (TRAP, 20 µm) or adrenaline/collagen (250 µM/25 µg/mL) were assessed using flow cytometry. C57BL6 mice were treated IV with Que (6 mg/kg, n=5), DiOHF (6 mg/kg, n=5) or vehicle (n = 3) 30 mins before 20% FeCl induced carotid injury and blood flow measured by Doppler flow.

Results
Que and DiOHF inhibited in vitro platelet aggregation in a concentration dependant manner with maximum inhibition achieved at 1 mM. Dense granule release was inhibited by both Que and DiOHF (1 mM) (Que 94.7 ± 3.9 %, DiOHF 95.0 ± 2.1% inhibition). Que, and DiOHF (1 mM) inhibited PAC-1 binding induced by ADP (Que 30.0 ± 9.5%, DiOHF 19.8 ± 9.7%, P < 0.01), TRAP (Que 32.0 ± 10.5%, DiOHF 17.8 ± 11.4%, P < 0.01), and adrenaline/collagen (Que 63 ± 6.8%, DiOHF 33.0 ± 11.8%, P < 0.01). Neither flavonol altered the expression of CD62P. Flavonol-treated mice maintained greater blood flow when compared to the vehicle-treated mice

Conclusions
These data demonstrate that flavonols have potent anti-aggregatory effects on platelets in vitro, involving selective inhibition of dense granule, but not alpha granule exocytosis. We show for the first time that flavonols improve arterial blood flow in acute thrombosis.

No conflict of interest to disclose
P168
Evaluation of the Vital APTT system for Lupus testing

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Aim
The 2009 ISTH guidelines for Lupus Anticoagulant testing have recommended dRVVT and aPTT with silica activator and low PL content. While the dRVVT has been the choice for Lupus testing, the aPTT method is new to most laboratories. The Vital aPTT system for Lupus testing appears to be suitable under the new recommendations. The purpose of this study is to evaluate this new kit and to compare it with our current methods, dRVVT and KCT.

Method
Cut-off for the Vital aPTT system was established with plasma from 40 normal individuals.
Routine samples for Lupus testing were collected by standard methods, double spun and aliquoted. One aliquot was filtered using 0.2um filters for KCT. All plasma aliquots were stored at -80°C until testing.
The following tests were performed within 2 weeks of collection with the STA-R.
1. dRVVT using STALCOT Screen and Confirm.
2. KCT using 0.5% Kaolin (Sigma) and 0.025M CaCl₂ with the filtered plasma aliquot.
3. Vital aPTT system with Lupus Sensitive and Lupus Resistant aPTT reagents and 0.025M CaCl₂.

105 samples with positive results in one or more of the above tests were used for analysis.
70 random Lupus aliquots were tested with 2 different Vital aPTT batches for batch to batch variation.

Results
77.14% dRVVT positive.
57.14% KCT positive.
61.90% aPTT positive.
Between the 2 batches of aPTT reagents, the correlation coefficient for LS, LR and LS/LR ratio were 0.988, 0.967 and 0.977 (p<0.01) respectively.

Conclusion
The dRVVT remains to be the most sensitive for LA. The Vital aPTT system was shown to pick up more LA than KCT. Batch variation is at an acceptable level for the Vital system.

No conflict of interest to disclose
Heparin-Induced Thrombocytopenia and Thrombosis - A New Emerging Therapeutic Strategy

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Heparin-induced thrombocytopenia and thrombosis (HITT) is a life-threatening disorder that affects 1% to 5% of patients receiving heparin therapy. A low platelet count is usually recorded (<150,000 per cubic millimeter). In HITT, an IgG antibody is produced against a complex formed between platelet factor 4 (PF4) and heparin. The immune complex activates platelets via the FcgammaRIIa receptor. Activation induces PF4 release which leads to the formation of more heparin-PF4-antibody complexes. This further promotes platelet activation, leading to complications in HITT patients. A neutralizing anti-FcgammaRIIa murine monoclonal antibody (termed IV.3) has been shown to abolish HIT IgG binding to the Fc receptor and prevents platelet activation. Thus, the aim of the project is to develop a humanized single-chain variable fragment (scFv) based on the IV.3 monoclonal antibody. We propose that this scFv would bind and neutralize the FcgammaRIIa receptor on platelets, and serve as a treatment for HITT.

The heavy and light chain regions of IV.3 were amplified and coupled with a linker (Gly4-Ser)6. Restriction sites, secretion signal and tags were introduced for cloning, detection and purification purposes. The fragment was cloned into the pET-11a plasmid and transformed into Escherichia coli BL-21 strain. The protein (34kDa) was expressed, purified and confirmed by immunostaining. The created scFv exhibits functional activity by binding to the FcgammaRIIa receptor on human platelets as determined by flow cytometric analysis and confocal microscopy. In addition, the scFv protein prevents platelet aggregation during heparin-induced platelet aggregation assays conducted in vitro.

The created scFv successfully demonstrated binding activity against the FcgammaRIIa receptor on human platelets. Most importantly, it is able to prevent platelet activation and aggregation. This implies that this protein may be applicable in ameliorating the unwanted side effects observed during heparin therapy and may serve as a potential therapeutic drug for HITT patients.

No conflict of interest to disclose.
Perioperative sCD40L and PFA-100® Profiles During Major Noncardiac Surgery

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Introduction
Adverse cardiovascular events can occur during noncardiac surgery. Plasma soluble CD40 ligand (sCD40L) concentration is a marker of platelet activation, and has been found to predict adverse cardiovascular events. sCD40L is elevated after cardiac surgery, but whether this elevation occurs after noncardiac surgery is unknown. The variation of PFA-100® measured platelet function during noncardiac surgery is also unknown. Profiling sCD40L and PFA-100® during the perioperative period may allow us to identify high risk periods in the general surgical patient.

Methods
With IRB approval and informed consent, 70 patients scheduled to undergo elective major orthopedic surgery were recruited. Patients receiving NSAID or other drugs that may affect platelet function were excluded. Blood was collected for complete blood count, plasma sCD40L, PFA-100®, fibrinogen and vWF:Ag at different time points in the perioperative period. Measurements at different time points were compared using paired t-test or repeated-measure ANOVA as appropriate, and correlations using Pearson’s correlation. \(P<0.05\) was considered significant.

Results
sCD40L was significantly elevated immediately after surgery (0.093±0.066 ng/ml postoperative vs 0.069±0.040 ng/ml preoperative, \(P<0.01\)). Both ADP and EPI closure times on PFA-100® were prolonged intraoperatively and returned to baseline values postoperatively. VWF:Ag was closely correlated with PFA-100® (\(r=-0.50\) vs ADPCT, \(r=-0.43\) vs EPICT; both \(P<0.001\)), but they were not correlated with sCD40L. sCD40L was correlated with patient’s BMI (\(r=0.31, P<0.01\)).

Conclusion
In patients undergoing noncardiac surgery, there is moderate elevation in sCD40L immediately after surgery. These changes are not correlated with changes in PFA-100® which suggests these measurements are independent and may supplement each other. Further studies are required to assess the implication of these changes on clinical outcome.

No conflicts of interest to disclose
Low Concentration Detergent Sclerosants Induce Platelet Activation but Inhibit Aggregation in vitro

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Aim
Sclerotherapy to treat varicose veins is associated with thromboembolic and ischemic neurological adverse events, but the effects on platelet activation and aggregation are unknown. The aim of this study was to investigate the in vitro effects of detergent sclerosants Sodium Tetradecyl Sulphate (STS) and Polidocanol (POL) on platelet activation and aggregation.

Methods
Platelet counts were measured using an automated cell counter for whole blood (WB) and by flow cytometry using CD41a for WB and platelet rich plasma (PRP). Platelet-derived microparticle (PMP) counts in PRP were measured by flow cytometry using annexin V and lactadherin. Platelet activation was examined by ELISA for soluble (s) factors (sP-selectin, von Willebrand factor, sCD40L and serotonin) and by flow cytometry for membrane-bound markers (CD62p, CD63) and intra-cellular calcium expression. Platelet aggregation was assessed by PFA-100®, light transmission and impedance (Multiplate®) aggregometry, and by flow cytometry for glycoprotein (GP) IIb/IIIa heterodimer and activated GPIIb/IIIa (PAC-1 binding).

Results
In WB, platelet lysis occurred at ≥0.3% STS and ≥0.6% POL. In PRP, STS lysed platelets at ≥0.15% and POL at ≥0.15%. Both agents induced PMP release at ≥0.075% but destroyed PMPs at higher concentrations. Low (0-0.1%) concentration sclerosants activated platelets as demonstrated by a rise in all soluble and membrane-bound markers and intra-cellular calcium. At 0.1%, STS induced a reversible platelet aggregation. Aggregation was otherwise progressively suppressed by both agents due to inhibition of GPIIb/IIIa activation. The sclerosants did not destroy the GPIIb, GPIIIa subunits and did not dissociate the GPIIb/IIIa heterodimer.

Conclusion
Low concentration sclerosants activated platelets and released microparticles but inhibited platelet aggregation. At high concentrations, both agents lysed platelets and PMP.
No conflict of interest to disclose
The TTP Registry: Initial Data from a New National Registry

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Background and Methods
The Thrombotic Thrombocytopenic Purpura (TTP) Registry was established in 2009 to determine incidence and natural history of TTP, a rare disease with significant morbidity and mortality. The true incidence in Australasia is unknown and national and international consensus is lacking regarding diagnosis and management. The register will analyse factors influencing outcomes, aiming to improve management and guide research. Inclusion of atypical haemolytic uraemic syndrome (aHUS) patients will begin in late 2011.

Results
To date 37 sites are participating; 19 have completed registration and ethics. Data have been received from 11 hospitals on 30 episodes in 26 patients (24 initial presentations, 6 relapses). Patients were aged 22-83 years and 14 (54%) were female. Patients presented with manifestations including: neurological (16), gastrointestinal or genitourinary (13), haemorrhagic (8), fever (7), thrombotic (5), or other (8). Clinical context and possible precipitants included infection (12), autoimmune disease (7), malignancy (3), medication (3), allogeneic stem cell transplant (1) and postpartum (1). ADAMTS13 levels were requested in 10 (38%) patients. Of 30 episodes, 28 received plasma exchange (PEx), initially at least daily. 25 (77%) received additional therapy, either to augment PEx (12, 48%), because of suboptimal response (5, 20%) or other reasons (8, 32%), including corticosteroids (21, 84%), rituximab (7, 28%), and other (9, 36%).

At time of entry on register, 4 (15%) patients were in ongoing therapy, 13 (50%) were in complete remission (CR) without long term impairment, 2 (8%) were in CR with persisting impairment, and 7 (27%) had died.

Conclusions
Cases reported demonstrate that there is still significant morbidity and mortality, despite rapid access to plasma exchange. Most patients received steroids and nearly 30% were given Rituximab. Conclusions are limited at this point, and will be enhanced by the anticipated increase in case reporting.

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P173
Retrospective Observational Study Assessing the Utility of the D-Dimer Assay in the Elderly Population for the Diagnosis of VTE

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Background
We sought to assess the sensitivity and specificity of the d-dimer assay for the diagnosis of venous thromboembolic (VTE) in different age groups.

Methods
A retrospective observational study of patients who presented to the emergency department and had a d-dimer assay performed between 1ˢᵗ January and 31ˢᵗ March 2009 was performed. There were four patient groups; Group 1: >60 years, normal d-dimer levels; Group 2: >60 years, elevated d-dimer; Group 3: <60 years, normal d-dimer; Group 4: <60 years, elevated d-dimer. D-dimer performance was assessed against the diagnosis of Pulmonary embolus (on a Computed Tomography Pulmonary Angiogram or Ventilation/Perfusion scan) and Deep Venous Thrombosis (on duplex venous ultrasound) using sensitivity, specificity and the area under the ROC curve.

Results
A total of 434 patients were identified. VTE were found in 6/188 in Group 2 and 13/119 in Group 4. There were no VTE in the Group 1 (0/26) and 3 (0/187) where the d-dimer was negative. At a d-dimer cut off level of 0.4mg/L, the sensitivity of the assay for the whole population is 100% and specificity is 63.39% [ROC area 0.89 (95%C.I. 0.84-0.93)]. In patients > 60 years, at a d-dimer cut off of 0.4mg/L the sensitivity is 100% with a specificity of 36.11%. Shifting the level to >1.51, the sensitivity remained 100% and the specificity improved to 63.89% [ROC area 0.85 (C.I. 0.73-0.96)]. In patients < 60 years, at a d-dimer cut off of 0.4 the sensitivity is 100% with a specificity of 70.83% [ROC area 0.91 (C.I. 0.87-0.96)].

Conclusions
The d-dimer cut-off level of 0.4mg/L is discriminatory for evaluating VTE disease in a young population. In patients over 60 years however, a higher level may have greater specificity and hence utility in VTE diagnosis.

No conflict of interest to report
P174

A Study on Appropriate Use of Plasma-Reduced Cryoprecipitate in the Management of Haemophilia A

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Background
Haemophilia A is a genetic disease that results in a lifelong tendency for hemorrhage because of a deficiency of functional defect of coagulation factor VIII. In classical haemophilia A, provision of some safe and affordable coagulation factor concentrate is essential for major bleeding and surgery. Some life-threatening bleeds cannot be adequately managed without advanced coagulation factor concentrates. Low dose strategies for surgery and other bleeding manifestations or low dose advanced factor treatment with cryoprecipitate can be used in developing countries. Cryoprecipitate is the only affordable treatment option in many developing countries in the management of Haemophilia A.

Objective
To assess the appropriate use of plasma reduced cryoprecipitate in the management of Haemophilia A.

Material and Methods
A twelve month retrospective study on use of cryoprecipitate was conducted from January to December 2009 to assess its appropriate use in the management of Haemophilia A in the haemophilia centre in the Lady Ridgeway Hospital for Children in Colombo Sri Lanka.

Results
367 diagnosed pediatric patients received 2766 units of pooled cryoprecipitate in additions to commercially available factor VIII.

Discussion
Cryoprecipitate is prepared by slowly thawing fresh frozen plasma at 4°C to 6°C. This results in the formation of an insoluble precipitate (cryoprecipitate) that can be resuspended in about 10 to 15 mL of plasma to be stored (at -18°C or colder) for up to a year. Each concentrate of cryoprecipitate prepared from a single donor unit of plasma contains 80 to 100 U of factor VIII. 367 children with hemophilia A were treated with cryoprecipitate in this study in addition to commercially available factor VIII, and cryoprecipitate has been used at this haemophilia centre for this diagnosis for many years. This is because commercially available factor VIII is no longer an available product as it is unaffordable and not available some months of the year, and even though the product of choice for treatment of hemophilia A. is commercially available factor VIII, cryoprecipitate has been used appropriately for the treatment of haemophilia A. However, in many developing countries, cryoprecipitate may be the only available source of factor replacement, and this should be considered in establishing indications for cryoprecipitate use.

No conflict of interest to disclose
Recombinant Activated Factor Seven (Novoseven) Succeeds its Haemostasis Achievement in the Management of Uncontrolled Massive Bleeding Due to Liver Laceration Following Road Traffic Accidents

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Background
Uncontrolled massive bleeding is the leading cause of early in-hospital mortality and the second leading cause of pre-hospital death in victims of road traffic accidents. Massive hemorrhage after traumatic injury is frequently a combination of surgical and coagulopathic bleeding. To arrest bleeding, both surgical measures and corrective surgical measurements are equally important to enhance the patient’s life expectancy.

Methods
The medical records of five surgical patients who were admitted with severe bleeding due to liver lacerations following road traffic accidents were retrospectively reviewed for blood products used before and after treatment with recombinant activated Factor seven (rFVIIa). The number of transfused units of red cells, platelets, fresh frozen plasma, cryoprecipitate, were determined both before and after the administration of rFVIIa and mortality and morbidity were analyzed.

Results
There was a statistically significant decrease in blood products used following treatment with rFVIIa in each case of the study group and reduced some complications such as Disseminated Intravascular Coagulopathy and renal failure with other multiple organ failure which is associated with massive blood transfusion and median mortality was 2 weeks and in each case and morbidity was 20% (one patient died 3 weeks after from renal failure during dialysis).

Conclusion
rFVIIa successfully used in the management of uncontrolled massive bleeding and is effective in decreasing blood products use and promoting haemostasis in patients with intractable bleeding.

No conflict of interest to disclose
P176
Prothrombin Mutation Gene (G-A 20210) in Thrombophilic Patients and General Population

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Background
Prothrombin mutation (FII 20210A) is a congenital defect which is present in 2% of the Caucasian population, 6% of patients with first episode of venous thrombosis and up to 18% of patients in the selected groups with rethrombosis.

Material and methods
Study population consisted of 2274 patients and 303 healthy individuals (voluntary blood donors). The assessment of prothrombin mutation G20210A was performed by the PCR analysis of the chromosomal DNA, which was isolated from the leukocytes gained from the peripheral blood of subjects.

Results
FII 20210A mutation was present in 157 patients (6.9%) and in 6 patients of the control group (2.6%). Among the patients there were 92 (59%) women and 65 (41%) men. Mean age of the first thrombotic event was 35.9 years (median: 34 years). Venous thrombosis was present in 122 (78%) and arterial thrombosis in 22 (14%) patients. Pulmonary embolism was found in 27 (17%) and recurrent miscarriages in 14 patients (15% of female patients). Three women with recurrent miscarriages showed combined mutations (factor V Leiden + FII G20210A). The use of oral contraceptives was associated in 11 patients (12% of female patients) with thromboembolic events (venous thrombosis in 9 women and pulmonary embolism in 2 patients). Recurrent venous thrombosis developed in 23 male patients (35%) and 32 female patients (35%). The coexistence of both FII G20210A and factor V Leiden was present in 39 patients (25%). Fourteen patients with a presence of both mutations were men (21%) and 25 patients were women (27%).

Conclusion
Based on our results we can conclude that FII 20210A mutation is a relatively frequent risk factor contributing to the occurrence of especially venous thrombosis in the Slovak population of patients with clinical signs of thrombophilia.

No conflict of interest to declare

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P177
Effect of Low Dose Omega-3 on Fibrin and Thrombin Generation in Healthy Volunteers and Patients with Cardiovascular Disease

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Aim
Hypercoagulability plays a significant role in the development of cardiovascular disease (CVD). High dose omega-3 has been shown to modify coagulation parameters, but low dose therapy has not been well studied. The aim of this study was to investigate the effect of low dose omega-3 on fibrin and thrombin generation in healthy volunteers and patients with CVD over a 4 week period.

Methods
Twenty-nine healthy volunteers (mean age 30.3yrs; range 22-64yrs), with mean BMI 23.63 kg/m² (range 18.93-35.92 kg/m²) and fifteen patients with CVD (mean age 68.1yrs; range 50-83yrs) with mean BMI 26.99 kg/m² (range 20.42-32.53 kg/m²) were tested pre- and post-omega-3 (DHA 520mg, EPA 120mg daily) for four weeks. Global coagulation activity was measured by overall haemostatic potential (OHP) and thrombin generation (CAT). Statistical analyses were performed using Wilcoxon’s matched pair tests and T-Test.

Results
Changes in clot formation were observed in the global clotting assay OHP for both healthy subjects and patients with CVD. This was evident as increased delay to fibrin formation (8:55±2:46 vs. 9:38±2:04min, P=0.021) and decreased maximum velocity (156.7±35.1 vs. 134.27±31.1 maxOD/min, P=0.010). There was also reduced overall coagulation potential (OCP) (41.0±7.1 vs. 38.2±6.9, P=0.053) and OHP (8.7±3.2 vs. 7.4±2.6, P=0.009). In patients, there were significant reductions in OCP (56.4±8.6 vs. 48.4±10.8, P=0.002), OHP (23.5±12.8 vs. 19.0±11.0, P=0.011) and maximum velocity (218.3±73.9 vs. 180.9±60.8 maxOD/min, P=0.010). In healthy volunteers the CAT assay showed no change in thrombin generation while the CVD patients showed a significant increase in lagtime (4.52±1.83 vs. 4.96±2.34min, P=0.009).

Conclusion
The results of this study suggest that low dose omega-3 fish oil has a mild hypocoagulant effect in both healthy subjects and those with CVD. This may be a mechanism for its cardioprotective effect.

No conflict of interest to disclose
NOTES
Effect of Very Long Term (16-25 years) Cryostorage on Haematopoietic Progenitor Cell Numbers and Function: Implications for Graft Quality

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Aim
To investigate the issue of human haematopoietic progenitor cells (HPC) deterioration during cryostorage and the effect of very long term cryostorage on HPC number and function.

Method
In 1999 we performed viable CD34+ enumeration and CFU-GM assays on samples that had been subjected to 5-14 years of cryostorage and found retention of engraftment potential. Using the same methodology, the present study re-analysed the 1999 study cohort of 29 reference vials (from 26 patients) that have now been in continuous cryostorage for 16 – 25 years (median 18 years). Cryopreserved grafts were re-assessed by enumeration of viable CD34+ HPC by single platform flow cytometry and CFU-GM assays using Methocult H4534 (Stem Cell Technologies). Statistical analyses were performed using Students paired t-test.

Result
There is no difference in the number of CD34+ HPC present at the 1999 and 2010 assays for each sample (p > 0.05, Students paired t-test). However there is a significant decrease in clonogenic activity between the 1999 and 2010 assays for each sample (p < 0.05, Students paired t-test). Overall during the 11 year testing window, while the viable CD34+ HPC content has not changed, the CFU-GM content has decreased significantly and may represent product deterioration during long term cryostorage. Applying the reference vials results to the entire harvest allowed determination of whether the product in cryostorage would meet our current institutional minimum requirements for transplant. The majority (15/23) of harvests that contained adequate HPC numbers for transplant in 1999 continue to be assessed as adequate in 2010.

Conclusion
HPC retain viability and function during very long term cryostorage, however the decreased clonogenic activity may be indicative of product deterioration. Laboratories should validate a time interval of storage after which product testing must be repeated before issue for transplant.

No conflict of interest to disclose
Predicting Overall Viability of Cord Blood Harvests

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Aim
Cord blood (CB) is a product rich in primitive adult stem cells used in haematopoietic stem cell transplantation. After collection, the CB is generally transported to a facility where the unit is processed and then frozen up to 48hrs after collection. These processes can lead to compromised overall white cell (WC) viability of the CB product. This study investigates the factors that affect overall WC viability before freezing of the cells cryogenically.

Method
Results from 9918 CB collections were studied. The relationship between collection volume and elapsed time to freezing, on the overall viability of CB product was analysed.

Results
The collected CB units had a mean volume of 77.1 ± 31.3 ml, a mean WC count of 10.5 ± 5.6x10^8, a mean total CD34+ cell count of 4.0 ± 3.7x10^6 and a mean overall WC viability of 91.7 ±6.5%. Overall WC viability was most significantly affected by the volume of CB collected and the time to freezing. As collection volumes increased, overall viability increased, with average viability of 95.0 ± 3.5% in CB collections of >120 ml. Decreased viability was associated with smaller volumes of <60ml and increased time to freezing of >24hrs. From this data we have developed decision tables that estimate overall WC viability based on CB volume and time to freezing.

Conclusion
This study identifies optimal time to freezing for different collection volumes in order to maintain optimal overall WC viability of the collected CB.

No conflict of interest to disclose
**PI81**

**Planer Kryo 560-16 Controlled Rate Freezer Validation**

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**Aim**

The aim of this validation was to determine the suitability of the Planer Kryo 560-16 controlled rate freezer for cryopreservation of Haemopoietic Progenitor Cell (HPC) products in 50ml and 250ml Cryocyte freezing containers and 0.5ml reference ampoules.

**Method**

A freezing program was optimised for the small volumes encountered in the paediatric setting that yielded a cooling rate of 1 – 2.5°C with the routinely used HPC product bags and reference ampoules. The optimised freezing program was run 3 times to assess: the reproducibility, the effect of the product volume and the position of the product within the freezing chamber on the freezing profile.

Five fresh HPC-Cord blood products, designated for research, were sourced from the Bone Marrow Donor Institute Cord Blood Bank. These products were assessed for: Total Nucleated Cell (TNC) and viable CD34. The HPC-Cord blood products and reference ampoules were cryopreserved using the Planer Kryo 560-16 controlled rate freezer with the verified freezing program and stored in a liquid nitrogen storage tank according to standard operating procedures.

**Results**

The cryopreserved HPC-Cord blood products and reference ampoules were thawed according to standard operating procedures. Standard assays were performed to determine TNC, viable CD34 and CFU-GM content. Non-parametric tests were used throughout the validation. A P value of < 0.05 was taken to infer significance. GraphPad version 5.0 for Windows (GraphPad Software, San Diego California, USA) was used for statistical analysis.

The TNC and viable CD34 content of the HPC products and reference ampoules were not significantly altered after freezing and thawing (P=0.08 and 0.18 respectively). The CD34 recovery (%) in the thawed bags (mean 91.6%) was significantly higher than the historical mean 79.6%, P=0.04.

The Planer Kryo 560-16 controlled rate freezer is comparable or superior to the currently used Planar controlled rate freezer.

*No conflict of interest to disclose*
A Single Centre Analysis of the Impact of Body Mass Index (BMI) on Treatment Related Toxicity and Survival in Autologous Stem Cell Transplantation (ASCT)

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Previous studies have suggested that elevated BMI, an accepted indicator of overweight, is associated with excess treatment-related toxicity and mortality where conditioning is dosed on actual body weight (ABW) during ASCT. We assessed the role of BMI in predicting toxicity and survival outcomes in ASCT patients (pts) undergoing BEAM conditioning at a single centre. Between 1/04 and 1/09, 47 pts, aged 26-70 yrs (median 46 yrs) undergoing BEAM conditioning for ASCT were divided according to BMI 20-25kg/m² (LO-BMI, n = 21) or >25kg/m² (HI-BMI, n = 26) with BMI calculated according to ABW used to dose conditioning chemotherapy. In LO-BMI vs HI-BMI groups there was no difference in frequency of WHO grade II-IV mucositis (47.6% vs 38.5%, p=0.56), use of parenteral narcotics (9.5% vs 15.4%, p=0.68), rates of proven infection (33.3% vs 46.2%, p=0.37), or use of IV antibiotics > 7 days duration (70.0% vs 53.8%, p=0.27). Median neutrophil and platelet engraftment for the cohort was 11(9-23) and 22(12-180) days respectively, with no differences between the LO-BMI vs HI-BMI groups (neutrophils, 11(10-23) vs 12(9-22) days, p=0.36 and platelets, 21(16-180) vs 23(14-61) days, p=0.49 respectively). Estimated 3 yr Disease Free (DFS) and Overall Survival (OS) for the cohort was 76.7% and 81.4%. Again, no difference could be identified in DFS or OS according to BMI group (77.8% vs 76.0%, p=0.98 and 88.9% vs 76.0%, p=0.34, respectively). While numbers in this retrospective study are small, no significant differences could be identified in treatment related toxicity or survival outcomes between LO- and HI-BMI cohorts dosed according to ABW who received BEAM conditioning for ASCT. Further studies are required to assess the role, if any, of dose adjustment in HI-BMI pts receiving high dose chemotherapy prior to ASCT.

No conflict of interest to declare
P184
Comparison of the Cobe Spectra and Optia Apheresis Systems for the Collection of Peripheral Blood Stem Cells

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Introduction
In November 2009 SCGH introduced the Caridian BCT Optia system into service for collection of peripheral blood stem cells. The collection of PBSC was previously undertaken on the COBE Spectra. As part of this transfer procedure we evaluated the Optia’s ability to collect PBSC compared to the COBE Spectra.

Methods
A formal audit was carried out on a series of 81 aphereses for PBSC procurement from 46 patients where the Optia and COBE Spectra systems were employed. Forty collections were performed on the COBE Spectra while 41 collections were performed on the Optia. PBSC were mobilised on the back of chemotherapy using Filgrastim. PBSC were collected according to the documented protocols in use with each apheresis system. PBSC were cryopreserved in 10% DSMO using a rate controlled freezer. All PBSC were stored in vapour phase liquid nitrogen below minus 160C.

Results
The Optia system collected significantly less total leukocytes during an apheresis procedure compared to the COBE Spectra (median 2.92x10¹⁰, range 6.78x10⁹ to 6.79x10¹⁰ vs median 4.5x10¹⁰, range 1.05x10¹⁰ to 1.68x10¹¹; p=0.0007). However the total number of CD34+ cells collected concurrently was not significantly different between the two systems. The apheresis collections on the Optia also were found to contain significantly less red blood cell contamination (p=0.001). There was no significant difference in the number of days of collection to achieve CD34 targets. Following apheresis bag processing the average cryopreserved bag volume was significantly lower for those obtained on the Optia (74.1±2.9mls; range50 – 123 mls ) compared to those obtained on the COBE Spectra (99.6 ± 4.4 mls; range 63–190 mls; p<0.0001). Recovery and viability of cryopreserved CD34+ PBSC was not significantly different between the two different systems.

Conclusions
The Optia system resulted in stem cell collections in a smaller volume with less leucocytes and less red cells compared with the Cobe Spectra. This has the advantage of reduced patient exposure to DMSO and free haemoglobin during re-infusion of stem cells.

No conflicts of interest to disclose
**P185**  
*Comparison of Granulocyte Content in Haematopoietic Progenitor Cells by Automated and Manual Differential Methods*  

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**Aim**  
White cell counts and differentials are usually performed using an automated haematology analyser. However these analysers are designed to analyse peripheral blood and therefore may not be accurate for peripheral blood stem cell products (PBSC) which have high white cell counts. The granulocyte content of the PBSC product is used to assess the quality of the collection process and stem cell product. This study was performed to compare and validate the percentage of granulocytes obtained by automated and manual differential methods to determine whether it is appropriate to use the white cell differential obtained by a haematology analyser.

**Method**  
The percentage of granulocytes comprised of neutrophils, bands and immature granulocytes was determined by manual and automated differential methods on 14 PBSC products from 8 patients. The cell count and differential was performed on a routine automated hematology analyser (Cell Dyn Sapphire™) which enumerate a 10 white cell differential (including nucleated red blood cells and immature white blood cell forms) and a slide was prepared for each sample within 1-2 hours of collection for the manual differential which was performed by a single experienced operator on all samples to avoid inter-operator variability.

**Results**  
The median white cell count of the PBSC product was 201.6 (range 71.7-441). The median percentage of granulocytes obtained on the automated analyser was 54.5% (range 25-88) as compared to 57.5% (range 16-90) by the manual differential. Paired t-test analysis of manual and automated differentials did not show a significant difference between the percentage of granulocytes (p= 0.891). There was no significant difference on Bland-Altman analysis (p=0.61).

**Conclusion**  
The granulocyte percentage results obtained by Cell Dyn Sapphire™ analyser are comparable to those obtained by manual differential performed by an experienced operator and therefore can be used to assess the granulocyte content of PBSC products.

*No conflict of interest to disclose*
P186
Effect of HPC-A Freeze WBC Concentration on Post Thaw Cell Viability and HPC Recovery

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Aim
To assess the relationship between increasing WBC concentration of HPC-A at cryopreservation and post thaw leucocyte viability and absolute viable CD34 cell recovery.

Method
WBC and viable CD34 recoveries were determined in all autologous HPC-A collections cryopreserved by our laboratory between May 2005 and August 2010 (n=442). Leucocyte viability was assessed in those cryopreserved between August 2009-August 2010 (n=74). Plasma reduction of HPC-A by centrifugation was performed, followed by controlled rate freezing. The freezing solution comprised DMSO/HBSS without additional plasma, at a final DMSO concentration of 10%.

WBC was determined by haematology analyser. Leucocyte (CD45) and CD34 assays were performed by flow cytometric analysis using single platform ISHAGE methodology. 7AAD was utilised in post thaw assessments conducted approximately 7 days post archival vial storage in gaseous LN2.

Statistical analysis included Pearson correlation coefficients (r and r2), associated p values by t-test statistic, slope and intercept of the regression lines.

Results
Statistically significant negative correlations were established between WBC and absolute CD34 recovery and WBC and leucocyte viability. The strength and significance of the correlation was strongest for leucocyte viability (r -0.39, p 0.0003). In contrast WBC was weakly correlated with CD34 recovery (r -0.14, p 0.0016). On average, each 100 x 10^9/L increment in WBC resulted in only 1% decrease in CD34 recovery compared to nearly 5% leucocyte viability. WBC median was 525.6 x 10^9/L (range 78.4- 818.4) and 561.2 (range 78.4-752.6) respectively for each data set.

Conclusion
HPC-A products may be frozen at a wide range of WBC concentrations, including relatively high counts, with little effect on HPC recovery post thaw. Increasing WBC has a negative impact on leucocyte viability, however no relevance to engraftment is expected. Findings support the robustness of HPC populations during the freeze-thaw process, even in the absence of additional plasma added to the freezing solution.

No conflict of interest to disclose
P315
A BMT Laboratory Processing Solution Following a Blockage During an Optia Apheresis System Collection

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The Spectra Optia is a new apheresis instrument that is based on the COBE Spectra. Recently, blockages have been observed in the Optia processing kit that prevents transfer of the HPC to the collection bag. As a result, HPC-A product may remain in the chamber and/or a belt. The aim is to share our experiences in maximising the retrieval of HPC-A cells from the chamber and/or belt.

A sterile connecting device (SCD) was used to connect the tubing of the Optia belt to a female adapter. A 60mL syringe, the belt and transfer pack were connected to a 3-way tap. The product was drawn from the belt into the syringe; the volume recorded and then transferred into a transfer pack. Ethanol was used to wipe clean both ends of the chamber's access tubes and scissor blades. Both ends of the chamber's tubes were cut and the belt disconnected from the 3-way tap and replaced with a blunt needle. The needle was inserted into the narrow internal bore of one end of the chamber tubing facilitating transfer into the syringe, volume recording and placement into the transfer pack.

The retrieved volume from the belt was 42mL and 7mL from the chamber. The residual volume was less than 1mL. The retrieved product total TNC was $1.3 \times 10^9$. A fresh CD34 count was not available due to weekend processing. A pilot vial thaw showed a viable CD34 $81 \times 10^6$. Microbial screening was negative following 14 days of culture.

Retrieval of HPC-A from the blocked Optia processing kit is possible. It is recommended a SCD should be available in the laboratory if the Optia system is in use for collections.
P183
Co-Amplification of ABL and JAK2 Together in CML Patient

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The presence of the JAK2 V617F mutation is now part of c myeloproliferative neoplasms (MPNs) clinical diagnostic algorithms, and JAK2 status is routinely assessed when BCR/ABL– chronic are suspected. Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell malignancies characterized by hypersensitivity of hematopoietic progenitors to numerous cytokines and by clonal proliferation of one or several myeloid lineages. In addition to thrombotic and hemorrhagic complications, leukemic transformation can occur. Typically, the classic MPNs encompass 4 related entities: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).

Method
Chronic myeloid leukemia (CML) is characterized by the presence of a t(9;22)(q34;q11.2), which leads to the well-known BCR-ABL1 fusion protein. We describe a patient who was diagnosed clinically with a typical CML. RT-PCR showed that the BCR gene locus spanned the breakpoint at band 22q11.2. By means of a candidate gene approach, the JAK2 gene, at 9p24, was identified as the fusion partner of BCR in this case. The BCR-JAK2 fusion protein contains the coiled-coil dimerization domain of BCR and the protein tyrosine kinase domain (JH1) of JAK2. The patient's disease respond to Imatinib, and this responsiveness was most likely a result of the BCR-JAK2 fusion protein.

Result
In JAK2 positive we need ph–chromosome check also for rule out other MPD (cml) with other specific treatment planning.

Conclusion
We recommended check up all MPD suspected patient with two most useful test.

No conflict of interest to disclose
The Effect of -24CT, 1249GA, 3972CT ABCC2 Gene Polymorphisms on Methotrexate Serum Levels and Related Toxicity in Acute Lymphoid Leukemic Children Under Treatment

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Background
Acute lymphoblastic leukemia patients under treatment with methotrexate have differences in methotrexate serum levels and toxic side effects.

Aim
The aim of this study was the evaluation of effect of -24CT, 1249GA, 3972CT ABCC2 (ATP Binding Casset Transporters) gene polymorphisms on serum level and toxic effects of methotrexate (BFM 2002 protocol) in acute lymphoblastic leukemic patients. In addition, the frequency of these polymorphisms were studied in Iranian subjects for the first time.

Method
No significant relationship was detected between -24CT and 1249GA polymorphisms with methotrexate serum level and related toxic side effects. A reverse relationship however was detected between 3972T allel and toxicity (odds ratio 0.244, 95% confidence interval 0.077-0.777). There was no significant relationship between these variants and other methotrexate toxic side effects and serum level. 1249GA and 3972CT genetic variants had more prevalence in Iranian subjects compared to the other ethnic groups (38.46% and 41.50% respectively).

Results
No significant relationship was detected between -24CT and 1249GA polymorphisms with methotrexate serum level and related toxic side effects. But, a reverse relationship was detected between 3972T allel and toxicity (odds ratio 0.244, 95% confidence interval 0.077-0.777). There was no significant relationship between these variants and other methotrexate toxic side effects and serum level. 1249GA and 3972CT genetic variants had more prevalence in Iranian subjects compared to the other races (38.46% and 41.50% respectively).

Conclusion
Genotype for 3972CT, ABCC2 gene variants maybe useful in acute lymphoblastic leukemia to optimize methotrexate therapy and reducing the associated toxicity.

No conflict of interest to disclose
**PI88**

**FLAG-Amsacrine as Salvage Therapy for Relapsed and Refractory AML**

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**Aim**

To evaluate factors predicting outcome after FLAG-Amsacrine salvage chemotherapy for patients with relapsed/refractory AML.

**Methods**

Between December 2004 and March 2011, 46 patients (24 male, 22 female) with relapsed or refractory AML were treated at The Alfred Hospital with FLAG-Amsacrine (Fludarabine 30mg/m²/day days 1-5; Cytarabine 2g/m²/day days 1-5; G-CSF 300mcg/day days 1-6; Amsacrine 100mg/m²/day days 1-3). Toxicity, response and survival data was obtained through retrospective chart review. The Kaplan-Meier method was applied in analysis.

**Results**

Median age was 57.5 years (range 18-70). 15 patients were primary refractory, 31 patients had relapsed (median relapse free interval 10.5 months) including four following allograft. Median neutrophil and platelet recovery occurred at 30 and 36 days from treatment commencement respectively. 2 treatment related deaths were observed. The overall CR/CRi rate was 65.2% (primary refractory 53%; relapsed 71%). 30% were refractory to FLAG-Amsacrine. Median EFS and OS from treatment commencement were 7 and 17 months respectively (median follow-up 9.5 months, range 1-53). 12 patients (3 refractory, 9 relapsed) remain in continuous CR with median duration of 13 months (range 3-53). There was no significant difference in EFS according to cytogenetic (p=0.33) or FLT3-ITD (p=0.53) status, previous HiDAC exposure (p=0.79), prior allograft (p=0.22) or number of previous lines of therapy (1 vs 2; p=0.26). Relapse within 6 months of previous therapy was associated with shorter EFS after FLAG-Amsacrine (p=0.005). 10 patients in CR had one cycle of consolidation FLAG-Amsacrine. 31 patients received subsequent therapy, including autologous (3 patients) and allogeneic stem cell transplantation (15 patients), with median time to treatment of 70 days.

**Conclusion**

FLAG-Amsacrine is effective salvage chemotherapy for relapsed/refractory AML and can be used as a bridge to transplantation. Alternative investigational approaches should be considered for patients with relapse free intervals of <6 months.

*No conflict of interest to disclose*
Reduced-Intensity Allogeneic Stem Cell Transplantation in Older Patients with Acute Myeloid Leukaemia

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Aim
In patients with acute myeloid leukaemia (AML) older than 55 years, long-term survival rates after chemotherapy alone are typically 10-15%, while conventional allogeneic stem cell transplantation (allo-SCT) poses unacceptable risks of morbidity and mortality. We conducted a retrospective review of older AML patients undergoing allo-SCT using reduced-intensity conditioning (RIC), in order to assess tolerability and efficacy.

Method
Sixteen patients transplanted between September 2008 and October 2010 were included who were preferentially more than 55 years of age and in CR1 at the time of transplantation. The median age was 60 years (range, 42-66). Eight had a matched related donor (MRD), and 8 a matched unrelated donor (MUD). Conditioning consisted of fludarabine (30 mg/m² IV [n = 15] or 48 mg/m² PO [n = 1] for 3 days) and low-dose TBI (2 Gy on day 0). Patients were preferentially managed in an outpatient setting.

Results
The median follow-up time was 13.1 months (range, 3.5-28.3). Acute GVHD (Grade 2-4) occurred in 5 patients (31%). Chronic GVHD occurred in 7 patients (44%). There was one case of graft failure with autologous count recovery; otherwise, engraftment was robust, with median CD3+ chimerism at 3 and 6 months of 89% and 99%, respectively.

Treatment-related mortality occurred in 1 patient (6%). Relapse was responsible for 4 of 6 deaths. OS at 1 and 2 years was 74% and 59%, respectively, and the median OS was 28 months. Progression-free survival (PFS) at 1 and 2 years was 61% and 30%, respectively, and the median PFS was 22 months.

Conclusion
We have demonstrated that RIC allo-SCT for older patients with AML can be delivered safely in an outpatient setting, with a low rate of treatment-related mortality. There may be superior survival when compared with historical data for such patients treated with standard chemotherapy, suggesting a significant graft-versus-leukaemia effect.

No conflict of interest to disclose.
A Retrospective Analysis of the Use of Azacitidine and Valproic Acid With or Without Tretinoin in Patients with Acute Myeloid Leukemia Not Suitable for Standard Intensive Chemotherapy

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Aim
Patients with acute myeloid leukemia (AML) who are unsuitable for intensive chemotherapy and receive supportive care only have very limited life expectancy. The use of DNA methyl-transferase and histone deacetylase inhibitors with low toxicity is an attractive therapeutic option for these patients. In our institution, patients with AML unsuitable for intensive chemotherapy received combination therapy with azacitidine and valproic acid with or without tretinoin. Herein we analyzed the efficacy of this combination therapy in our AML patients.

Method
A total 22 AML patients deemed not suitable for intensive chemotherapy received azacitidine-based combination therapy from December 2009 to May 2011. Clinical, haematological and cytogenetic data were collected and analyzed.

Results
Patients received azacitidine and valproic acid (AZA/VPA, n=11) or azacitidine, valproic acid and tretinoin (AZA/VPA/ATRA, n=11) combination. They had newly-diagnosed AML (n=14) or relapses (n=8) prior to azacitidine-based therapy. Median age of the cohort was 69 years (range 20-78). Cytogenetic risk groups were favourable (n=2), intermediate (n=15) and poor-risk (n=5). Median number of treatment cycles was 3 (range 1-18). Overall response rate was 45% and was not significantly different among the 2 treatment groups (AZA/VPA 55% vs. AZA/VPA/ATRA 36%, p=0.34). There was no significant difference in attainment of complete remission (CR) or CR with incomplete count recovery (CRi) in these 2 groups (27% vs. 18%, p=0.5). Best responses were achieved at a median of 3 cycles. At 4 months median follow-up, median survival has not been reached. There was no survival difference between the 2 treatment groups (p=0.68).

Conclusion
Our results indicate that treatment combination of azacitidine and valproic acid with or without tretinoin gives a good overall response rate of 45% and overall CR/CRi rate of 23% in AML patients unsuitable for intensive chemotherapy. The inclusion of tretinoin in the treatment regimen did not appear to have any impact on the overall outcome in this small series.

No conflict of interest to disclose
Azacitidine in Elderly Patients With Acute Myeloid Leukemia: A Single Center Experience

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Aim
Acute myeloid leukemia (AML) is associated with dismal prognosis in the elderly. Older patients often have a higher burden of co-morbid illnesses and poorer performance status which result in increased mortality during induction chemotherapy. Those who received best supportive care (BSC) have a life expectancy of only a few months. Since 2010, azacitidine and valproic acid with or without tretinoin was offered to some patients deemed unsuitable for intensive chemotherapy in our institution. We performed a retrospective analysis of the outcomes of elderly patients with AML who received azacitidine-based treatment compared with those who only received BSC.

Method
Patients 60 years or older diagnosed with AML between January 2010 and May 2011 were included in our review. Those who received only intensive chemotherapy were excluded. We analyzed the difference in clinical outcome between the patients who received azacitidine or BSC.

Results
29 patients were included in the analysis. 17 received azacitidine-based therapy and 8 received BSC. Baseline characteristics were comparable between the two groups. Mean age was 71 in the azacitidine group and 75 in the BSC group. Median bone marrow blast counts were 47% in the azacitidine group and 46% in the BSC group. At 10 months, median survival was 2.4 months in the BSC group and 8.9 months in the azacitidine group (p = 0.03). 3 patients (17%) attained CR or CR with incomplete recovery of counts (CRi). In the intention to treat cohort, young age, attainment of CR/CRi was associated with longer survival. Bone marrow blast counts, performance status, WHO classification and karyotype were not associated a difference in overall survival in this analysis. No treatment-related mortality was observed.

Conclusion
Azacitidine combined with valproic acid with or without tretinoin appears to be well tolerated in elderly patients with AML who cannot receive intensive chemotherapy. Survival in patients who received azacitidine appears to be improved compared with those who only received BSC.

No conflict of interest to disclose
**P192**

Use of Antithrombin (AT) Concentrate (Thrombotrol®-VF) to Reduce Thromboembolic (TE) and Bleeding Complications from Asparaginase (Asp) Therapy in Adult Acute Lymphoblastic Leukemia (ALL)/Lymphoblastic Lymphoma (LBL) Patients at Peter MacCallum Cancer Centre

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**Aim**

Review AT concentrate (Thrombotrol® VF) as primary thromboprophylaxis for Asp-related AT depletion.

**Method**

Patients were identified from a database at PeterMac from 2005-2011. Data collected included disease characteristics, coagulation, thrombotic complications and AT administration. AT administration was guided by monitoring of plasma AT levels. VTE occurring within 30 days of L-ASP was considered related.

**Results**

13 patients, 10 males, median 33 years (range 16-53): 10 ALL (5 pre-B-ALL, 5 T-ALL), 3 T-LBL. 17 courses of chemotherapy administered; Hyper-CVAD (n=9), FLAG-Ilda (n=2), paediatric protocols (n=3), salvage regimens (n=3). L-ASP was administered weekly; 16 courses incorporated L-Asp (median dose 20000 IU (range 9000-20000) median doses 4 (range 3-14)). 5 incorporated PEG-Asp (median dose 4500 IU (range 1875-5250), median doses 1 (range 1-4)). Nine patients (total 55 Asp doses) received regular AT monitoring with AT doses guided by monitoring of AT levels. AT administration resulted in normalization (>80%) of plasma AT levels in 70% (31/44) of doses, failure to achieve normal levels in 11% (5/44) and undetermined in 18% (8/44). Failure related principally to insufficient dose. Five episodes of L-asp anaphylaxis requiring medical intervention and 1 episode PEG-asp-related pancreatitis, with no documented AT administration side-effects. Six patients developed TE, 3 were within 30 days of Asp, all were CVAD-related, 2 with active ALL at time of thrombosis. None had received regular prophylactic anticoagulation. Two had sub-therapeutic AT levels (53 and 59%), one of whom was receiving replacement. The third patient had not undergone AT monitoring for 20 days prior to thrombosis.

**Conclusion**

AT concentrate normalizes plasma AT levels and appears to protect against Asp-associated TE providing active monitoring and replacement is undertaken. The role of prophylactic anticoagulation is undefined. These findings support our AT replacement strategy. Prospective studies are required to confirm these preliminary results. No conflicts of interest to disclose
PI93
Atypical Features in a Patient with Acute Promyelocytic Leukemia – A Case Report

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Background
Acute promyelocytic leukemia (APML) requires prompt treatment and is considered a medical emergency. APML has characteristic clinical, morphological, immunophenotypic and molecular features. In patients with acute leukemia, a high index of suspicion is required to exclude APML, since early diagnosis and treatment is mandatory. In this case report, an atypical presentation of APML is discussed.

Case report
A 44 year old female presented with general weakness and a few petechial spots in the skin for 2 weeks. She had pancytopenia with a few blasts in blood. The PT and APTT were normal with a slightly low fibrinogen (1.3g/L). Bone marrow showed 50% blasts, most of which appeared like myeloblasts - large sized agranular cytoplasm with large nucleus, fine chromatin and prominent nucleoli. There were no Auer rods or no bilobed nucleus. The immunophenotype was CD33+, CD13+, CD34+, CD117+, HLA-DR+. Based on these features, a diagnosis of AML was made and Big-ICE induction therapy was commenced. After 2 days, a surprise call came from the cytogeneticist that BM cytogenetics showed a t (15:17)(q,22;q21). The PML/RARα rearrangement was also confirmed by FISH testing. The diagnosis was revised as APML and Big-ICE chemotherapy was ceased and ATRA introduced.

Discussion
APML generally presents with pancytopenia with coagulopathy +/- DIC. The most common morphological type is the hypergranular APML. Hypogranular and hyperbasophilic forms are rarer variants. The blasts in APML express CD33+, CD13+ and are usually negative for HLA-DR, CD34 and CD117 markers. These features, if present will prompt fast-tracking for FISH or RT-PCR for PML/RARα. ATRA + chemotherapy is standard induction therapy for APML. Coagulopathy will worsen during the first few weeks of induction requiring close monitoring and management with blood products. There were a few case reports about atypical morphology in APML patients. This case report highlights an APML patient who had atypical morphology and immunophenotype with an unusual clinical presentation (no major coagulopathy / DIC at presentation and even after chemotherapy).

No conflict of interest to disclose
P194
Does Transient Abnormal Myelopoiesis Occur in the Absence of Down Syndrome, or Is It Really AML?

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Case history
A well 1 day old preterm male neonate, born at 34 weeks gestation with a birth weight of 2740 g, was noted to have a petechial rash. Full blood examination revealed anaemia (Hb 109 g/L), thrombocytopenia (22 x 10^9/L), and a large proportion of pleomorphic blasts (40%). Immunophenotyping confirmed these as myeloblasts (CD13, CD33, CD56 and some expressing CD117). The differential diagnosis included transient abnormal myelopoiesis (TAM) associated with Down syndrome (DS) or acute myeloid leukaemia (AML). The infant had no compelling clinical features of DS. Mild hypotonia was consistent with his stated gestation and epicanthic folds were in keeping with the South-East Asian background of his mother. Cytogenetic analysis on unstimulated cells in the peripheral blood confirmed the sole abnormality as trisomy 21 in the blast cell line. A phytohaemagglutinin (PHA) stimulated culture of T lymphocytes and analysis of 100 cells from a buccal smear both revealed a normal male karyotype in all cells (46,XY). A provisional diagnosis of non-DS TAM was made and supportive treatment included red cell transfusion and multiple platelet transfusions. Two weeks after diagnosis, the platelet count had not improved with the blast count remaining at approximately 10%. At four weeks post diagnosis, there were no circulating blasts, the platelet count had recovered (106 x 10^9/L) and the child was discharged home.

Discussion
Although the current World Health Organisation classification of haematological malignancies describes TAM as a unique disorder of DS, it is clear from this case and other cases in the literature that TAM can occur in infants in the absence of DS. In this case trisomy 21 was identified only in the peripheral blast line. The most appropriate long-term follow-up for infants with TAM remains unclear. Whilst there is a well recognized increased risk for subsequent development of leukaemia in these infants there is currently no treatment to lower this risk. The longer-term follow up of this case and a systematic review of the literature will be presented.

No conflict of interest to disclose
P195
Excellent Outcomes for Young Adults with Acute Lymphoblastic Leukaemia (ALL) Without Allogeneic Haematopoietic Stem Cell Transplantation (alloHSCT) – The FRALLE 93 Protocol Paediatric Protocol. A Multicentre Case Series Review

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Background
Adolescents and young adults with acute lymphoblastic leukaemia have better outcomes when treated using paediatric protocols compared to adult protocols. We present the progress and outcomes of 19 adolescent and young adult patients treated with the FRALLE 93 paediatric protocol.

Methods
We identified all patients diagnosed with ALL and treated with the FRALLE 93 protocol at 3 centres. Their clinical histories, bone marrow biopsies, cytogenetics and molecular studies were reviewed. Patients were not routinely offered alloHSCT unless high risk features, other than age, were present.

Results
19 patients were included from 3 centres with a median age of 23 (17 to 45) years. 11 were male. 14 patients had pre-B-ALL, 5 had T-ALL or lymphoblastic lymphoma. 17 patients had intermediate risk disease, 2 had high risk features. All patients reached a CR with induction chemotherapy. 1 patient was refractory and went onto salvage but died of progressive disease. 4 patients underwent alloHSCT, 2 due to poor risk factors, 2 on physician request. Of these, 1 is alive in CR at 1431 days, and 3 have died of progressive disease. The remaining 14 patients not transplanted, are all alive without relapse. 9 patients are in CR greater than 2 years post diagnosis and 7 greater than 3 years post diagnosis. Median survival was 857 (72 to 2049) days. Overall relapse free survival was 79%.

Conclusions
The FRALLE 93 paediatric protocol was comparatively well tolerated and showed excellent survival without alloHSCT in this cohort of young adult patients with newly diagnosed ALL. AlloHSCT may not need to be considered for standard risk patients in CR1.

No conflict of interest to disclose
P196

Case Report of Philadelphia Chromosome Positive T-cell Lymphoblastic Lymphoma

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Aim
The clinical features, diagnostic investigations, treatment and outcome of a 44-year old male patient with chronic myeloid leukaemia and Philadelphia chromosome positive T-cell lymphoblastic lymphoma.

Method
A previously healthy 44-year old man presented with rapidly progressive cervical lymphadenopathy without B symptoms. At presentation he had mild splenomegaly, cervical lymphadenopathy and a peripheral blood white cell count 282 x 10⁹/L, Hb106 g/L, platelets 256 x 10⁹/L, with increased granulocyte precursors, a myelocyte peak and 5% myeloblasts. Bone marrow aspirate was consistent with chronic phase of chronic myeloid leukaemia. Cytogenetics confirmed that all cells demonstrated t(9;22) translocation and PCR detected the BCR ABL1 rearrangement. Core biopsy of the cervical lymph node demonstrated T lymphoblastic lymphoma with positive expression of CD3, 5, 7, TdT. Interphase FISH analysis of these cells demonstrated the presence of BCR ABL1 fusion signal with evidence of amplification with 2-3 additional copies of this fusion gene. The patient received HyperCVAD with 600mg imatinib daily throughout induction therapy.

Results
Interim PET analysis demonstrated complete response of the lymphoma after 1 cycle of therapy. Induction therapy was complicated by severe bacterial sepsis requiring inotropic and ventilatory support. The patient completed a further 2 cycles of HyperCVAD Cycle A and 1 of Cycle B before proceeding to matched sibling allogeneic transplantation.

Conclusion
Philadelphia chromosome-positive acute lymphoblastic leukaemia comprises up to 50% of older B-ALL cases, carrying poorer prognosis and improved outcomes with tyrosine kinase inhibitor-based therapy. Transformation of chronic myeloid leukaemia to acute T-cell lymphoblastic disease is rare. This case demonstrates good response to a combination of induction chemotherapy and tyrosine kinase inhibition.

No conflict of interest to disclose
Identification of PML-RARA Rearrangement by RT-PCR Without t(15;17) on G-Banding and FISH in Acute Promyelocytic Leukaemia: A Case Study and Review of the Literature

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Introduction

Acute promyelocytic leukaemia (APL) is characterised by the reciprocal translocation, t(15;17)(q22;q21), resulting in the fusion of the promyelocytic leukaemia (PML) gene on chromosome 15 and the retinoic acid receptor α (RARA) gene on chromosome 17. Rarely in APL, the t(15;17) can not be detected by conventional cytogenetic analysis nor fluorescence in situ hybridisation (FISH) due to a masked or cryptic t(15;17). Diagnosis must be made based on clinical, morphological, and immunophenotype evidence and molecular confirmation of the PML-RARA fusion by reverse transcriptase polymerase chain reaction (RT-PCR). Thirteen such cases have been reported in the literature. Here we report the first case at our institution of an APL case confirmed only by positive PML-RARA RT-PCR.

Case Report

A 27 year old male presented with pancytopenia and DIC. Bone marrow morphology suggested APL. Flow cytometric analysis identified a population of cells expressing CD13, CD33 and CD117 but negative for CD34, HLA-DR and CD11b. Cytogenetic analysis revealed a normal karyotype and FISH studies were negative for the PML-RARA fusion using the VYSIS dual fusion probe. Subsequent molecular testing by RT-PCR revealed PML-RARA transcripts. The patient achieved a complete molecular remission with Idarubicin and all-trans-retinoic acid (ATRA) induction and consolidation. Quantitative molecular studies for PML-RARA transcripts remain negative after 8 months of standard maintenance therapy.

Conclusion

Rapid and accurate diagnosis of APL is important as it mandates targeted therapy with ATRA improving patient outcomes. The diagnosis of APL is generally based on clinical and morphological analysis and the confirmation of t(15;17) by conventional cytogenetics or FISH. This case emphasises the importance of RT-PCR for molecular confirmation of APL in the analysis of rare cryptic rearrangements. RT-PCR for PML-RARA fusion transcripts should be performed when strong morphological and clinical evidence exists of APL despite negative cytogenetic or FISH analysis for t(15;17). No conflict of interest to disclose.
P198
Outcome of Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin and Dexamethasone (Hyper-CVAD) Regimen in Acute Lymphoblastic Leukaemia - A Single Institution Experience

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Aim
Hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (Hyper-CVAD) chemotherapy has been commonly used for patients with acute lymphoblastic leukaemia (ALL) in our institution. Herein we report our data on the efficacy of this chemotherapy regimen and the characteristics of our patients with ALL.

Methods
A total of 86 patients who were diagnosed to have ALL from January 1998 to July 2007 received Hyper-CVAD chemotherapy. Clinical, haematological and cytogenetic data were collected, and treatment response and survival outcome were analyzed. Relapses occurred in 24 patients. Thirty-two patients underwent allogeneic stem cell transplantation. Their data were censored at the time of salvage chemotherapy or transplant.

Results
Median age of the cohort was 38 years (range 15-67). FAB subtypes were ALL-L1 (n=65), L2 (n=15) and L3 (n=6). Eleven patients had T-cell phenotype. Cytogenetic risk groups were standard (n=40), high (n=42) and unknown (n=4). Amongst the high-risk group, 24 patients had the Philadelphia (Ph) chromosome. Sixty-eight patients (79%) achieved complete remission (CR) after 1 to 2 induction cycles of Hyper-CVAD. Median time to CR was 21 days (range 13-149). Median overall survival was 19.3 months. The median survival was significantly influenced by the presence of Ph chromosome (Ph+ 9.7 vs. Ph- 21.3 months, p=0.035), cytogenetic risk category (standard 47.8 vs. high-risk 9.7 months, p=0.005) and the response status post induction cycles (remission 24.8 vs. refractory disease 9.7 months, p<0.001). However age, FAB classification, white blood cell counts at presentation and T-cell phenotype did not have any significant impact CR rate and survival in our cohort of patients.

Conclusion
Hyper-CVAD gives a good CR rate of nearly 80% in our patients with ALL. However the median survival was disappointing (19.3 months) except in the standard-risk cytogenetic subgroup (with median survival of 47.8 months). This could be explained by the large proportion (nearly 50%) of our patients having high-risk karyotypic features.

There is no conflict of interest to disclose.
PI99
The Mito-Flag Regimen in the Induction Treatment of Refractory or Early Relapsed Acute Myelogenous Leukemia

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Aim
To assess the efficiency of the Mito-FLAG regimen for induction treatment in patients with refractory or early relapsed acute myelogenous leukemia.

Method
32 patients with diagnosis of refractory or early relapsed acute myelogenous leukemia from the Blood Tranfusion and Hematology Hospital at Ho Chi Minh city – Vietnam were analysed. The statistical results were analysed by software SPSS 11.5 for windows. Student-t and Chi-squared test were used with the p value < 0.05 for the statistical significance.

Result
From December 2006 to October 2009, in the Blood Tranfusion and Hematology Hospital at Ho Chi Minh city, 32 patients with diagnosis of refractory or early relapsed acute myelogenous leukemia received the Mito-FLAG regimen consisting of fludarabine 30mg/m², high-dose aracytine 2g/m² intravenously daily for 5 days, mitoxantrone 12mg/m² daily for 3 days and G-CSF 5ug/kg daily from day 6 until neutrophils> 0.5x10⁹/l. Overall 23 patients (71.9%) had a complete remission, 5 patients (15.6%) had resistant disease whereas 4 (12.5%) died during induction therapy. Most nonhematological drug-related side effects were moderate and manageable. The recovery of neutrophils >0.5x10⁹/l and platelets >20x10⁹/l required a median of 21 and 22 days from the start of therapy.

Conclusion
Preliminary results showed that the Mito-FLAG regimen with high complete response and acceptable toxicity is an effective one for patients with refractory or early relapsed acute myelogenous leukemia.

No conflict of interest to disclose.
P200
The Use of HRM Technology to identify IDH Mutations, and their Prognostic Implications in NPM1+/FLT3- AML Patients

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Aim
To assess High Resolution Melt (HRM) technology as a method to screen NPM1+/FLT3- Acute Myeloid Leukaemia (AML) patients for Isocitrate Dehydrogenase (IDH) mutations.

Background
The IDH gene encodes an enzyme involved in the Citric Acid Cycle. Both IDH1 (R132) and IDH2 (R140 and R172) mutations are associated with myeloid malignancies, particularly NK-AML (Normal Karyotype). Recent studies suggest IDH mutations adversely affect outcome in “good molecular-risk” NPM1+/FLT3- AML patients.

Method
HRM is a post-PCR screening method that enables rapid fluorescence-based identification of mutations, based on amino acid changes altering the melting temperature of a DNA amplicon of interest. An electronic database was used to identify our cohort of patients. Stored DNA was accessed, and ethics approval attained as per Institutional guidelines.

Results
8 NPM1+/FLT3- AML patients comprising 5 females and 3 males, with median age 65 years (range 57-83), diagnosed between 2003 and 2009, were tested. 7/8 patients had de novo NK-AML and one, AML with preceding MDS. The IDH1 R132H mutation was identified in 1/8 patients (13%). The IDH2 R142Q mutation was identified in 3/8 patients (38%), while the IDH2 R172 mutation was not found. Of the three IDH mutation-positive treated patients, median remission duration was 13 months, and median overall survival, 27 months. One patient was palliated.

Of the three IDH mutation-negative treated patients, the median remission duration was 38 months, and median overall survival, 51 months. One patient was not actively treated.

Conclusion
HRM is an inexpensive and rapid screening method that can be used in identifying the IDH mutation status of AML patients. This small cohort appears to support the adverse prognostic impact of IDH mutations in NPM1+/FLT3- AML. Further large prospective studies will be required for validation. No conflict of interest to disclose.
P201
Ursolic Acid Induced Apoptosis Involving Upregulation of Expression of PTEN Gene and Inactivation of PI3K/Akt Pathway in K562 Cells

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Aim
Ursolic acid (UA), a pentacyclic triterpenoid derived from many kinds of medicinal plants, exhibits potent anticancer activity in many kinds of cancer cells. However, the anticancer mechanism of UA is not clearly understood.

Method
The toxic action of UA in K562 cells was assessed using CCK-8. K562 cells treated with UA were incubated with FITC-conjugated Annexin V and counterstained with PI in order to allow exclusion of necrotic cells, then were subsequently analyzed using a flow cytometer. Pro-caspase-3, cleaved caspase-3, Pro-caspase-9, cleaved caspase-9, Akt and Akt phosphorylation were analyzed by Western Blotting. The expression of PTEN gene was analyzed by Quantitative real-time RT-PCR.

Result
Here we showed that UA could cause growth inhibition, and induce apoptosis in human chronic myelogenous leukemia cell line K562 cells. The expression of PTEN gene is rejected in many kinds of cancers including leukemia, then the PI3K/Akt pathway is actived. So the PTEN gene and PI3K/Akt pathway become the central target for cancer therapy. UA treatment can up-regulate the expression of PTEN gene, inhibit the activity of Akt kinase, change the mitochondrial transmembrane potential, then induce the release of cytochrome C and activation of caspase family.

Conclusion
These results suggest that UA might exhibit its strong antitumor effects via the up-regulation of PTEN gene and inhibition of the activity of the PI3K/Akt pathway.

No conflict of interest to disclose
P202
Local Experience with Single-agent Ofatumumab as Salvage Treatment for Patients with Heavily Pre-treated Chronic Lymphocytic Leukaemia (CLL)

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Background
Ofatumumab is an anti-CD20 monoclonal antibody with greater *in vitro* potency than rituximab against CLL cells and promising clinical activity in refractory CLL¹. (Wierda, J Clin Oncol 2010).

Methods
We report the outcome of 9 patients with CLL treated with ofatumumab at our centres. (Peter MacCallum 8, Traralgon 1).

Results
Patients characteristics: median age 71 years (57-79), median disease duration 10 years (3-17) and median number of treatments 4 (3-6). 7 patients (78%) had received FCR with 5/7 (71%) refractory to fludarabine by standard definitions. Two patients had not previously been treated with fludarabine. 6/9 had Rai stage IV disease, 6/9 had bulky lymphadenopathy (nodal diameter >5cm) and 4/7 with data had adverse cytogenetics by FISH [del(11q)]. Ofatumumab was given weekly x 8 then monthly x 4 (dose 1, 300 mg, dose 2-12, 2000 mg). Six patients completed all planned doses. The remaining three patients experienced progressive disease and discontinued after 8, 9 and 9 weeks respectively.

Infusion reactions were noted in 4/9 (44%) of patients, all but one mild. Haematological toxicity was modest. 3/7 patients experienced anaemia (grade 2 in 7 cycles, grade 3 in 2 cycles). There was no new thrombocytopenia or neutropenia. Non-haematologic adverse events included cognitive impairment and diarrhoea. Four patients experienced grade 3/4 infections, including one toxic death (pneumonia & sepsis).

The ORR was 44% (all PR), SD 22% and PD 33%. 2/3 patients who progressed had del(11q) and bulky lymphadenopathy. 4/8 patients manifest an increase in platelet count (median 35 x 10⁹/L to 72 x 10⁹/L). Of those deriving clinical benefit (PR or SD), the median TTP was 2 months (1-6 months). One patient remains in ongoing remission (1 month after completion).

Conclusion
Ofatumumab has modest single agent activity in a highly pre-treated, refractory setting with a favorable toxicity profile.

*The authors have no conflicts of interest to declare. The drug was supplied under compassionate access by GlaxoSmithKline Australia.*
P203
Relative Gene Expression of the Bcl2 and Bax Isoforms in Patients Diagnosed with Chronic Lymphocytic Leukaemia and Healthy Control Individuals

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Aim
The Bcl-2 family of apoptosis regulating proteins have been known to play a critical role in the regulation of apoptosis in chronic lymphocytic leukaemia (CLL). From the current GenBank entries it is known that the Bcl-2 and Bax transcripts are expressed in the form of α and β isoforms whose biological functions have yet to be studied in detail. In this study, we examine the relative gene expression levels of the pro-apoptotic Bcl-2α and Bcl-2β isoforms and the anti-apoptotic Baxα and Baxβ isoforms in healthy controls and patients with CLL.

Methods
Peripheral blood was obtained from 10 healthy controls (HC) and 36 patients diagnosed with CLL. Total RNA was purified from CD19+ B lymphocytes isolated using an antibody coupled magnetic bead isolation system. Following cDNA synthesis, the relative levels of gene expression were examined by quantitative real-time PCR using primers specific for each isoform.

Results
- Bcl-2α and Bcl-2β are both expressed at higher levels in CLL however the Bcl-2α/Bcl-2β ratio remains the same between HC and CLL patients (Table).
- Baxα expression was similar in both states while Baxβ expression was raised in CLL.
- Bcl-2α and Baxα isoforms are expressed at higher levels than the β isoforms in both HC and CLL patients.
- The Bcl-2α/Baxα ratio is increased in CLL patients compared to the HC.

Conclusion
Of the isoforms examined, Bcl-2α and Baxα appear to be the dominant pro- and anti-apoptotic isoforms in CLL, with the Bcl-2α/Baxα ratio increasing in the disease state compared with healthy controls. Both β isoforms are expressed at lower levels; further investigation to elucidate their biological function is warranted.

No conflict of interest to disclose
P204
Treatment of Acute Renal Failure Secondary to Light Chain Secreting Disease with High Cut-Off Haemodialysis and Chemotherapy

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Background
Acute renal failure requiring renal replacement therapy is a frequent complication of myeloma, and approximately 85% of cases subsequently require long term haemodialysis. High concentration of free light chains (FLCs) in the patient’s sera causing cast nephropathy is the principle cause. Plasma exchange and conventional dialysis do not increase the likelihood of renal recovery. A new technique, high cut-off dialysis (HCO-HD), has been shown to remove significant quantities of light chains and data is emerging that when combined with effective chemotherapy, rates of renal recovery are increased.

Aim
To report a single centre’s experience of HCO-HD and chemotherapy in patients with acute renal failure due to light chain secreting disease.

Method
Retrospective analysis of the first four cases utilising HCO-HD at the Royal Perth Hospital. Data on serum light chain concentrations, renal function, disease response and survival were recorded.

Results
The four patients (52-74 years old) all had renal biopsy proven light chain nephropathy. Three patients had myeloma (two newly diagnosed, one relapsed) and one had a FLC secreting lymphoplasmacytic lymphoma. They received between 11 and 17 sessions of HCO-HD. Three were concurrently treated with bortezomib based regimens, and one with VAD. Mean peak FLC levels were 14105 mg/l. Each session of HCO-HD caused a 20-92% fall in serum FLC, although they often rebounded to near previous levels by the next dialysis. Prior to the last HCO-HD, FLCs had fallen to a mean 34% of presenting levels. Following HCO-HD no patient became dialysis independent and none had achieved a complete remission. Two have died, 9 and 21 months after presentation.

Conclusion
Despite reduced FLC levels, HCO-HD and chemotherapy did not result in renal recovery in any patient in this small single centre sample. Factors accounting for this response rate include late presentation, high risk disease and poor response to drug therapy.

No conflict of interest to disclose
P205
Bcl-2 and Bax Isoforms as Prognostic Markers in Chronic Lymphocytic leukaemia

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Aim
To assess the role of the anti-apoptotic protein bcl-2, the pro-apoptotic bax, and their respective isoforms, in prognosis of Chronic Lymphocytic Leukaemia (CLL) by comparing their expression to traditional prognostic parameters.

Methods
A group of 36 patients with CLL (drawn from our institution’s tissue bank) were assessed alongside 10 controls. Each sample was tested for bcl-2 expression, bax expression and their respective isoforms (bcl-2\textsubscript{α}, bcl-2\textsubscript{β}, bax\textsubscript{α} and bax\textsubscript{β}). This data was correlated with clinical and laboratory parameters. Data obtained included: demographic data, clinical stage, CD38 expression, LDH, β2-microglobulin, lymphocyte count, haemoglobin, platelet count, CLL FISH panel results, lymphocyte doubling time and requirement for therapy. Statistical analysis was undertaken to compare patients and controls for bcl-2, bax and isoform expression, as well as expression of traditional prognostic parameters in the patient cohort.

Results
Bcl-2\textsubscript{α} and Bcl-2\textsubscript{β} expression were increased in CLL (2.12 times controls, \( p=0.0051 \)). Similarly, the bcl-2:bax ratio was also increased in these patients (3.29, compared with controls 1.66, \( p=0.0012 \)). Bax\textsubscript{α} was expressed at similar levels in CLL and controls, while Bax\textsubscript{β} was increased in the CLL cohort. There was no association between Bcl-2 and Bax isoforms, or the Bcl-2/Bax ratio, and the stage at diagnosis, need for therapy, expression of CD38 or lymphocyte doubling time.

Conclusion
Our study reaffirmed results from prior studies, which demonstrated increased bcl-2 expression, and an increased bcl-2:bax ratio in CLL patients. The notable further finding in our study was of increased bax\textsubscript{β} isoform expression, an anti-apoptotic isoform known to be active in-vitro. There was no association between Bcl-2 and Bax isoforms, or the Bcl-2/Bax ratio, and the stage at diagnosis, need for therapy, expression of CD38 or lymphocyte doubling time. As the Bcl-2 and Bax analyses were undertaken at different time points during the CLL course (not all at diagnosis), we cannot definitively conclude they are not correlated with traditional prognostic markers.

No conflict of interest is declared
P206
Simultaneous Occurrence of Lymphoproliferative and Myeloproliferative Disorders in Two Patients – Single Centre Experience

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Background
The association of lymphoproliferative and JAK 2 positive myeloproliferative disorders is extremely rare and such a coexistence have been reported in literature very rarely. We would like to present two patients.

Aims
65 years old man with cerebral stroke, with proved Jak 2 positive Essential Thrombocytopenia, who developed Non Hodgkin lymphoma - Chronic lymphocytic leukemia, treated so far with anagrelide. And - 59 years old woman, with 7 years history of myeloproliferative disorder JAK 2 positive Polycythemia Vera, who developed Non Hodgkin lymphoma - Follicular lymphoma G1, with extranodal propagation to soft tissues.

Methods
Both diagnosis (myeloproliferative neoplasm and lymphoproliferative disorders) was diagnosed by complete hematological examination including morphology, histology, immunohistochemie and immunophenotypization of blood marrow, lymph node biopsy, cultivation of hemopoetic progenitors, etc. according WHO criteria for each diagnosis.

Results
1-st case: Patient is treated for ET and trombophilia, CLL is on watch and wait strategy without progression. 2-nd case: Patient was successfully treated with combination of immuno- chemotherapy R-COP and reached remission.

Conclusions
It is not known yet whether some common mechanism can explain both processes' pathogenesis and the control mechanisms of one process over the other. There are different theories to explain the process, some are based on common cell origin of both lines, other implicate a humoral or immune mechanism caused by first disorder leading to the second clonal expansion, or there are several gene studies trying to explain the coexistence of the diseases. Possible ethiopathogenetic relationships between both disorders are discussed.

No conflict of interest to disclose
P207
A Retrospective Audit of Outcomes and Risk Factors for Relapse in Patients With Thrombotic Thrombocytopenic Purpura (TTP). A Single Centre Review

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Background
Thrombotic thrombocytopenic purpura (TTP) is an uncommon disorder with a high mortality if untreated. Plasma exchange (PE) is the mainstay of treatment with a response rate of 80% but 30-60% of patients relapse and in these patients the mortality is high (15-20%). A recent study suggests adjunctive high dose methylprednisolone reduces relapse rate in TTP.

Aim
To review the response and relapse rates among patients with TTP following PE and adjunctive therapies (e.g. methylprednisolone) in a tertiary hospital.

Methods
A retrospective review of TTP patients treated at Southern Health with PE. Patients were identified from 2001 to 2011 using ICD codes for TTP, PE and haemolytic uraemic syndrome. Patients were then categorised as being consistent with or probable TTP, an alternative diagnosis or TTP (unlikely) based on their presenting clinical and laboratory profile.

Results
Twenty two cases were reviewed of whom, 16 received PE. Nine were judged as having TTP with another two probable cases. Two patients had a clear alternative diagnosis and three patients considered “unlikely” TTP. The median age of TTP subjects was 37 years (23-72), four were female and the median number of PE received was 14. Nine out of eleven patients achieved remission. Two were initially refractory but responded to further PE. Only two patients received methylprednisolone (variable doses). Four patients relapsed, all responded to further PE and only one patient received rituximab. The three patients who died were in remission from TTP at the time of death - two while in hospital due to existing renal failure and diabetes and one eight months later secondary to metastatic disease.

Conclusion
The response and relapse rates for TTP at our institution following initial PE are similar to that in the literature. Few patients received high dose methylprednisolone in our series suggesting significant room for improvement.

No conflict of interest to disclose
P208
Chronic Myeloid Leukaemia in Chronic Phase in the Real World - Ten Years Experience in the Hunter

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Aim
Identify a comprehensive regional data set of patients with chronic myeloid leukaemia in chronic phase (CML-CP) for insight into the disease, it’s management and outcome, to compare with patients in clinical trials (CT).

Methods
Using the bone marrow data base of the Hunter Area Pathology Service (Pathology North) cytogenetics service, we identified patients from Jan 2000 to Dec 2009 who had t(9;22), in CP and treated within the Hunter region. Treatment in the first year, enrolment on clinical trial (CT), survival, and cause of death were obtained from medical records.

Results
83 patients were identified, mean age 56 years (range 15-83), 57% male and 22% on CTs.

Management in First Year

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The table shows expected transition from interferon (IFN) to tyrosine kinase inhibitors (TKIs). Allogenic BMT was used in patients < 18 years due to unknown long term side effects of TKIs. To date 20 patients have died, with only 4% CML related. Although only 1/18 patients on CTs died compared with 19/65 not on CT (p=0.05, Fisher’s exact test) this may be misleading because CTs exclude patients with significant comorbidities.

Conclusion
1) New patients presenting with CML-CP was stable over 10 years 2) IFN has been superseded 3) Death due to CML is low at 4%, consistent with published literature, even though a minority of patients are on clinical trials. Collection of molecular genetic and additional treatment data is ongoing.

No conflict of interest to disclose.
P209
BCR-ABL DNA PCR for the Monitoring of CP-CML Patients Treated with Imatinib: A TIDEL II Sub-study

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Aim
Conventional monitoring of CML using RNA-based PCR (RQ-PCR) measures the number of BCR-ABL transcripts in a sample relative to a control gene. DNA-PCR measures the number of copies of genomic BCR-ABL, which is directly proportional to the number of leukaemic cells. In the setting of early treatment response, we hypothesized that a DNA-based approach for BCR-ABL quantification might provide novel insights into the kinetics of leukaemic cell depletion.

Method
TIDELII is an ongoing clinical trial of imatinib (IM) in newly diagnosed CP-CML patients. In this sub-study we collected sequential blood samples and performed DNA-PCR for BCR-ABL in a cohort of 30 patients. Patient selection was based only on sample availability. The level of BCR-ABL detected by RQ-PCR and DNA-PCR was compared.

Results
The level of BCR-ABL measured by DNA-PCR was higher than by RQ-PCR at 1, 2 and 3 months after the initiation of imatinib. Subsequent measurements (6, 9 and 12 months) showed no significant difference between the methods (Table 1). In 4/7 patients who reached RQ-PCR negativity, BCR–ABL could still be detected by DNA-PCR.

Table 1: Comparison of sequential BCR-ABL levels detected by DNA and RNA in IM treated CP-CML patients.

<table>
<thead>
<tr>
<th>Month post imatinib start</th>
<th>RQ-PCR</th>
<th>DNA-PCR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>2.5</td>
<td>0.025</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>0.09</td>
<td>0.71</td>
</tr>
<tr>
<td>12</td>
<td>0.04</td>
<td>0.04</td>
<td>0.893</td>
</tr>
</tbody>
</table>

Conclusion
DNA-PCR measures the proportion of leukaemic cells in a background of normal cells, while RQ-PCR is a composite of leukaemic cell number and RNA expression level. These data suggest that a reduction in the level of the BCR-ABL transcript precedes a reduction in the proportion of leukaemic cells following imatinib treatment. While it is currently impossible to identify conclusively the exact mechanism at play we hypothesize that the initial fall in transcript on imatinib is due to a reduction in transcriptionally active cells.

This research was supported by Novartis Oncology. The company had no role in analysing the data or preparing the abstract.
P210
Dasatinib Versus Imatinib in the Treatment of Newly Diagnosed Chronic-Phase Chronic Myeloid Leukaemia (DASISION): 24-Month Follow-Up

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Aim
In the phase 3 DASISION trial, first-line treatment with dasatinib induced higher and faster rates of complete cytogenetic response (CCyR), confirmed CCyR (cCCyR) and major molecular response (MMR) than imatinib in patients with newly diagnosed chronic-phase chronic myeloid leukaemia (CML) after 12 months. The aim of this study was to assess the efficacy and safety of dasatinib in the DASISION trial after 24 months.

Method
Informed, consenting patients with newly diagnosed chronic-phase CML were randomly assigned to received 100 mg dasatinib once daily (n=259) or 400 mg imatinib once daily (n=260). The primary endpoint was cCCyR, confirmed by two consecutive assessments at least 28 days apart. Secondary endpoints included rates and times to CCyR and MMR, duration of CCyR, progression free survival (PFS) and overall survival (OS).

Result
After a follow-up of 24-months, the rate of cCCyR continued to be higher with dasatinib than imatinib (80% versus 74%). The rate of CCyR was also higher for dasatinib (86% versus 82%). The MMR rate was higher for dasatinib than imatinib (64% versus 46%, P<0.0001). Furthermore, the median time to MMR was less for dasatinib than imatinib (15 months versus 36 months). Fewer patients receiving dasatinib transformed to accelerated/blast phase than patients receiving imatinib (6 patients (2.3%) versus 13 patients (5.0%)). Pleural effusion was experienced by 14.3% of patients (2% grade 1, 9% grade 2, <1% grade 3), although this did not appear to impact efficacy. The frequency of many of the most common non haematologic adverse events was comparable or lower than imatinib and most cytopenias occurred within the first year, indicating that dasatinib was well-tolerated.

Conclusion
Longer follow-up continues to support the use of 100 mg dasatinib once daily as first-line treatment for newly diagnosed chronic-phase CML.

Conflict of interest statement
This research was supported by the following commercial companies: SS, MBBG and CZ are employees of Bristol-Myers Squibb. AH has received research funding from Bristol-Myers Squibb and Novartis. NS has acted as a consultant for Bristol-Myers Squibb, Novartis and ARIAD. JC has acted as a consultant for Bristol-Myers Squibb, Novartis and Pfizer, and has received research funding from Bristol-Myers Squibb, Novartis, Pfizer, ARIAD, Deciphera and Chemgenex. MB has acted as a consultant for and received honoraria from Bristol-Myers Squibb and Novartis, and has received research funding from Novartis. HK has received research funding from Novartis, Bristol-Myers Squibb and Pfizer, and has acted as a consultant for Novartis. Bristol-Myers Squibb also provided medical writing and editorial assistance.
Dasatinib 100 mg Once Daily is Associated with Long-Term Efficacy and Safety in Imatinib-Resistant or -Intolerant Chronic-Phase Chronic Myeloid Leukaemia: Five Year Follow-up

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Aim
Dasatinib is an approved therapy for patients with imatinib-resistant or -intolerant chronic myeloid leukaemia (CML), and a recently approved therapy for newly diagnosed chronic-phase CML. The recommended dose of dasatinib is 100 mg once daily. The CA180-034 trial provides the longest follow-up of patients with chronic phase CML treated with a more potent BCR-ABL inhibitor. The aims of this study were to assess the five-year results of the CA180-034 trial and determine the long-term efficacy and safety of dasatinib 100 mg once daily in patients with chronic phase CML with resistance or intolerance to prior imatinib therapy.

Method
Informed, consenting patients with chronic-phase CML resistant or intolerant to imatinib (n=670) were randomised using a 2x2 factorial design to one of four dasatinib dosing regimens: 100 mg once daily, 50 mg twice daily, 140 mg once daily, or 70 mg twice daily.

Result
After five years, 57 patients (35%) remained on a once daily dosing of dasatinib. Major molecular response (MMR) was 44%, progression-free survival (PFS) was 57%, overall survival (OS) was 78%, and transformation to advanced disease on study was 5% after five years of treatment with 100 mg dasatinib once daily. Non-haematologic adverse events (AEs) generally occurred within the first 24 months of treatment and included headache (33%), diarrhoea (28%), fatigue (26%). Pleural effusion occurred in 24% of patients, generally within the first 36 months of treatment. Grade 3/4 hematologic AEs generally occurred within the first 12 months of treatment and included neutropenia (36%) and thrombocytopenia (24%). Response to dasatinib at 6 and 12 months was predictive of progression-free survival and overall survival at five years.

Conclusion
Five-year follow-up of CA180-034 demonstrates that dasatinib 100 mg once daily is associated with durable efficacy in responding patients with chronic-phase CML following imatinib therapy, and a tolerable long-term safety profile.

This research was supported by Bristol-Myers Squibb. SS, AC, AL and DH are employees of Bristol-Myers Squibb. JC has acted as a consultant for Bristol-Myers Squibb, Novartis and Pfizer, and has received research funding from Bristol-Myers Squibb, Novartis, Pfizer, ARIAD, Deciphera and Chemgenex. NS has acted as a consultant for Bristol-Myers Squibb, Novartis and ARIAD. Bristol-Myers Squibb also provided medical writing and editorial assistance.
P212
DNA-based Monitoring in Chronic Myeloid Leukemia (CML)

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We have investigated monitoring of CML using DNA in order to overcome the disadvantages of RT-qPCR. The BCR-ABL sequence was determined as previously described and a library of 531 primers was synthesised to obviate synthesising specific primers for each patient. Minimal residual disease (MRD) was quantified using one or two round PCR in 356 assays on 126 samples from 44 patients, down to a level of approximately 10⁻⁶.

Results
The results of DNA-qPCR and RT-qPCR were highly correlated (p < 0.001) but DNA-qPCR could detect MRD 1.5 logs lower than RT-qPCR. The SD of an estimation by DNA-qPCR was 0.25 log units but increased at low MRD levels. Analysis of multiple samples obtained from each of 28 patients showed systematic inter-individual differences between the results of RT-qPCR and DNA-qPCR. These differences were most likely due to differences in gene expression, were evident in approximately 30% of patients and could alter expression by up to 10-fold

Conclusions
1. The results of DNA-qPCR are broadly comparable to RT-qPCR when MRD is > 10⁻⁶.
2. DNA-qPCR is more sensitive than RT-qPCR by approximately 1.5 log units.
3. DNA-qPCR provides a more accurate measure of leukaemic cell number than RT-qPCR as it is not influenced by the level of BCR-ABL expression.
4. DNA-qPCR is more expensive than RT-qPCR but the initial expense can be amortised over serial MRD estimations.

This research was supported by Monoquant Pty Ltd. The company had no role in analysing the data or preparing the abstract.
P213
Double Trouble – Two Cases of Imerslund-Gräsbeck Syndrome

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Introduction
A three-year-old girl presented at a regional hospital with severe anaemia, poor weight gain and small for age. The full blood count results showed pancytopenia.

Case Report
The blood film showed megaloblastic changes but there was also a question of possible acute leukaemia so a bone marrow aspirate and trephine were ordered. When she presented for the procedure along with her identical twin sister, there was confusion as to which was the patient, as her sister was also very pale and small for age. The bone marrow was performed and with B12 assays confirmed megaloblastic anaemia, but intrinsic factor antibodies were not detected and thiamine diphosphate was not reduced. The twin was also investigated and showed similar results. Both also had significant proteinuria.

Discussion
Imerslund-Gräsbeck syndrome is an autosomal recessive disorder caused by the selective malabsorption of cobalamin in the ileum. There are mutations in the cubulin and amnionless genes which encode two subunits in the cobalamin-IF receptor in the ileal mucosa. These proteins are also components of the receptor mediating tubular reabsorption of protein from primary urine.

Conclusion
The twins were commenced on B12 injections with normalisation of their haemoglobins and B12 levels within three months and both have gained weight and nearing the normal height for age.

No conflict of interest to disclose.
P214
Clinical Utility of Blood Film Reviews in a Tertiary Hospital Setting

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Aim
There is a paucity of literature regarding the clinical impact of manual blood film reviews outside haematology practice. This study examines whether blood film reports are clinically useful to non-haematology clinicians in a large metropolitan hospital.

Method
A cross-sectional, anonymous, Internet-based survey of medical staff at Westmead Hospital (Sydney, Australia) was conducted. The 12-item questionnaire asked about demographics, average patient load, frequency and main reasons for accessing blood film reports, and perceived clinical utility of these reports. Wilcoxon Signed Ranks test was used to compare the within subject responses.

Result
The survey was completed by a total of 77 respondents giving a nominal response rate of 16%. A clear majority of respondents (75%) referred to fewer than 10 blood film reports over the study period of four weeks. The two most frequent reasons for viewing blood film report were to obtain diagnostic information and in response to a numerical FBC abnormality. There was no significant difference in perceived overall clinical utility (median score = somewhat helpful) between film reports issued by morphologists or those issued by haematologists (p = 0.291). In terms of usefulness of report components, morphological description was rated as significantly less helpful than differential diagnoses (p = 0.002), which was scored comparably to suggested further investigations (p = 0.103). Changes in diagnostic and therapeutic approach were rated as most frequent outcomes in response to viewing a blood film report. Flagging reports containing significant abnormalities, and emphasising diagnostic features of the report were identified by the respondents as the two strategies most likely to improve the clinical utility of blood film reports.

Conclusion
Hospital clinicians underuse blood film reports. Educational initiatives to inform clinicians how to access the full range of full blood count features should be considered. The format and focus of the blood film reports could be improved to increase the clinical utility.

No conflict of interest to disclose
Resurgence of Lead Poisoning Cases with Imported Complementary Medicine

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Introduction
With the ban on lead-based paints, use of unleaded petrol in industrialised countries, and monitoring of persons working in the mining industry for heavy metal levels, a reduction in the human body lead burden would be expected. There does however appear to be a resurgence in the number of cases reported.

Regulation
Whilst there is strict regulation by the Therapeutic Goods Administration (TGA) of complementary medicines manufactured in Australia and of commercial importation of herbal medications, individuals are still able to purchase such drugs overseas or via the internet without prescription or health professional input. A perception of the safety of these “natural” therapies is prevalent and the products are thus widely utilised.

Reported Cases
The diagnosis of recent cases of lead poisoning by our laboratory reveals a common factor. The cases have used complementary including Ayurvedic medicine, contaminated with or containing lead as an active ingredient. The patients had excessively high lead levels and overt features of lead poisoning on their blood films and had used Ayurvedic medicines imported from India.

Pathology
In addition to the well recognised haematological effects because of the multiple enzymatic systems disrupted by lead, a number of serious neurological, cardiovascular, renal and fertility effects are well documented. Treatment may be complicated and often the effects are not reversible.

No conflict of interest to disclose
P216
Greater Opportunities for the Diagnosis of Immune Related Neutropenia in Paediatrics, Using Small Sample Volumes

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Aim
Harvesting neutrophils from whole blood by conventional density gradient methods is a time consuming procedure that may require up to 30ml of patient's blood. A new method has been established that reduces both the required blood sample volume and cell preparation time. This procedure makes paediatric testing feasible, and autoimmune neutropenia testing practicable within the same day of collection.

Method
A red cell lysing solution was used to directly purify leucocytes in whole blood. Immunofluorescence testing was then performed to detect neutrophil-reactive antibodies, with analysis by flow cytometry. The various leucocyte populations were gated based on forward and side scatter characteristics, and confirmed by monoclonal antibodies. The often complex identification of neutrophil antibodies was facilitated by the reaction patterns of neutrophils, lymphocytes, and monocytes. Parallel testing of 25 clinical samples and ten known neutrophil reactive anti-sera was performed using the density gradient and direct erythrolysis methods.

Results
Within 60 minutes, the lysis method yields approximately 1mL of leucocytes at 10x10^9/L from 3mL of whole blood. This is a 90% reduction in required volume when compared to the density gradient technique, and reduces processing time by 90 minutes. 100% concordance was demonstrated for the samples tested in parallel. Neutrophils obtained by direct erythrolysis detected the NIBSC anti-HNA-1a minimum potency standard at a 1 in 32 dilution in comparison to 1 in 16 with the density gradient recovery.

Conclusion
Utilising a heterogeneous leucocyte population with flow cytometry to screen for neutrophil antibodies, has allowed our laboratory to develop a harvesting method that requires only 3ml of whole blood. This reduced volume will prove invaluable for the investigation of immune-related neutropenia of childhood and infancy. In addition to detecting neutrophil-specific antibodies, this procedure can be used to detect the presence of HLA Class-I and -II antibodies, providing more comprehensive test results than presently available.

No conflict of interest to disclose
The Online Display of Virtual Haematology Slides for Morphology Training: A Pilot Survey

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Aim
Blood and bone marrow morphology is an essential skill for haematologists. We present the results of a survey to evaluate the acceptability of online access of virtual slides for morphology training.

Method
An area of 10mm x10mm of each glass slide was scanned at X100 magnification (oil) with a Scanscope OS® system. The slides consisted of blood and bone marrow smears and a bone marrow trephine biopsy (BMTB). The slides were mounted on a RCPA QAP server and viewed online with a Webscope® viewer. Specialist haematologists with no previous experience of online virtual slides were invited to review the slides and complete a survey, using a 5-point Likert scale and open-ended comments.

Results
There were 28 respondents out of 105 haematologists polled (27%). The majority of respondents used a networked hospital computer with the Internet Explorer browser. Respondents gave above average scores for image resolution (sharpness and detail), quality of colours, ease of panning, ease of zooming and the speed of display of the images. Five respondents (18%) found that the speed of display was inadequate. Four respondents (14%) experienced difficulty with the BMTB slide (mostly with a lack of focusing), three respondents (11%) with the lack of preset zoom levels and 2 respondents (7%) with perception of nuclear detail. Respondents favoured the use of virtual slides for haematology applications in the following order of preference: teaching, quality assurance, secondary consults, morphology examinations and routine diagnosis. Overall, the experience of online virtual microscopy received ratings of average and above from 23 respondents (82%).

Conclusion
The online display of X100 virtual slides was judged by specialist haematologists to be an acceptable alternative to glass slides for morphology training. Issues that affected some users included an inadequate speed of display, lack of preset zoom levels and a lack of focusing.

No conflict of interest to disclose
P218
Folate Deficiency Is Very Uncommon Following the Introduction of Compulsory Folate Fortification in Australia

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Aim
From 13 September 2009, all bread-making flour in Australia has been fortified with folate. The study aimed to measure the incidence of folate deficiency before and after fortification, and determine the factors predicting low levels.

Method
All red cell folate tests performed at a single Melbourne tertiary teaching hospital laboratory in two cohorts were identified: pre-fortification (1/9/08 – 31/8/09) and post fortification (whole of 2010). A retrospective review of pathology results and medical records was performed in 51 of the 53 patients with low folate levels to determine causation. Groups were compared using the chi-squared test.

Results
Folate deficiency was found in 50 patients (0.77% of 6495 tests) in 2008/9 but only 3 (0.04% of 7239 tests) in 2010 (P<0.0001). Supranormal levels were increased post folate fortification: 1007 tests (15.5%) compared with 1959 tests (27%) post-fortification (P<0.0001). 23 of 50 (46%) of the folate deficient were anaemic; only 17 of 50 (34%) had macrocytosis &/or a megaloblastic blood film. 21 of 51 pts (41%) with low folate levels had co-existing low B12. Folate therapy was only begun in 20 of 50 pts (41%) (1 result unavailable). The most common cause of low folate was being elderly with social factors leading to poor nutrition (32%), followed by severe systemic illness (20%), malabsorption (11%), alcoholism (11%), documented poor diet (9%) and psychosocial dysfunction (9%). In only 5 pts was no cause found.

Conclusion
Compulsory folate supplementation has markedly reduced the incidence of folate deficiency. A much higher clinical suspicion of deficiency should be present before initiating testing.

No conflict of interest to disclose
P219
Bone Marrow Aspiration and Trephine With Methoxyflurane

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Aim
Our aims are:
1. To audit patients’ experience, including the level of pain relief/degree of comfort, during the bone marrow procedure.
2. To determine the ease of use and safety of Penthrox
3. To compare the reported experiences with the perceptions of staff performing the procedure
4. To assess the adequacy of the bone marrow specimen

Method
The audit was undertaken at Concord Repatriation General Hospital. Twenty consenting patients scheduled for outpatient bone marrow biopsies received the Penthrox inhalation device. Prior to using Penthrox, each patient underwent a risk assessment.

Following the procedure, the patients completed a questionnaire, regarding the pain experienced at consecutive stages of the biopsy. The patients were further questioned regarding any side effects and their preference for subsequent biopsies. The doctors and assisting nurses completed a separate questionnaire regarding the perception of the patient’s pain during the procedure, the ease of use of Penthrox, the difficulty of the biopsy (including the quality of the samples obtained), and any complications.

Results
The majority of patients preferred Penthrox to previous forms of analgesia, and all would prefer Penthrox again if having further bone marrow biopsies. Penthrox is mostly well tolerated by the patients and also appears to be easy to use. Doctors and nurses observed patients to be mostly pain free and cooperative. They were seen to be euphoric rather than sedated and the recovery time was approximately 5 minutes. 10% of patients experienced a fall in oxygen saturation during the procedure, however recovery with oxygen was prompt. The average time for the procedure was 20 minutes, with mostly good quality trephine samples obtained.

Conclusion
From this audit we conclude that Penthrox is a safe, easy to use and effective form of sedation for bone marrow biopsies, provided patients are carefully screened and monitored during the procedure.

No conflict of interest to disclose
P220
Failure of Point of Care Derived INR to Detect Venom Induced Consumptive Coagulopathy Following Snake Bite – A Case Report and Establishment of a State-wide Quality Assurance Activity

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Background
Point-of-care testing devices are increasingly used in remote locations and emergency departments to provide rapid access to test results, which are used to guide initial patient management. We report a recent case in which the iSTAT® point of care device grossly underestimated the severity of venom induced coagulopathy (VICC) following snake bite.

Case report
A 36 year old man presented to the emergency department of a regional hospital following a snake bite 30 minutes prior to presentation. On arrival he had spontaneous mucosal bleeding, including gum and gastrointestinal bleeding. He had not applied a pressure dressing, nor had the bitten limb been immobilised. Initial assessment demonstrated a skin wound consistent with snake bite. He was haemodynamically stable and there were no specific localising signs on examination.

An iSTAT® INR was performed in an attempt to provide a rapid assessment of the status of his coagulation system. The result was returned with an INR 3.2, PT 37 seconds. A snake venom detection kit revealed the bite was from a Brown snake, and he received appropriate doses of anti-venom. A full coagulation profile was returned; INR >10, PT >100secs, APTT >200secs, fibrinogen not available, D-dimer 512. He was admitted to the ICU for monitoring. His coagulation profile normalised over 18 hours.

Discussion
This is the second reported case of failure of iSTAT® INR to detect venom induced consumptive coagulopathy. Reasons for this may include snake venom directly acting on the chemical substrate used in the iSTAT® device, or possibly the depletion of fibrinogen. As a result of this case and the ongoing use of an iSTAT® derived INR to screen for VICC in rural and regional Queensland we have implemented a state-wide quality assurance activity to determine the validity of the iSTAT® derived INR following snake bite.

No conflict of interest to disclose
P221
Investigating the Use of a Pre-operative Haematinic Screen in Cardiac Surgery Patients

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Introduction
In patients undergoing cardiac surgery, pre operative anaemia has been shown to predict post operative transfusion requirements and to be an independent mortality risk factor. A preoperative haematinic screen was trialed to identify reversible causes of anaemia pre operatively.

Method
150 patients attending an anesthetics review for planned cardiac surgery underwent a haematinic screen. Anaemia was defined as a haemoglobin below 115g/l in females and below 130g/l in males. Iron deficiency was defined as a ferritin below 11 µg/l in females and below 24 µg/l in males in the absence of raised inflammatory markers CRP and ESR. Intervention was implemented where possible to correct iron deficiency with or without anaemia. Intra-operative and post operative transfusion data was completed for 92% of patients over a 12 month period from July 2010 to July 2011.

Results
Table one summarizes the incidence of iron deficiency, anaemia and the rate of post operative transfusion of red cells by these parameters. Comparable rates of iron deficiency, anaemia and red cell transfusion were seen in male and female patients. Iron deficiency was a better predictor of transfusion requirement in female patients compared to male patients, whereas anaemia was a better predictor of transfusion requirement in male patients compared to female patients. Iron replete and non anaemic patients required red cell transfusion at a rate between 19-27%.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Iron deficiency</th>
<th>Anaemia</th>
<th>Post operative rate of transfusion of red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>All patients</td>
</tr>
<tr>
<td>Male</td>
<td>5%</td>
<td>19%</td>
<td>33%</td>
</tr>
<tr>
<td>Female</td>
<td>6%</td>
<td>18%</td>
<td>37%</td>
</tr>
</tbody>
</table>

Table one: incidence of iron deficiency and anaemia by gender and post operative red cell transfusion rate for each parameter.

Conclusion
The low rate of iron deficiency identified in this population of patients undergoing cardiac surgery has not provided evidence to recommend a haematinic screen as a pre operative tool. In addition, although iron deficiency was predictive of post operative red cell transfusion in women, the same association was not seen in men. Pre operative anaemia remains a strong indication for transfusion, reflecting this multi factorial contribution to morbidity and mortality in cardiac surgery.

No conflict of interest to disclose
P222
High-Dose Methotrexate for the Treatment Of Relapsed CNS Erdheim Chester Disease

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Introduction
Erdheim-Chester disease (ECD) is a rare non-Langerhan’s histiocytosis with multistystem involvement including central nervous system (CNS) disease which confers a poorer prognosis. There is no definitive treatment for ECD, though interferon-alpha may be useful for non-CNS disease, if given for more than 3 months.

Case Report
A 60-year old lady with a 5-year history of stable non-CNS ECD presents with 4 days of diplopia and right arm numbness. Neurological examination revealed a horizontal gaze palsy and right arm paraesthesia. Her MRI brain showed an extensive brainstem/cerebellar lesion but her PET/CT scan revealed stable systemic disease. CSF analysis showed raised protein (3.12g/L) but no evidence of infection or malignancy. A brain biopsy was not performed due to the risk of permanent neurological damage and a presumptive diagnosis of CNS relapse of ECD was made. During the first 72-hour period, our patient rapidly developed dysarthria and ataxia, rendering her bed-bound. This necessitated urgent treatment, but interferon-alpha was not ideal due to its slow onset of action and poor CNS penetration. We chose high-dose methotrexate (8g/m²), due to its excellent CNS penetration and known therapeutic effect on CNS lymphoma. This treatment arrested the rapid progression and led to a significant improvement in her speech and ataxia. A post-induction MRI brain showed a reduction in the size of her brainstem/cerebellar lesion and her CSF protein reduced to 0.53g/L. She remained stable with ongoing high-dose methotrexate for 4 months, but subsequently developed new right-sided weakness and an increase in the size of her brainstem lesion. She is currently being treated with interferon-alpha.

Conclusion
We describe a case of CNS relapse of ECD in the setting of well controlled systemic disease. High-dose methotrexate was an effective initial salvage agent but further systemic treatment (e.g. interferon-alpha) may be necessary for a sustained long-term response.

No conflict of interest to disclose
P223
AKT Inhibition – A Novel Therapeutic Agent in Refractory Langerhans Cell Histiocytosis

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Langerhans cell histiocytosis (LCH) is a rare clonal disorder of Langerhans cells with a broad disease spectrum. Trialled therapies include steroids, chemotherapy, radiotherapy and stem cell transplantation, but for patients with disease refractory to these modalities there are few options available.

AKT (a serine/threonine) is a protein kinase involved in oncogenesis with key roles in cellular survival and proliferation. AKT inhibition is a novel therapeutic approach under evaluation in a variety of haematological malignancies. We present the case of an adult with heavily pretreated, refractory mediastinal and pulmonary LCH who achieved a sustained response after treatment with an oral AKT-inhibitor (GSK2110183) as part of a phase I, open-label study.

A 44 year old woman presented with cough, weight loss and sweats. CT & whole-body PET imaging revealed a large pulmonary and hilar mass accompanied by extensive mediastinal lymphadenopathy. Tissue biopsy confirmed LCH. Six cycles of conventional therapy consisting of 2-chlorodeoxyadenosine (0.14mg/kg/day for 5 days) and subsequent adjuvant local radiotherapy resulted in a partial response based on PET criteria. Within 12 months there was disease progression with additional mediastinal lymphadenopathy and pulmonary involvement confirmed by rebiopsy. Treatment with multi-agent conventional chemotherapy (“Third International Study for Langerhans Cell Histiocytosis protocol”) for 6 months resulted in a partial PET response, but within 6 months, disease progression was evident with dysphagia secondary to external compression of the gastro-oesophageal junction. At this stage GSK2110183 was commenced (150mg daily, in 21-day cycles) with a rapid resolution of her dysphagia and cough after 2 cycles. CT and PET demonstrated reduction in size and activity of known lesions. No significant adverse events were experienced. She has now completed 25 cycles of therapy and remains asymptomatic with a sustained near complete response.

The dramatic and sustained response to AKT inhibition in this case of refractory LCH provides a rationale for the re-evaluation of the pathogenesis of LCH and the mechanisms underlying the response.

This research was supported by Octagon Research Solutions, Inc. The company had no role analysing the data or preparing the abstract.
P224
Hybrid CD99-Friend Leukaemia Integration 1 Transcription Factor 1 (FLI1) Immunohistochemistry to Detect Bone Marrow Metastasis in Ewings Sarcoma in Two Cases

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Background
Bone marrow metastasis in Ewing Sarcoma (ES) is associated with a poor prognosis. Immunohistochemical (IHC) staining of trephine specimens with CD99 has been shown to double the rate of detection of occult bone marrow metastasis in patients with ES, however, interpretation is challenging due to significant non-specific staining. Due to the characteristic reciprocal translocation (11;22), ES cells also show aberrant FLI1 expression. We describe two cases of ES where hybrid CD99-FLI1 IHC was used to detect bone marrow metastasis.

Case reports
Case 1 – A 17 year-old female was diagnosed with ES involving the proximal femur. Staging BMAT showed extensive involvement with large, primitive non-haematopoietic cells on H&E staining. Trephine sections were stained with FLI-1(254M-16, clone MRQ-1, dilution 1/100, Cell Marque) and CD99 (M360, clone 12E7, dilution 1/200, Dako). Slides were run using Ventana Ultra Instruments and Ventana ultraview DAB and Alkaline Phosphatase Detection reagents. The non-haematopoietic cells showed strong CD99 membrane staining with simultaneous nuclear staining for FLI1. A BMAT was performed after chemotherapy which was initially thought to be uninvolved on H&E staining. However CD99-FLI1 hybrid staining clearly demonstrated a nest of 4-5 ES cells.

Case 2 – A 30 year-old man was diagnosed with ES involving his left kidney. A BMAT performed at first relapse showed effacement of normal haematopoiesis with large, primitive non-haemopoietic cells. The cells were strongly positive with CD99/FLI1 hybrid staining.

Both patients had t(11;22) detected by FISH on bone marrow specimens and in the original tumour specimens.

Conclusion
CD99-FLI1 hybrid staining is a useful technique for identifying bone marrow metastasis in ES with t(11;22). This technique has the ability to detect occult bone marrow involvement not appreciated with H&E morphology alone and may help overcome the problems of non-specific staining when using CD99 IHC alone.

No conflict of interest to disclose
P225
Massive Fetomaternal Haemorrhage (FMH) Complicated by Intravascular Haemolysis of Fetal Red Cells in Response to Intravenous Anti-D Without the Prevention of Isoimmunisation

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Introduction
FMH is uncommon with an approximate incidence of 1:5000. We present a case where the use of high dose anti-D use was complicated by significant intravascular haemolysis and iso-immunisation was not prevented.

Case
A 30 year old full-term primiparous, O Rh(D) negative woman underwent emergency Caesarian section for fetal distress. Blood-stained liquor and severe neonatal anaemia (Hb 84g/L) suggested a large silent placental abruption. Kleihauer acid elution test demonstrated FMH of 174 mls (6.8% of maternal RBCs). The neonate was A Rh (D) positive. The exact timing of FMH was uncertain, but felt to be approaching 72 hours prior and 29 vials of WinRho (120mcg/600IU) were estimated necessary to prevent Rh(D) isoimmunisation.

24 vials were immediately available and were infused in normal saline over 1 hour. Immediately prior to, and during, the infusion, the patient was well. 30 minutes after the infusion the patient developed fever, severe lower back pain, hypertension, nausea, tremulousness and haematuria. The patient was transferred to a high dependency observation area, administered oxygen, intravenous normal saline and paracetamol. All symptoms resolved after 2 hours.

LDH, bilirubin and haptoglobin changes were consistent with haemolysis. A transient increased level of Hb F noted in serum suggested the haemolysis was of fetal cells. The next day when clinically stable further dosing (5 x 625IU vials) of Rh (D) immunoglobulin (CSL) was given in divided IM doses without reaction. On serial Kleihauer testing, fetal cells became undetectable. Anti-D antibody subsequently developed and has persisted.

Conclusion
The administration of intravenous WinRho for massive FMH resulted in transient symptomatic intravascular haemolysis of fetal red blood cells. Divided dosing, prehydration and close observation are recommended when high doses of anti-D are given IV. Despite prompt Anti-D treatment and brisk haemolysis of fetal RBCs isoimmunisation may not be prevented.

No conflict of Interest to disclose
P226
Retrospective Audit of Red Cell Transfusion in Elective Cardiac Surgery in Fremantle Hospital

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Introduction
Blood transfusion in the setting of cardiac surgery is increasingly recognised as an independent adverse risk factor for patient outcomes including longer hospital stay and even death. As a part of the Patient Blood Management Programme in Fremantle Hospital, we assessed the use of red cells in a retrospective cohort of elective cardiac surgical patients. We wished to determine a baseline level of blood product use and patient factors associated with transfusions.

Method
We performed a retrospective audit of all elective cardiac surgery for six months from July 1st 2009. Baseline patient characteristics recorded including pre-op haemoglobin, creatinine, body-mass index & use of anti-platelet therapy. Operative factors including complexity of surgery, cardiac bypass time, intra-operative fluid were also recorded. Transfusion of red cells within 48 hours post-op were taken and correlated with patient and operative factors.

Results
94 patients were included in this audit with a median age of 66 yrs and 80% of patients were male. 64 patients underwent CABG surgery and 30 patients underwent valve/combination surgery. 51% of patients were taking anti-platelet therapy within 1 week of surgery. 39% of CABG patients and 57% of patients undergoing valve/combination surgery received red cell transfusions post-op. Patient factors associated with transfusion were pre-op Hb<120g/L, creatinine>120mmol/L & use of clopidogrel within 7 days of surgery. The use of aspirin within 5 days of surgery did not increase transfusion rate. Intra-operative factors also did not strongly influence transfusion in this cohort.

Conclusion
A significant proportion of patients received red cell transfusions post-cardiac surgery. A number of patient-related factors appeared to be strongly associated with transfusion. In Fremantle Hospital we have instituted a pre-operative clinic to optimise these risk factors and reduce red cell transfusion. This current cohort will form a baseline for future comparisons.

No conflicts of interest to disclose
P227
A Novel ELANE Gene Mutation in a Korean Girl with Severe Congenital Neutropenia

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Case Report
An 8-month-old girl was transferred to our hospital with prolonged fever and recurrent cervical lymphadenitis. Her initial laboratory tests revealed severe neutropenia (90/µL) and increased acute phase reactants (erythrocyte sedimentation rate 97 mm/hr and C-reactive protein 7.9 mg/dL). She had a past history of admission to the neonatal intensive care unit at another hospital due to omphalitis. The initial absolute neutrophil count (ANC) was also low (100/µL) at that time. Her course of development was normal and she had no congenital malformation suggestive of specific syndromes associated with neutropenia. Family history was nonspecific, and she had no siblings.

The cervical computed tomography was performed to exclude abscess formation or deep neck infection which needs incision or aspiration. There was no definite drainable lesion. Bone marrow (BM) findings showed early-stage maturation arrest of myelopoiesis (cellularity 80~100%, myeloblasts 3.0%, promyelocytes 0.7%, myelocytes 2.2%, metamyelocytes 0.5%, band neutrophils 0.2%, and segmented neutrophils 0.0%), with M:E ratio 0.43:1. There was no evidence of malignant involvement in the BM. The chromosome study was normal (46, XX). Direct DNA sequencing analysis of the ELANE gene was demonstrated a substitution of the 607th base (G to C) in exon 5, resulting in a change of the 203rd codon (glycine to arginine), which is a novel variation of SCN. Her parents’ tests were negative. She had no increase in ANC despite daily administration of subcutaneous 5~10 µg/kg granulocyte colony stimulating factor (G-CSF). After management with an increased dose (15 µg/kg/day) of G-CSF for 6 consecutive days and intravenous antibiotics, she recovered and was discharged with a normal ANC (2260/µL).

However, her ANC was again decreased at 130/µL after cessation of G-CSF injection. For the past 8 months, she has suffered 4 episodes of febrile illness, including septicemia and a urinary tract infection.

No conflict of interest to disclose
The Detection of Iron Deficiency in Obese Young Australian Women

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Aim
Recent opinion has suggested that there may be a chronic inflammatory state in obesity which increases ferritin and obscures the diagnosis of iron deficiency (ID). There is also evidence suggesting hypoferraemia in obesity may be due to inflammation and increased hepcidin in a process analogous to the anaemia of chronic disease. Few studies in this population have addressed these questions. We identified young women through our local obesity service and tested for iron status and inflammatory markers to ascertain the frequency of ID with and without evidence of coexisting inflammation.

Methods
18-25 year old women with BMI >27.5 kg m⁻² and no significant comorbidities were recruited. Haemoglobin (Hb), serum ferritin, plasma soluble transferrin receptor (sTfR) and C-reactive peptide (CRP) were analysed. Simple iron deficiency was defined by ferritin <15 µg L⁻¹. Iron deficiency with coexisting inflammation was defined by sTfR >2.39 mg L⁻¹ or sTfR/log ferritin <0.8 (sTfR-F index).

Results
Of 118 participants the mean age was 22.2 ± 2.3 y. Mean BMI was 33.7 ± 4.4 kg m⁻². Mean Hb was 130 ± 8.7 g L⁻¹ with anaemia (Hb < 115 g L⁻¹) identified in 4.2% (n = 5). Mean serum ferritin was 43.9 ± 34.9 µg L⁻¹ with ferritin <15 µg L⁻¹ in 16.9%. Mean CRP was 5.72 ± 7.11 mg L⁻¹ with 14% having a CRP > 10 mg L⁻¹. Mean sTfR was 1.61 ± 0.44 mg L⁻¹ (ref. range 0.74-2.39 mg L⁻¹). An elevated sTfR was identified in 5.9% (n = 7). This identified 3.4% (n=4) with ID and a normal range ferritin. None of these individuals had a CRP > 5 mg L⁻¹. The sTfR-F Index failed to identify any further patients with ID.

Conclusions
There was a relatively low prevalence of anaemia and ID compared to non-obese young females. Measurement of sTfR identified few women with ID and normal range ferritin and an elevated CRP did not predict for this. The sTfR-F index failed to identify any further patients with ID. The absence of significant numbers of individuals with evidence of coexisting ID and inflammation may be explained by the younger age, more modest obesity or exclusion of comorbidity from the study group. This supports current practice with ferritin appearing the most appropriate measure of ID in this group.

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A Fulminant Case of HIV-negative, HHV8-negative Multicentric Castleman’s Disease

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Introduction
Multicentric Castleman’s disease (MCD) is a rare non-clonal lymphoproliferative disorder which may present with a systemic inflammatory syndrome, and generalised lymphadenopathy with distinct nodal histopathology. There is a known association with human herpes virus 8 (HHV-8), which has been reported in almost all cases of human immunodeficiency virus (HIV)-associated MCD and approximately 50% of HIV-negative MCD. HHV-8 is thought to mediate lymphoid hypertrophy and inflammation through the interleukin-6 pathway.

Case Report
A 57 year old man presented with flu-like symptoms, generalized lymphadenopathy, and splenomegaly. He demonstrated cytomegalovirus seroconversion and was managed conservatively, with some symptomatic improvement over the following three months, but with persistent lymphadenopathy and splenomegaly. The patient declined lymph node biopsy, and two months later presented with pyrexia of unknown origin, mild pancytopenia and worsening lymphadenopathy. A cervical lymph node was excised with non-specific findings of necrosis/haemorrhage with a surrounding lympho-histiocytic infiltrate, and a bone marrow biopsy revealed non-specific nodular follicular dendritic cell hyperplasia. The presentation worsened with hypercalcemia and renal impairment. Repeat lymph node biopsy revealed Castleman’s disease of a mixed hyaline and plasma cell variant, with prominent atypical follicular dendritic cell hyperplasia, and was notably negative for HHV-8. He was commenced on oral steroids 1mg/kg/d with minimal improvement. Over the following month he developed profound pancytopenia, end stage renal impairment requiring dialysis, and required admission to intensive care. Following induction with CHOP chemotherapy, intensive blood product support, and dialysis, the patient gradually improved. After six courses of chemotherapy there was a complete resolution of the lymphadenopathy and hepatosplenomegaly, with restoration of normocalcaemia, haemopoietic, and renal function.

Conclusion
MCD can be difficult to diagnose, and extremely aggressive resulting in multiorgan failure. Unfortunately, there are no guidelines or large studies to guide treatment of MCD, but here we report a good response to combination chemotherapy. Case pooling and prospective trials are required to provide a platform for evidence-based therapy of this rare disease.

No conflict of interest to disclose
P230
Development of a Training Network for Haematology Trainees across a Large Geographical Area in NSW/ ACT

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Aims
The NSW Haematology training network was developed in 2005 and extended in 2009 to include ACT. It provides for 18 weekly morphology and lecture sessions by rostered tutors from February to June every year. These are attended by trainees from all centres but logistic issues often hamper participation from remote locations. Our strategies to address these issues were:
1. Encouraging attendance of trainees and tutors from remote locations by providing funding for travel.
2. Providing trainees and tutors with around-the-clock online access to Morphology cases using Virtual Microscopy (VM) via the RCPA Education Portal.

Methods
Funding was obtained from the Commonwealth Specialist Training Program initiative for the period Jan-July 2010. This included travel funding for trainees and tutors, and funding for a trial of 5 VM sessions. Four tutors agreed to participate in the VM trial, and were asked to select and mark areas on 10-12 slides of blood and bone marrow specimens which were scanned by the RCPA QAP, mounted online, and distributed in DVDs to trainees a week before the morphology sessions.

Results
Six trainees, and 2 tutor sessions were funded through the grant with advantages in attendance, greater support from local departments, and reduced isolation of trainees. Five VM sessions were conducted with the advantages of inclusion of rare cases, distribution of DVDs to more centres than was possible with glass slides, access to cases after the session, and their inclusion on the RCPA Education Portal (education.rcpa.edu.au). A further grant has been obtained to extend the project with inclusion of general networking support for webcasting lectures.

Conclusion
Project implementation has been successful in 2010-2011 and has created a ripple effect with collaborations across the network for a successful teaching program. Ongoing surveys will ensure the programme continues to grow.

No conflicts of interest to disclose
Three Cases of Haemophagocytosis in Adults

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Aim
A secondary cause for Haemophagocytosis is common in adults. Three cases of adult Haemophagocytosis are reviewed and we propose an approach for investigating this syndrome.

Method
Three patients diagnosed with Haemophagocytosis at Westmead Hospital are presented. The investigations required to confirm the diagnosis of Haemophagocytosis included bone marrow biopsies, full blood counts, ferritin, triglyceride and fibrinogen levels. Investigations performed in assessing a secondary cause included imaging, microbiological and autoimmune studies. Flow cytometry and gene mutation testing were also employed. We performed a literature review to identify the described secondary causes of Haemophagocytosis in adults. In conjunction with our three cases this was used to devise a suggested diagnostic paradigm.

Results
The first patient, a 49 year-old female was diagnosed with relapsed inherited Haemophagocytic syndrome. The second, a 44 year-old male was found to have HIV associated Haemophagocytosis and later diagnosed with Multicentric Castlemans Disease. The third, a 32 year-old male was diagnosed with Gamma Delta Hepatosplenic T-cell Lymphoma.

Timely investigations to elicit a secondary cause of Haemophagocytosis included: bone marrow morphology and flow cytometry, to assess for lymphoproliferative disorders. Serologic or molecular testing for Herpes viruses and HIV, bacterial cultures and serology for parasitic infections. Systemic Lupus Erythematosus and Adult Onset Stills Disease should be excluded with serological markers. Gene mutations have been associated with Haemophagocytosis and mutation identification can be considered.

Conclusion
Three unique cases of Adult Haemophagocytosis are presented. Early treatment of Haemophagocytosis is recognised to impact prognosis. Prompt investigation should be undertaken to identify the cause. A broad list of aetiologies should be considered in adult patients.

No conflict of interest to disclose
P232
Performance Evaluation of the Abbott CELL-DYN® Emerald™ for Human and Mouse Blood Samples

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Aim
To evaluate the bench top haematology analyser, Abbott CELL-DYN® Emerald™, which provides 18 parameter results, including a 3-part differential, to the larger Abbott CELL-DYN® Sapphire™ analyser. The CELL-DYN® Emerald™ was evaluated as a point of care analyser for human blood, and in a research laboratory processing murine blood samples.

Method
100 human (normal and abnormal) and 30 mouse (normal and high white cell count) EDTA samples were analysed for equivalent results on the two analysers.

Results
Precision was good for all levels for the routine cell count parameters, with CV% ≤ 1.5% for white cell count and haemoglobin, CV% ≤ 3% for platelets. Linearity (R² ≥ 0.99) was excellent and carryover was minimal (< 1%). Overall inter-instrument agreement for most parameters was acceptable for both human and mouse samples. For human samples, the majority of parameters showed good correlation (R² ≥ 0.95) for haemoglobin, white cell count and platelets. As a screening tool, The CELL-DYN® Emerald™ was able to detect abnormalities that would flag a sample to have a blood film made for review. For the analysis of murine samples, the analyser required re-calibration for the white cell differential count. For white cell count, neutrophils, lymphocytes, and haemoglobin, R² ≥ 0.90; whereas for platelets R² = 0.78. The main drawback was in the analysis of red cell parameters. Apart from haemoglobin, other parameters required a dilution step due to the higher red cell count in mice and linearity limitations of the analyser.

Conclusion
The Abbott CELL-DYN® Emerald™ was generally comparable to the larger reference analyser. It appears suitable for small-sized laboratories, use as a point of care system or as a backup system in larger laboratories. It was able to analyse murine full blood count sample reasonably well, with limitations regarding red cell parameters due to higher red cell count.

No conflict of interest to disclose
Introduction
Over-reliance on blood transfusion to “treat” blood loss/iron deficiency has been recognised as a contributor to increased patient morbidity, mortality and health care costs for over fifty years. General clinical practice has not kept pace with advances in iron physiology and development of safe IV iron formulations over the past two decades. The multidisciplinary patient blood management (PBM) approach to anaemia management, blood conservation and transfusion minimisation involves maximising red cell mass, minimising blood loss and exploiting physiological tolerance of anaemia. “Iron Myths” are a barrier to PBM implementation in Australian hospitals and community settings.

Aim
To promote an evidence-based PBM approach through development of “Iron Myths” educational material for Australian health care providers.

Method
Literature review, international exchange and clinical experience were used to design educational posters and supporting information, which are intended to complement the NSW Clinical Excellence Commission’s “Blood Myths” poster series.

Results
Iron Myth 1: “Any iron will do…”
Iron Myth 2: “But isn’t IV iron dangerous?”
Iron Myth 3: “My patient’s haemoglobin is fine – they’re not iron-deficient”
Iron Myth 4: “The ferritin is normal – no iron needed…”
Iron Myth 5: “Just eat more spinach!”

The “Iron Myths” posters include basic iron studies interpretation, with iron dosing/administration suggestions for various patient groups. Clinical practice concerns to be addressed include:
- formulary and/or clinical decisions influenced by poorly-designed studies/outdated beliefs about oral versus IV iron risk-benefit and cost-benefit relationships
- limited availability of iron infusion facilities for pre-surgical/other outpatients
- product information which encourages persistence of IM iron use in some practice settings

Conclusion
“Iron Myths” educational materials complement the successful “Blood Myths” series. These will be made available to Australian PBM programs, haematologists and hospital pharmacists for health care provider education, “busting” barriers to evidence-based patient care.


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P234
Evaluation of the Reticulated Haemoglobin, a New Parameter on the Sysmex XE 2100 by Comparison With Bone Marrow Stores

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Background
Laboratory diagnosis of iron deficiency has traditionally been based on iron studies including low serum iron, low percent transferrin saturation and low ferritin. Combination of ferritin and soluble transferrin receptor (sTfR) (sTfR/log ferritin) falls short because of acute phase reactant changes in the setting of inflammation\(^1\). Bone marrow (BM) iron stores is the long established gold standard but suffers from inter-observer variability and impractical for quick assessment. A new parameter available on the Sysmex XE2100 automated analyser, is the reticulated haemoglobin (ret Hb). It measures the haemoglobin (Hb) content in reticulocytes. The aim of this study was to evaluate the reticulated haemoglobin function on the Sysmex and see whether it correlated with markers of iron stores.

Methods
We evaluated 104 BM samples, performed as part of our routine haematology practice and assessed iron stores, independently by 2 investigators. BMs were performed for a variety of conditions. Peripheral blood samples were obtained at the time of BM acquisition and evaluated for full blood count, iron studies, sTfR and the ret Hb. Reference range was established using 70 females and 75 males with normal Hb and MCV.

Results
The average ret Hb from our normal cohort was 34.615+/− 0.8526 pg. The normal range established was 30.15-39.07 pg. This is not dissimilar from published ranges.\(^3\)
Iron deficient bone marrow stores was identified in 23/104 patients (22.1%). Using ROC (receiver operating characteristic) analysis, taking all patients into account, the ROC area was 0.649±0.0639 for ret Hb, 0.740±0.071 for sTfR/log ferritin ratio and 0.658±0.071 for mean cell volume (MCV). The overall sensitivity and specificity for ret Hb was 78.3% and 51.9%, 68.4% and 74.4% respectively for StFr/log ferritin ratio and 65.2 and 69.1% for MCV.

Conclusions
Based on these small numbers, mixed cohort, and allowing for the problems associated with BM iron stores as a gold standard, we found that the sTfR/log ferritin ratio was the most useful parameter for identifying iron deficient erythropoiesis. The overall sensitivity of ret Hb for iron deficiency was comparable to published results although the specificity was lower. The diagnosis of iron deficiency requires a composite of several factors and results.

Reference
No conflict of interest to disclose
P235
A Very Unusual Presentation of Heparin-Induced Thrombocytopenia Syndrome – A Case Report

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Introduction:
Heparin-induced thrombocytopenia syndrome (HITS) is an immune-mediated disorder characterized by the formation of antibodies against the Heparin-PF4 complex. HITS is characterized by thrombocytopenia +/- thrombosis. The diagnosis of HITS is mainly clinical. Although laboratory tests are helpful in diagnosing HITS, they have many limitations. We present a case of HITS with a very unusual and complex presentation.

Case report
An 84 year old Caucasian lady was admitted in the hospital with a surgical wound infection, which responded well within a few days of starting IV antibiotics. She received daily prophylactic doses of unfractionated heparin (UFH). On day 9, the platelet count started falling <50% of baseline with a further rapid drop to 3x10^9/L and multiple large ecchymoses. HITS was in the list of differential diagnosis. Heparin was ceased and PaGIA card screening test was strongly positive. Simultaneously she also developed large lower limb DVT + massive PE, confirmed by imaging and severe coagulopathy with a DIC picture. Since false positive results are common with PaGIA test, serotonin release assay (SRA) was sent. The patient succumbed to PE progression in a few days, despite treating her with Danaparoid. The SRA result, which came a month later was strongly positive (89% - normal range 0-20%).

Discussion
The clinical criteria (4Ts) are commonly used for diagnosing HITS. Many conditions like drugs, sepsis, DIC and immune disorders can mimic HITS. Immunoassays (ELISA, PaGIA) are highly sensitive, but are less specific. SRA is considered gold standard due to high sensitivity and specificity (>95%), when performed in experienced laboratories. This patient had many atypical features like HITS following prophylactic doses of UFH, severe thrombocytopenia, coagulopathy, DIC and bleeding. She also presented a clinical challenge with difficulty in the managing simultaneous massive VTE, severe coagulopathy and bleeding. Such a picture is very unusual in HITS and it is difficult to find similar case reports in literature.

No conflict of interest to disclose.
P236
A Paradigm Shift in the Treatment of Patients with HFE-Associated Haemochromatosis

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Background
It has been common practice in our institution to venesect all patients confirmed to be homozygous for the C282Y mutation or compound heterozygous for the C282Y and H63D mutations who have a raised ferritin and/or a high transferrin saturation to a low-normal or iron deficient state to prevent organ damage, particularly liver cirrhosis. Recent studies have demonstrated variable clinical penetrance. Venesection may exacerbate iron accumulation by increasing gut absorption and the rate of iron re-accumulation after depletion is variable.

Aim
To develop a new algorithm for the management of HFE-associated haemochromatosis and assess the impact of adopting this in practice.

Methods
The Haematologists at our centre agreed to adopt the following approach to manage these patients.

- Ferritin of >1000ug/L: ultrasound and consider liver biopsy, aggressive venesection and specialist follow up if cirrhosis or fibrosis is present. The target serum ferritin is <50ug/L, to be maintained by ongoing venesection indefinitely.
- Ferritin of 700-1000ug/L: aggressive venesection until ferritin<50ug/L. This group may discontinue venesection and be monitored unless the ferritin increases to >500ug/L.
- Ferritin <700ug/L will be monitored only, regardless of saturation. Lifestyle factors and other causes of abnormal LFTs, if present, will be considered.

Results
56 patients received 368 venesections for the year up to January 2010 in our centre. The compliance and effect of applying this algorithm will be assessed in terms of staff time, cost and patient satisfaction in January 2011.

Conclusions
It is anticipated that the numbers needing to be venesected will fall considerably, with the majority of patients being under surveillance by their general practitioners. This algorithm is likely to be suitable for a nurse led clinic.

No conflict of interest to disclose
The Use of MLPA and PCR Sequencing to Confirm (HBB:G.1197-1816del620), A Partial β-Globin Gene Deletion Associated with the β-Thalassemia Trait

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Aim

Beta thalassaemia is most commonly a result of point mutations or microdeletions within the beta globin gene. Larger deletions are less common and, until recently, have been difficult to detect, requiring cumbersome techniques such as Southern Blotting. In populations where specific deletions are present at high frequency, allele specific PCR is a useful tool for the detection of these deletions. Western Australia has a very diverse population however, and we have adopted the MLPA (multiplex ligand dependent probe amplification) assay to screen for deletions in the beta globin locus. This study was undertaken to further characterise a beta globin deletion detected using the MLPA assay.

Methods

The patient demonstrated typical indices of β-Thalassaemia trait (Hb 124 g/L, MCV 59 fL, HbA2 5.6%, HbF 0.5%). Beta globin sequencing was normal. MLPA was performed using the commercial kit from MRC Holland (P102-B1 HBB probe mix v9). To confirm the breakpoint, a series of PCR primers was designed to prime on each side of the deletion, and the PCR product was sequenced.

Results

MLPA (P102-B1 HBB probe mix v9), showed that the patient has a deletion including probes 26 and 27 which are located in exon 3 of the β-globin gene, with the 5’ and 3’ breakpoints lying upstream and downstream of the region respectively. To confirm the finding as well as to identify the breakpoints of the deleted HBB allele, a series of PCRs was conducted with primers that primed outside this region confirming a wild type product size of 1629bp and a second product size of 1000bp. Subsequent DNA sequencing performed on the smaller PCR product confirmed the 620bp deletion as HBB:g.1197-1816del620.

Conclusion

This highlights the value of using MLPA in conjunction with PCR and DNA sequencing when a comprehensive molecular evaluation of unusual haemoglobin patterns is needed.

No conflict of interest to disclose
P238
A Novel Point mutation of Alpha1 Globin Gene (IVSII-147 [HBA1:c.303-3 C>G]) Causing Mild alpha Thalassemia Phenotype

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Aim
The aim of this work is to report and characterize a novel alpha (α-) thalassemia caused by a point mutation of the α1-globin gene (HBA1:c.303-3 C>G) found in an adult female patient living in Western Australia. It is believed to cause a mild α-thalassemia phenotype.

Methods
Clinical data were generated during investigation of the patient for thalassemia. These include CBC, HPLC, Iron studies, Gap-polymerase chain reaction (gap-PCR) for the common alpha globin gene deletions, and direct sequencing of the alpha globin genes. Experimental work involved generation of an expression vector carrying the HBA1:c.303-3 C>G mutation followed by transfection, RNA purification, reverse-transcription polymerase chain reaction (RT-PCR) and cDNA sequencing. Protein detection was performed using Immunohistochemistry (IHC) with antibodies to the N- and C-terminal of the alpha globin protein.

Results
In vitro molecular characterization of this mutation showed a cryptic splice site formation at intron 2 of the pre-mRNA leading to an aberrant splice variant. As a result, a shift in the reading frame causes a premature termination codon (PTC) at codons 101/102 and thus generation of a truncated protein. Using IHC, the generated α-globin showed positive reaction to the N-terminus antibody while negative reaction to C-terminus one confirming the production of a truncated α-globin.

Conclusion
We identified a novel nondeletional α-thalassemia caused by point mutation of α1-IVS II -147 C>G and provide experimental evidence that this mutation causes an aberrant splice variant leading to a premature termination codon (PTC) and truncated protein.

No conflict of interest to disclose
Investigation of the Impact of Point Mutations in the Transcription Initiation Site of the Alpha-1 globin Gene

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Aim
The majority of cases of alpha thalassaemia are due to deletions of varying size within the alpha globin locus, however, with access to sequencing an increasing number of point mutations have been discovered and postulated to contribute to the thalassaemic phenotype in affected individuals. It can be difficult to determine whether the sequence variation is, in fact, pathologic or clinically silent. During the course of investigation of a patient with microcytosis, a base substitution was detected at the transcription initiation site of the alpha1 globin gene (HBA1:c-37A>C). This study was undertaken to investigate the impact of this on alpha globin transcription levels.

Methods
Site directed mutagenesis was used to generate base substitutions (HBA1:c-37A>C, HBA1:c-37A>T, HBA1:c-37A>G) in an expression plasmid containing the alpha globin gene. Following transfection, the level of alpha globin mRNA was assessed by quantitative PCR. Protein expression was assessed by immunohistochemical technique.

Results
The real time-quantitative polymerase chain reaction analyses revealed that transcript levels generated from HBA1:c-37A>C and HBA1:c-37A>T mutations showed higher expression than those produced from the wild type. The HBA1:c-37A>G transversion showed a slight reduction in expressed α1-globin transcripts. The subsequent immunohistochemistry analyses revealed no detectable difference in the α1-globin protein levels between the mutants and the wild type.

Conclusion
Our findings provide experimental evidence that mutations at the transcription initiation site of the α1-globin gene have minimal effects on the level of transcription, and would not be expected to cause a thalassaemia phenotype.

No conflict of interest to disclose
P239
Investigation of the Impact of Point Mutations in the Transcription Initiation Site of the Alpha-1 Globin Gene
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The majority of cases of alpha thalassaemia are due to deletions of varying size within the alpha globin locus, however, with access to sequencing an increasing number of point mutations have been discovered and postulated to contribute to the thalassaemic phenotype in affected individuals. It can be difficult to determine whether the sequence variation is, in fact, pathologic or clinically silent. During the course of investigation of a patient with microcytosis, a base substitution was detected at the transcription initiation site of the alpha1 globin gene (HBA1:c-37A>C). This study was undertaken to investigate the impact of this on alpha globin transcription levels.

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Conclusion
Our findings provide experimental evidence that mutations at the transcription initiation site of the α1-globin gene have minimal effects on the level of transcription, and would not be expected to cause a thalassaemia phenotype.

No conflict of interest to disclose.

P240
Prevalence of Hemoglobinopathies in Kermanshah, Iran
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Aim
Hemoglobinopathies are the most common single gene disorder worldwide with a considerable frequency in certain areas particularly Mediterranean and Middle Eastern countries. Hemoglobinopathies include structural variants (such as Hb S, Hb C, Hb E), and thalassaemias which are inherited defects in globin chains synthesis. The aim of this study was to determine the prevalence of hemoglobinopathies in western Iranian patients.

Method
From 2010 to 2011 a total of 221 patient (120 male, 101 female) were entered to this study for anemia and a lower than normal MCV and/or MCH. RBC, Hb, Hct, Serum Iron, TIBC, Serum Ferritin and Mentzer index were determined. Hb A, A2, F and S band were assayed with cellulose acetate electrophoresis. S band indicate hemoglobin S, G or D.

Result
66 patients (29.8%) had only Iron deficiency anemia, 41 patients (18.5%) had Iron deficiency anemia and minor alpha-thalassemia, and 48 patients (21.7%) had Iron deficiency anemia and minor beta-thalassemia. 17 patients (7.6%) had minor alpha-thalassemia and 5 patients (2.2%) had trait alpha-thalassemia. 36 patients (16.2%) had minor beta-thalassemia. 8 patients (3.6%) had S, G or D hemoglobinopathies.

Conclusion
Our results shows that hemoglobinopathies is an important problem in this area. Previous studies indicated that alpha-thalassemia in Iran had a low prevalence. So, in screening tests beta-thalassemia were paid more attention. But our result shows that alpha-thalassemia has a high prevalence in our area, so it needs more attention in screening tests.
P241
Causes and Clinical Significance of Prolonged Activated Partial Thromboplastin Times in Thalassaemia Major

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Aim
15% of regularly transfused thalassaemia major patients at our centre have an unexplained prolongation of the activated partial thromboplastin time (APTT). There is a paucity of published data regarding its relevance. We aimed to investigate the cause and the clinical consequences of these prolonged APTTs.

Methods
19 patients with thalassaemia major on a regular transfusion program who had a prolonged APTT were identified. Blood samples were taken immediately pre-transfusion for coagulation profile, factor VIII, IX, XII, XII assays and lupus anticoagulant testing. Relevant clinical data including bleeding symptoms, frequency of transfusion and history of splenectomy was obtained.

Results
The median prolonged APTT was 35 seconds (reference range 22-32secs). Factor VIII, factor IX, factor XI and factor XII was low in 1, 3, 16 and 13 patients respectively. 11 patients had 2 or more factor deficiencies. A lupus anticoagulant was not detected in any of the patients. The median transfusion requirement was 3 units every 3 weeks. 4 patients had been splenectomised and only 1 patient had a history of bleeding, however this appeared to be the result of a previously diagnosed platelet function disorder.

Conclusion
Thalassaemia major patients receiving regular transfusions who have a prolonged APTT appear to have decreased factor XI and XII levels more commonly, but low factor VIII and IX levels are also observed. This is unlikely of clinical significance in the absence of bleeding symptoms. Further studies are needed to evaluate whether or not the cause of this is due to activation of the contact system from abnormal thalassaemic red blood cells that provide a source of negatively charged phospholipids.

No conflict of interest to declare
P242
Quality of Life in Parents of Children Living with a Clinically Significant Haemoglobinopathy in NSW, Australia

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Aim
To analyse the impact that having a child with Sickle cell disease (SCD) or Thalassaemia has on parental quality of life.

Method
The NSW-Haemoglobinopathy project is a comprehensive health needs assessment of patients (and parents of children) with a clinically significant haemoglobinopathy in NSW, Australia. In this subgroup analysis parents of children cared for at a single paediatric centre completed an in-house disease specific survey and a commercially available measure of health related quality of life (HrQoL) (SF36v2).

Result
16 parents responded (mean age children: 9.8 years (0.9-19)). There were equal numbers of children with SCD and Thalassaemia. 50% of parents reported the impact of their child’s illness on parental HrQoL as moderate to severe. This was not different between disease groups. Linear rating of HrQoL was not different between the groups with a mean of 6.83 on a 10 point scale. Factors thought to impact on quality of life were investigated. Of those parents of children with SCD; 12.5% report significant work absence, negative effects on career success (25%), and family and romantic relationships (25%, 37.5%). More parents of children with Thalassaemia reported significant work absence (37.5%), although less reported impact on career (12%) and family relationships (12.5%) and none reported impact on romantic relationships. Although due to the small sample size there was no statistically significant difference between the two disease groups. The parents had a lower than expected summary score on the SF36v2 for mental health (Mean MCS =35%) compared with the normal Australian population (Mean MCS=50%). Physical health summary scores (PCS) were similar to the normal population.

Conclusion
Some parents report a lower HrQoL compared with the normal population as well as with their own expectations. Such an impact requires investigation with regards to interventions for psychological support for parents of children with a haemoglobinopathy.

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P243
Molecular Testing for Haemoglobinopathies in Queensland

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Queensland has a unique mixture of ethnic backgrounds. There is a large Asian population as well as a large African refugee population. The Mater Hospital in Brisbane has a dedicated refugee clinic, a large obstetric hospital, and a dedicated haemoglobinopathy clinical unit. Thus, we have a diverse population of patients with haemoglobinopathies including a significant population with sickle cell disease, and other major haemoglobinopathies. We have established a comprehensive pipeline for the accurate molecular diagnosis of all \( \alpha \) and \( \beta \)-haemoglobinopathies. We employ a mixture of GAP PCR¹, MLPA², PCR-strip hybridisation assays and direct sequencing of the \( \beta \)-globin locus to determine the precise molecular lesion in women of childbearing age, or when otherwise clinically indicated or requested. Each of these assays has advantages and disadvantages with respect to reliability, false negative results and cost, which we will share and discuss. We have developed a panel of positive controls based on DNA samples supplied from the Coriell Institute. We also have developed a comprehensive pipeline for investigation of HPFH, whether linked or unlinked to the \( \beta \)-globin locus. This includes tests for deletional HPFH by GAP-PCR, strip hybridisation assays for point mutations in the \( \gamma \)-globin genes, direct sequencing and screens for mutations in the KLF1 gene³, the MYB upstream enhancer region⁴ and the BCL11A gene⁵. We will share our experiences with these assays and discuss a few interesting cases.

References:

No conflict of interest to declare
P244
Retrospective Study of the Causes of Microcytosis in a Community based Sample Population

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Aim
Microcytosis in the community is prevalent and aetiologies include iron deficiency, haemoglobinopathy and anaemia of chronic disease. Hypothyroidism is a well known cause of macrocytosis. We wished to review the aetiology of microcytosis seen in the community particularly assessing the contribution of hyperthyroidism to the aetiology.

Methods
A retrospective review of samples received by a community laboratory over a 12 month period between 1st Dec 2008 and 30 Nov 2009. Data obtained included: HB, MCV, Ferritin, transferrin, transferrin saturations, TSH, T4, T3 and High Performance Liquid Chromatography (BioRad Variant). Molecular studies included multiplex gap PCR for the common alpha globin deletions, and sequencing of the alpha1, alpha2 and beta globin genes where relevant.

Results
473 samples were received for haemoglobinopathy investigation and had haemoglobin DNA studies performed. Of these 93% (441 samples) were microcytic of which 67%(294 samples) were not anaemic and 33%(147 samples) were anaemic. Of the 441 microcytic samples the following results were obtained: no haemoglobinopathy (n=175), alpha thalassemia trait (n=233), HbH disease (n=3), beta thalassemia trait (n=12), compound heterozygote alpha/ beta thalassemia trait (n=5), HbE trait (n=5), compound heterozygote HbE/ alpha thalassemia (n=5), and compound heterozygote HbS/ alpha thalassemia (n=6). Of the 175 microcytic samples with no haemoglobinopathy, 88 were iron deficient, 62 were not iron deficient and 25 were unclassifiable (anemia of chronic disease was not able to be excluded). 3 samples were found to be hyperthyroid: 1 also iron deficient, 2 not iron deficient. All 3 had normal Hb. Of the samples with normal MCV, 8 patients had alpha thalassemia trait with a mean MCV of 82 fl.

Conclusions
Hyperthyroidism is a rare cause of microcytosis. The yield for specialist haemoglobinopathy investigation is high in a community based sample population. There is a small population of patients with haemoglobinopathy that are not microcytic.

No conflict of interest to disclose
P245
A Retrospective Analysis of Outcomes in HIV-associated Lymphoma

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Introduction
The treatment of HIV-associated lymphomas remains controversial despite the widespread use of combination antiretroviral infection (cART). Historically, patient outcomes have been poor compared to non HIV infected patients.

Aim
To investigate the outcomes of patients with HIV-associated lymphoma treated at a statewide referral service for HIV medicine.

Methods
A retrospective analysis of patients treated by the Alfred Hospital, Malignant Haematology and Stem Cell Transplantation Service over 5 years (from 2006 – 2010) was undertaken. Data obtained included lymphoma subtype, stage at diagnosis, chemotherapy regimen, the use of cART, response to treatment and overall survival.

Results
There were 13 patients diagnosed with high grade non-Hodgkin’s lymphoma from 2006-2010 (12 patients with Burkitt’s or Burkitt like and 1 diffuse large B cell lymphoma). The majority (10/13) of these patients presented with advanced (stage IV) disease. The median CD4 count at diagnosis was 120 cells/mL. All were treated with intensive chemotherapy regimens. Eleven patients received CODOX M/IVAC and four received CHOP. Additionally, all patients received Rituximab and cART. 77% (10/13) achieved a CR and the overall survival with a median follow up of 24 months was 69% (9/13). Of the 4 deaths, 3 patients died of infection and one of progressive disease.

Over the same period, 3 cases of Hodgkin’s lymphoma were diagnosed. Two patients had advanced (stage IV) disease and all were treated with ABVD chemotherapy and cART. All achieved a CR and remain disease free with a median follow up of 14 months.

Conclusions
This retrospective series from a single institution of HIV positive lymphomas treated with aggressive immunochemotherapeutic approaches and cART demonstrates outcomes equivalent to those seen with non-HIV patients.

No conflict of interest to disclose
P246
Hematologic Toxicity of Gemcitabine: A Comparison between Fixed-Dose Rate Infusion and Thirty-Minute Infusion in the Treatment of Malignant Tumor

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Aim
Fix dose rate therapy of gemcitabine has been used in pancreatic cancer treatment, but this kind of regimen has been limited because of controversial reports of hematologic toxicity. Our study compared the hematological toxicity of gemcitabine between “fix dose rate” (FDR) infusion and 30-minute standard infusion in the treatment of malignant tumors and investigated the possibility of high dosage chemotherapy of gemcitabine in solid tumors with the APBSCT (autologous peripheral blood stem cell transplantation) support.

Method
25 patients diagnosed with malignant tumor histopathology or cytology received chemotherapy with gemcitabine alone or combined with other chemotherapy agents. The patients were randomly assigned to receive gemcitabine 1000mg/m² on d1, d8 at a rate of 10mg·m²⁻¹·min⁻¹ (FDR group) or over 30 minutes (Standard group). The cycle was repeated every 21 days. Hematologic toxicity and other side effects were evaluated after each cycle.

Result
13 of 25 patients received FDR treatment and finished 28 cycles; others (12 of 25 patients) received standard treatment and finished 32 cycles. All patients were able to be evaluated for hematological toxicity. Effects of myelosuppression were compared between two groups (FDR and Standard groups). Our data showed that the III/IV degree inhibition of leukocyte was significantly different between two groups (14.3% versus 0.0%, p<0.05). No significance was observed between two groups about inhibition of III/IV degree neutrophil, platelet and hemoglobin (14.2% versus 3.1%, 10.7% versus 3.1%, 3.6% versus 9.4%, p>0.05, respectively), no IV degree of hemoglobin inhibition was caught in whole patients.

Conclusion
Hematologic toxicity with fix dose rate gemcitabine infusion is tolerated in the treatment of malignant tumors. However, to make a further conclusion as to hematologic toxicity of fix dose rate infusion of gemcitabine, although APBSCT supporting is not needed at gemcitabine FDR regimen but maybe one of the choices in higher dosage treatment, further and more clinical trials will be needed.

No conflict interest to disclose
Place of Residence and Socioeconomic Status Do Not Influence Survival of Patients with Diffuse Large B-cell Lymphoma (DLBCL) in Queensland

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Aim
Recent epidemiologic studies have shown that survival rates for patients with breast, lung and prostate carcinoma are lower in rural areas compared with urban areas in Australia. We performed a population-based analysis to see if this effect applies to adults with diffuse large B-cell lymphoma in Queensland.

Methods
We compared survival outcomes during a six year period from Jan 2000 to Dec 2005. Data was abstracted from a single consolidated statewide cancer database called the Queensland Oncology Repository. Residence was classified as either urban or rural following a modified Rural, Remote and Metropolitan Areas (RRMA) classification system. Socioeconomic status was based on the 2006 Socio-economic Indexes for Areas (SEIFA) Index of Relative Socio-economic Disadvantage (IRSD) (Australian Bureau of Statistics, 2008). The effect of rurality and socioeconomic status on survival was assessed by the log-rank test as well as through multivariate proportional hazards regression controlling for demographic variables, comorbidities and introduction of rituximab on the Australian Pharmaceutical Benefits Scheme schedule (1 July 2003 for patients ≥ 60yrs and 1 April 2005 for patients < 60yrs).

Results
Of 1210 patients with DLBCL, 54% were male and 66% were aged greater than 60. The median SEIFA-IRSD was 991. Of the 344 (28%) patients living in rural areas, the 2-year overall survival rates were 58% in the pre-rituximab era and 67% in the post-rituximab era, while the rates in urban residents were 60% and 70% respectively. On multivariate analysis, only age > 60 years (HR 2.39; p <0.001) and the presence of one or more comorbidities (HR 1.76; p<0.001) were associated with poorer survival; neither lower socioeconomic status nor area of residence (urban vs rural) significantly affected this outcome. Not surprisingly, the 2-year survival was significantly better introduction of rituximab significantly improved 2-year survival in this population (HR=0.69; p<0.001) after the introduction of rituximab.

Conclusions
In contrast to some solid organ malignancies, survival outcomes for patients with diffuse large B-cell lymphoma in Queensland are not affected by socioeconomic status or rural area of residence.

No conflict of interest to disclose
P248
A Retrospective Analysis of Methotrexate-based Combined Modality Therapy With or Without Rituximab in Primary Central Nervous System Lymphoma

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Background
Most cases of primary central nervous system lymphoma (PCNSL) are histologically identical to nodal diffuse large B-cell lymphoma (DLBCL). The addition of the CD20 monoclonal antibody rituximab to conventional cytotoxic chemotherapy in nodal DLBCL has demonstrated improved survival. There is limited data on the ability of rituximab to cross the blood-brain-barrier and the effect of the addition to chemoradiotherapy in PCNSL is unknown.

Aim
To determine the impact of the addition of rituximab to chemoradiotherapy in the treatment of PCNSL.

Methods
A retrospective analysis of patients with PCNSL treated by the haematology department at our institution was undertaken. Patient records were reviewed to obtain baseline characteristics, treatment and outcome data. The log-rank test was used to compare progression-free survival (PFS) and overall survival (OS) Kaplan-Meier curves for patients treated with and without rituximab.

Results
Between 2000 and 2011, 33 patients were identified with PCNSL. All cases were histologically confirmed as DLBCL and none were associated with positive HIV serology. 2 patients had less than 1 month follow-up post completion of therapy and were not included in the analysis. Median age 62 (range 42-85). All patients received methotrexate-based chemotherapy and 28 patients received radiotherapy. 52% (16/31) of patients received rituximab. Median follow-up time is 28 months (range 3-111). PFS in the rituximab group was 37 months compared to 35 (p=0.74). OS in the rituximab group was 45 months compared to 66 (0.94).

Conclusion
In this retrospective analysis, rituximab did not significantly influence PFS or OS when combined with chemoradiotherapy for PCNSL. Prospective analysis of the role of rituximab in PCNSL is required.

No conflict of interest to disclose
P249
IT meets MDT: Developing a Streamlined Operating Procedure and Proforma for Quality Peer Review

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Aim
To develop an efficient process for quality multidisciplinary peer review of patients with haematological malignancies.

Methods
Since August 2010, a haematology specific Standard Operating Procedure (SOP) and proforma have been under continuous development for the weekly Multidisciplinary Team meeting (MDT). Individual patients’ diagnosis, stage, prognostic factors (+/- response assessment) and proposed management plan are submitted electronically by the treating physician/haematology registrar. Care coordinators, pathologists and radiologists are alerted to the patient to be presented. During each meeting, the patient proforma is displayed on an audiovisual screen concurrent with imaging and pathology. Diagnoses and management plans are discussed and verified in real time. A PDF proforma is sent to the GP within 48 hours.

Results
Feedback at each meeting has enabled continuous process improvement to optimise efficiency. The current proforma format includes drop down menus, which facilitate accurate data entry, and provide disease specific staging criteria and prognostic scores. Improved proforma layout facilitates rapid absorption of patient data by all participants. We have successfully developed and can demonstrate a streamlined process for documented quality peer review.

Discussion
In achieving its primary aim of quality peer review this more structured MDT process has been welcomed by team members. Additional benefits include: less repetition of patient discussion across forums, improved patient records and GP correspondence, improved data quality for the NSW Cancer Registry, streamlined clinical trials screening, Medicare billing, with notable patient appreciation of peer review of their treatment, an improved teaching environment for junior medical staff, and a platform for ongoing audit. Planned future developments include a Webinar for PET-CT review; posting the verified MDT proforma on the electronic medical record; and collaboration with the IT department to develop a proforma that both maintains functionality and is downloadable directly to a database.

No conflict of interest to disclose
P250
CHOP Chemotherapy Plus Rituximab Compared With CHOP Alone in Patients With DLBCL CD 20 (+). A 6-Year Study at the Hematology and Blood Transfusion Hospital, Ho Chi Minh City Vietnam

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Aim
Diffuse large cell Lymphoma (DLCL) CD 20(+) responds generally well to initial treatment, but has a high rate of treatment refractory relapse and 5 years after initial therapy with the classic CHOP regimen, survival is only about 40%. In recent years, adding monoclonal antibodies in the treatment has lead to a spectacular improvement in the survival and quality of life of cancer patients. Rituximab is now a standard part of the treatment of DLCL CD20 (+). We conducted a randomized trial to compare CHOP plus Rituximab with CHOP alone in patients with DLCL CD20 (+)

Methods
Randomized controlled clinical trial (RCT): Patients over 16 years old, diagnosed of DLCL with CD20(+), stage II or higher (Ann Arbor), divided in 2 arms, one with and one without rituximab.

Results
From 2005 to 2011, we studied 40 cases DLCL CD20(+), 22 to 57 years old. There are 20 cases treated with CHOP + rituximab (50%) and 20 cases with CHOP alone (50%). Patients received 6 to 8 cycles of R-CHOP or CHOP, 21-day interval. The rate of complete response was significantly higher in the group that received CHOP + rituximab than in the group that received CHOP alone (85% vs 65% respectively). With a median follow-up of six years, 4 events (relapse) were observed in the CHOP+rituximab group and 9 in the CHOP group (in 20% and 45% of patients, respectively). DFS and OS are significantly higher in the CHOP+rituximab group (p<0.03 and p<0.04, respectively). Grade 1 or 2 adverse events related to rituximab were observed in 4 patients in the CHOP+rituximab group (20%), the most frequent of them being chill, fever, hypertension and hypotension. Our study had no HBV, HCV, CMV reactivation.

Conclusion
The addition of rituximab to the CHOP regimen increases the complete response rate and prolongs DFS and OS in patients with DLCL CD20 (+), without a significant increase in toxicity.

No conflict of interest to declare
P251
Pericardial Administration of Rituximab in a Patient with Cardiac Tamponade in the Course of Marginal Zone Lymphoma (MZL) - A Case Report

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A 85-year-old woman was admitted in May 2008 because of dyspnoea caused by fluid in the left pleural cavity. Cells present in this fluid have been found to have phenotype typical for MZL. No other foci of disease have been found at this time. Following four cycles of CVP the patient obtained CR. In the control PET/CT performed in October 2008, the uncharacteristic tracer accumulation in projection of the rear wall of the left ventricle was found. In January 2009 the patient suffered from chest pain, shortness of breath and oedema. The blood gas analysis revealed respiratory failure, ECG - atrial fibrillation and low voltage of QRS wave. ECHO showed the fluid (45mm) in the pericardium. The compression of the right heart cavities was observed. The patient underwent pericardiocentesis. 1000ml of bloody fluid was evacuated. Phenotype of cells present in this fluid was the same as earlier found in pleural cavity. Two days later 400 ml was removed from right pleural cavity and the cells present there had again the same MZL phenotype. Rituximab 100 mg in 50 ml 0.9% NaCl for 2 hours infusion to the pericardium and systemic chemotherapy CVP i.v. were administrated. The control ECHO showed no presence of fluid in the pericardial cavity. Mid February 2009, because of the fluid observed in the right pleural cavity visualized on chest X-ray, the pleural puncture was performed. The intrapleural therapy of Rituximab 400mg in 100ml 0.9% NaCl for 60 min. and systemic immunochemotherapy R-CVP were administered. The systemic chemotherapy 4xR-CVP was continued as well as the final treatment with 5 x Rituximab 375mg/m2 i.v every four weeks. The treatment was successfully finished in December 2009. The complete remission of lymphoma has been maintained until June 2011 and continues.

No conflict of interest to disclose
Bortezomib-based Combination Therapy for Multiple Myeloma in Southern Vietnam

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Aim
To investigate the efficacy and toxicity of bortezomib based combination therapy for Vietnamese patients with multiple myeloma (MM), and to determine the combination regimen, dosage and cycles in application of bortezomib for MM therapy.

Methods
Thirty patients with newly diagnosed, refractory or relapsed myeloma were treated with bortezomib (1.3 mg/m2) as an intravenous bolus twice weekly for 2 weeks on days 1, 4, 8, and 11 in a 3-4 week cycle, in combination with dexamethasone, dexamethasone plus thalidomide, or melphalan plus prednisone. Response to bortezomib was evaluated according to the criteria of the International Myeloma Working Group (IMWG) before initiation of each cycle. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0. Thirty matched patients who received melphalan plus prednisone and thalidomide (MPT) therapy were used as a historical control group.

Results
Among 28 of the 30 patients who could be evaluated, the overall response rate was 71.4% (the control group was 30%, P < 0.05), including complete response in 5 patients (17.8%), very good partial response in 10 patients (35.7%), and partial response in 5 patients (17.8%). The overall response rate after two and three cycles was 35.7% and 57.1% (P < 0.05), respectively. The frequent adverse events were thrombocytopenia (46.4%), neutropenia (50%), constipation (25%) and peripheral neuropathy (35.7%); all of the events could be tolerated. The most common adverse event in the control group was neutropenia (70%) and fatigue (60%).

Conclusions
Bortezomib based combination therapy is a new effective therapy in myeloma patients with a higher response rate and different toxicities than MPT group.

No conflict of interest to disclose
P253
Reduced-Intensity Allogeneic Stem Cell Transplantation in Older Patients with Acute Myeloid Leukaemia

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Aim
In patients with acute myeloid leukaemia (AML) older than 55 years, long-term survival rates after chemotherapy alone are typically 10-15%, while conventional allogeneic stem cell transplantation (allo-SCT) poses unacceptable risks of morbidity and mortality. We conducted a retrospective review of older AML patients undergoing allo-SCT using reduced-intensity conditioning (RIC), in order to assess tolerability and efficacy.

Method
Sixteen patients transplanted between September 2008 and October 2010 were included who were preferentially more than 55 years of age and in CR1 at the time of transplantation. The median age was 60 years (range, 42-66). Eight had a matched related donor (MRD), and 8 a matched unrelated donor (MUD). Conditioning consisted of fludarabine (30 mg/m² IV [n = 15] or 48 mg/m² PO [n = 1] for 3 days) and low-dose TBI (2 Gy on day 0). Patients were preferentially managed in an outpatient setting.

Results
The median follow-up time was 13.1 months (range, 3.5-28.3). Acute GVHD (Grade 2-4) occurred in 5 patients (31%). Chronic GVHD occurred in 7 patients (44%). There was one case of graft failure with autologous count recovery; otherwise, engraftment was robust, with median CD3+ chimerism at 3 and 6 months of 89% and 99%, respectively.
Treatment-related mortality occurred in 1 patient (6%). Relapse was responsible for 4 of 6 deaths. OS at 1 and 2 years was 74% and 59%, respectively, and the median OS was 28 months. Progression-free survival (PFS) at 1 and 2 years was 61% and 30%, respectively, and the median PFS was 22 months.

Conclusion
We have demonstrated that RIC allo-SCT for older patients with AML can be delivered safely in an outpatient setting, with a low rate of treatment-related mortality. There may be superior survival when compared with historical data for such patients treated with standard chemotherapy, suggesting a significant graft-versus-leukaemia effect.

No conflict of interest to disclose.
Detection of Chromosomal Aberrations in Multiple Myeloma by PCR – An Institutional Study

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Aim
This study explores the usefulness of PCR methodology to test for common genetic aberrations in multiple myeloma (MM). They include t(11;14), detected by the overexpression of cyclinD1 gene¹ (CCND1), t(4;14) and mutation of the TP53 gene. The clinical and biological significance of patients stratified according to the PCR results were evaluated.

Method
Sixty-six MM patients who required a bone marrow biopsy at our institution were included in this study. All genetic abnormalities were performed using cDNA generated from purified plasma cells; whole marrow was used when plasma cells were >80%. Real-time PCR (qPCR) was used to detect for CCND1 and t(4;14), while TP53 mutation was detected by conventional PCR. Statistics analysis was performed by Graph Pad Prism 5, a p value of <0.05 was considered significant.

Results
CCND1 was overexpressed in 8 patients, 3/8 being also FISH positive, 5/8 has no FISH performed. Two of five patients were t(4;14) positive by PCR, one of those was also FISH positive. Based on our PCR results, the prevalence of the genetic lesions t(11;14), t(4;14) and TP53 mutation in our patient cohort was 12.5%, 7.5% and 22.7% respectively. Patients with TP53 mutation have significantly higher levels of creatinine and b-2 microglobulin (p=0.048 and 0.024 respectively) compared to the relatively normal group. Patients with no genetic abnormalities have less aggressive diseases at diagnosis when compared to those who have a mutation (p=0.004). Patients with TP53 mutation have a significantly shorter progression-free survival compared with those who have no genetic lesions (18 months vs 56 months, p=0.03).

Conclusion
We have employed PCR to detect chromosomal translocation t(11;14), t(4;14), and TP53 mutation at cDNA level. There was a relatively high prevalence of TP53 mutation in our patient cohort. TP53 mutation is associated with less favourable clinical and biological features compared to the other groups that were investigated.

No conflict of interest to disclose

Presentation with Concurrent B-Cell Lymphoproliferative Disorder and Plasma Cell Myeloma – A Report of 3 Cases

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Background
The concomitant diagnosis of plasma cell neoplasm and lymphoproliferative disorder in the same patient is quite rare, with isolated cases described. The relationship of the two disorders to each other in terms of clonality is variable in described cases. We report 3 cases of concurrent presentation with myeloma and a B-cell lymphoproliferative disorder.

Cases
Case 1: A 72 year-old man presented for investigation of mild peripheral blood lymphocytosis. Flow cytometry revealed this population to be kappa restricted and positive for CD5, CD19, CD20, CD23 consistent with chronic lymphocytic leukaemia/small lymphocytic lymphoma. Further workup demonstrated a lambda bence jones protein of 6g/L. Imaging studies revealed no lymphadenopathy and a single lytic lesion. Bone marrow examination demonstrated concomitant bone marrow involvement with plasma cell myeloma and a B-cell lymphoproliferative disorder.

Case 2: An 84 year-old woman was referred for investigation of the incidental finding of an IgG lambda paraprotein (13g/L). Imaging studies were normal. She proceeded to bone marrow examination which demonstrated several paratrabecular and interstitial aggregates of small lymphocytes. There were scattered interstitial plasma cells accounting for 15-20% of cells. Plasma cells were lambda restricted. Flow cytometry detected a population of clonal B cells; CD19, CD20, CD23, kappa.

Case 3: A 76 year-old woman was investigated for an IgM lambda paraprotein (12g/L), found incidentally during workup for acute renal failure (unrelated cause). Imaging studies were normal with no lymphadenopathy or lytic lesions. Bone marrow examination revealed a modest plasma cell infiltrate and several moderately sized interstitial and paratrabecular lymphoid aggregates composed of small to medium sized mature cells, with abnormal nuclear folding. The immunophenotype was suggestive of follicular lymphoma.

Conclusion
We have described three cases of concurrent bone marrow involvement with a plasma cell neoplasm and B-cell lymphoproliferative disorder. In 2 of the 3 cases there is evidence the two disorders have a separate clonal origin, indicated by disparate light chain restriction.

No conflict of interest to disclose
P256
Carfilzomib and Dexamethasone Therapy in a Patient with Relapsed Refractory Multiple Myeloma: A Single Case Study

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Background
Carfilzomib is a next generation, selective, irreversible proteasome inhibitor that has demonstrated single-agent activity in patients with relapsed and or refractory multiple myeloma (MM). Patient X is a 55 year old man with multiple relapsed and refractory MM. Diagnosed in 2006 he has received multiple lines of therapy. At baseline Patient X presented with significant bone pain, pancytopenia, existing peripheral neuropathy and with clinical hyperviscosity requiring plasma exchange.

Aim
Deliver a regimen of Carfilzomib and dexamethasone to Patient X and obtain a response with minimal toxicity.

Methods
Ethics approval was received prior to administration of Carfilzomib. Patient X received Carfilzomib on days 1,2,8,9 and 15,16 of a 28-day cycle, with a total of 12 cycles of Carfilzomib and dexamethasone received to date. Dexamethasone was given at 4mg on days of Carfilzomib therapy and increased to 40mg (D1-4) from cycle 9 in response to a rise in PP level. Carfilzomib was delivered by intravenous infusion over 30mins at a dose of 20mg/m2 (40mg) and increasing to a dose of 36mg/m2 (72mg) by beginning of cycle 2. Anti-varicella prophylaxis was given due to increased risk of zoster associated with treatment with proteasome inhibition.

Results
Patient X received his 1st cycle of therapy as an inpatient and subsequent cycles as an outpatient. Three readmissions for short durations during outpatient treatment, twice for IV hydration and once for IV antibiotics. He achieved Partial Response (PR) after 4 cycles of therapy. No grade 3 or 4 toxicities or worsening of baseline neuropathy was observed. Additionally, no dose reductions due to toxicities were made. Patient X became blood product independent by end of cycle 4.

Conclusions
A sustained response was seen over 12 cycles to date. No significant toxicity has been demonstrated. Carfilzomib and dexamethasone was an effective and well tolerated therapy for Patient X with R/R MM.

No conflict of interest to disclose (Onyx Pharmaceuticals supplied Carfilzomib for patient X)
A Case of Multiple Myeloma in association with Vanishing Bile Duct Syndrome

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Vanishing bile duct syndrome (VBDS) refers to a group of acquired disorders associated with gradual destruction and disappearance of the intrahepatic bile ducts leading to cholestasis. Acquired forms have been reported in association with several haemopoietic disorders including Hodgkin lymphoma, histiocytosis X and chronic graft versus host disease. We report a case of VBDS in association with multiple myeloma (MM), which to our knowledge has never been described.

A 59 year-old male presenting with bone pain was diagnosed with IPSS stage 2 MM. Bone marrow biopsy demonstrated 60-80% plasma cells with areas of vascular amyloidosis. Pelvic CT revealed extensive bony disease necessitating prophylactic pinning of both femora and radiotherapy. Serum protein electrophoresis revealed mild immunoparesis with no paraprotein, however kappa free light chains were elevated at 139mg/L – with an abnormal kappa:lambda ratio of 7.90. There was no anaemia, hypercalcaemia or renal impairment.

Prior to commencing chemotherapy, he was noted to be jaundiced with cholestatic liver function tests (ALP 543U/L; GGT 249U/L; Bilirubin 281umol/ml). Ultrasonography and MRCP excluded extrahepatic biliary obstruction. Liver biopsy demonstrated marked canalicular cholestasis with small or absent bile ducts consistent with VBDS. There was amyloid deposition in the hepatic arteries, but none in the sinusoids. The pro-BNP was elevated suggestive of subclinical cardiac involvement however there was no evidence of amyloid at other sites.

Treatment was commenced with cyclophosphamide, thalidomide, dexamethasone and zoledronic acid resulting in a partial response with normalisation of bilirubin after four cycles. Autologous stem cell transplant is planned in the near future.

We believe this is the first reported case of VBDS in association with MM. Furthermore, the temporal association of VBDS and myeloma and the improvement with treatment in this case suggests causality. We speculate that immune responses to light chain deposition may play a role in VBDS pathogenesis.

No conflict of interest to disclose
P258
CNS Multiple Myeloma – A Multicentre Experience of a Rare Manifestation

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CNS involvement is a rare complication (1%) of multiple myeloma (MM) with a dismal prognosis (median survival 2 months). There is no consensus on treatment and anecdotally, its prevalence appears to be increasing in the era of novel therapy.

Aim
To describe clinical features, response to therapy and overall survival (OS) of patients with CNS MM.

Methods
Review of patient records from 1/2000-5/2011 at 4 tertiary referral hospitals identified 17 cases of CNS MM defined by monoclonal plasma cells in CSF and/or radiological evidence of cerebral parenchymal plasmacytoma. A retrospective analysis of clinical and treatment data was performed.

Results
Median age at initial diagnosis was 58 (41-70) years. Seven patients had ISS stage III disease, two presented with non-CNS extramedullary disease, and 3/10 evaluable had unfavourable cytogenetics. Lambda light chain restricted cases were predominant (11/17). Patients received a median of 3 prior therapies (1-4): autograft (n=16), allograft (n=2) and at least one of thalidomide (n=14), lenalidomide (n=4) or bortezomib (n=8). Median time to diagnosis of CNS MM was 36 months (1-114). Eight patients had concomitant progressive systemic disease, two were in CR. The most common clinical presentation was cranial nerve palsy (n=7). All patients received combinations of radiotherapy (RT) (n=12), intrathecal (IT) chemotherapy (n=8), systemic chemotherapy (n=3) and/or novel agents (bortezomib n=2, thalidomide n=5). Median OS from initial diagnosis was 47 (12-124) months. Survival from diagnosis of CNS MM was 4 (1-23) months. IT chemotherapy had superior OS [20 months vs. 2 months (p=0.02)]. There was no improvement in OS for RT (p=0.9). CSF clearance was documented in 2/5 patients who had positive CSF cytology and IT chemotherapy. Only 2 patients were alive at time of report (5, 10 months post-CNS MM diagnosis). Both received IT chemotherapy and bortezomib.

Conclusion
Little is reported on the impact of novel agents on CNS MM. IT chemotherapy and bortezomib may be of benefit in selected cases; larger prospective collaborative studies are required to test this observation.

No conflicts of interest to disclose.
P259
Plasmablastic Myeloma is Highly Associated with Increased CD45 Expression Compared to Morphologically Mature Multiple Myeloma

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Aim
The majority of cases of myeloma show aberrantly decreased CD45 expression compared to normal plasma cells. However, subpopulations of myeloma cells exist with bright CD45 expression which has been hypothesised to represent a distinct immature proliferating plasma cell compartment. Plasmablastic myeloma (PM) is characterised by immature plasma cell morphology and an aggressive clinical course, however, no studies to date have specifically assessed CD45 expression in PM. Our aim was to assess the difference in CD45 expression by flow cytometry between PM, myeloma with mature morphology (MM) and normal controls (NC).

Method
Cases of PM with available flow cytometry data were identified from an institutional database and were compared with randomly selected cases of MM from the same database. The morphological classification (PM/MM/NC) was confirmed by histopathological review by two haematopathologists (DW & NC). Cases were categorised as PM if plasmablasts accounted for at least 20% of total plasma cells. Samples had been analysed by quantitative four colour flow cytometry on a FACSCalibur flow cytometer (BD Biosciences). Malignant plasma cells were gated on using a CD138 vs. side scatter strategy with clonality confirmed on immunophenotyping using cytoplasmic light chain expression.

Results
17 cases of PM were compared to 26 cases of MM and 5 NC. The results are summarised below:

<table>
<thead>
<tr>
<th></th>
<th>Plasmablastic myeloma n=17</th>
<th>Mature myeloma n=26</th>
<th>p value (PM vs. MM)</th>
<th>Normal controls n=5</th>
<th>p value (MM vs. NC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45 Mean Median Fluorescence Intensity (MFI)</td>
<td>37.1</td>
<td>1.0</td>
<td>p=0.005</td>
<td>5.0</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Mean coefficient of variation of CD45 expression</td>
<td>155.2</td>
<td>422.7</td>
<td>p&lt;0.001</td>
<td>136.3</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusion
Plasmablastic myeloma shows significantly brighter and more homogenous CD45 expression than morphologically mature multiple myeloma. This previously undescribed phenomenon provides support for the hypothesis that CD45 expression in myeloma is associated with a more immature and proliferating plasma cell compartment.

No conflicts of interest to disclose
P260
Pre-existing Diabetes in Patients with Multiple Myeloma

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Aim
Multiple myeloma (MM) is prevalent in the elderly and type 2 diabetes is occasionally present at diagnosis. There are few studies regarding the impact of pre-existing diabetes on MM patients compared with other cancers.

Method
Newly diagnosed MM patients in Taipei Veterans General Hospital between 1999 and 2007 were enrolled and those with pre-existing diabetes were identified. The impact of pre-existing diabetes on MM patients was evaluated by comparing clinical features, treatments, and adverse reactions related to glycemic control and overall survival of patients with and without pre-existing diabetes.

Result
Out of 310 MM patients, 73% were male and 40 patients (12.9%) had pre-existing diabetes. Compared with their non-diabetic counterparts, MM patients with pre-existing diabetes had a significantly higher proportion of renal impairment (RI, serum creatinine >2.0 mg/dL), elevated serum beta-2 microglobulin (>5.5 mg/L), and International Staging System stage III at diagnosis and a significantly lower proportion of bisphosphonate use. During the course of the disease, the method of glycemic control was changed in 10 (29.4%) of 34 evaluable patients. Hyperglycemia and hypoglycemia of any grade was noted in 23 (67.6%) and 6 (17.6%) of these patients, respectively. Although the rate of RI reversibility was not significantly different, patients with MM and pre-existing diabetes had a significantly higher all-cause mortality risk (hazard ratio [HR], 1.509; 95% CI, 1.023–2.225, \(P = 0.037\)) compared with their non-diabetic counterparts.

Conclusion
To our knowledge, our study is the first to show the impact of pre-existing diabetes on clinical features and overall survival of MM patients.

No conflict of interest to disclose
Multiple Myeloma, Plasmacytoma and Extramedullary plasmacytoma: Incidence Within Sydney South West Area Health Service from July 2005 to June 2009

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Aim

Multiple myeloma (MM), plasmacytoma (PC) and extramedullary plasmacytoma (EMP) account for the third largest group of Haematological malignancies within Sydney South West Area Health Service (SSWAHS). Our objective was to investigate and describe the demographics, initial treatment and outcomes for newly diagnosed MM, PC and EMP cases within SSWAHS.

Method

Data on all new patients with MM, PC, and EMP diagnosed between July 2005 and June 2009 were obtained from the SSWAHS Clinical Cancer Registry. All patients identified were diagnosed and/or treated in SSWAHS public facilities. Data collected included gender, age, country of birth, residence at diagnosis, socio-economic status, initial treatment and death.

Result

267 new cases were identified of which 51\% were male. The median age of the population at diagnosis was 62 years. 91\% percent of cases were MM, 7\% were PC and 0.7\% were EMP. Demographics revealed that 46\% percent of patients were born in Australia. Of the immigrant groups, Southern and Eastern Europeans (19\%) were the most prevalent and only 5\% of patients were born in Asia. The majority (32\%) of patients belonged to the 3\textsuperscript{rd} and 4\textsuperscript{th} socioeconomic quintile and 13\% of the population belonged to the highest socioeconomic group. There were a range of patient treatment options. Chemotherapy followed by autologous stem cell transplantation was used only in 22\% of the patients. 73\% of patients received chemotherapy alone. Approximately 19\% of cases did not commence therapy at diagnosis. The overall survival at 24 months was 75\% with 10\% of deaths occurring within 3 months of diagnosis.

Conclusion

Patterns of incidence, demography, therapy and outcomes identified are similar to other studies but also hold unique local characteristics. This will influence local clinical practice and health resource distribution. Ongoing epidemiological studies will shed insight into the aetiology of MM, PC and EMP.

No conflict of interest to disclose.
P263
The Efficacy and Safety of Velcade (Bortezomib) + Dexamethasone (VD) as Salvage Treatment in Relapse/Refractory Multiple Myeloma Patients: A Retrospective Analysis

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Objective
The aim of this study was to determine the efficacy and safety of combined bortezomib and dexamethasone as salvage treatment in relapse/refractory multiple myeloma (MM) patients.

Methods
Thirty-four patients from January 2006 to March 2011 were analyzed. Velcade (bortezomib) (1.3 mg/m²) and dexamethasone 40mg/d was administered intravenously on days 1, 4, 8, and 11. This treatment was repeated every 3 weeks. The primary end point was clinical response. The secondary end point was safety. The objective response was evaluated after 2 cycles of chemotherapy. Toxicity was assessed according to the National Cancer Institute common toxicity criteria (NCI-CTC) scale version 2.0.

Results
Among the 34 patients, 18 were male, 16 were female, with a median age of 68 years, and a median of 6 cycles per patient (range 1-8 cycles). 19 in international stage system (ISS) III, 14 in ISS II, 1 in ISS I. 25 received the planned 8 cycles treatment. After 8 cycles, the overall response rate (ORR) was 88% (complete response 24%, near-complete response 24%, partial response 40%). The median survival time was not achieved. The main toxicities were hematologic (58.8%), gastrointestinal (44.1%), peripheral neuropathy (41.1%), fatigue (32.3%), and were usually mild (grades 1, 2). The grade 3-4 toxicities were peripheral neuropathy (8.8%), neutropenia (5.9).

Conclusion
The VD regimen shows a very high ORR and complete response rate and tolerable toxicity profile in the treatment of relapsed/refractory MM patients. Velcade in combination with dexamethasone is a very effective salvage regimen for relapsed/refractory MM patients.

No conflict of interest to disclose
Low Dose Lenalidomide Induction Followed by Autologous Transplantation in Untreated Patients with Myeloma is Associated with High Response Rates

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Aim
Lenalidomide 25mgD1-21 with dexamethasone 40mg weekly is effective in first line and maintenance treatment of myeloma. Higher doses of lenalidomide and dexamethasone are associated with more side effects. We examined the response efficacy of low dose lenalidomide and dexamethasone followed by consolidation HSCT in LITVacc, a phase II study of Lenalidomide Induction, autologous haematopoietic stem cell Transplantation (HSCT) and adjuvant Vaccination with autologous dendritic cells (DC) loaded with autologous tumour cell lysate (ATCL) and lenalidomide maintenance.

Method
Subjects: Newly diagnosed HSCT eligible myeloma patients. Induction: Lenalidomide 15 mg days 1-21 and dexamethasone 20 mg weekly, 4x28 day cycles. HSCT collection: using cyclophosphamide 2-4 gm/m² and G-CSF 10mcg/kg/day. HSCT conditioning: melphalan 140-200mg/m². Maintenance: commenced D21-35 post HSCT, consisting of lenalidomide 25 mg D1-21(minimum12x28 day cycles) ± DC vaccination (D1 C1-6) (n=10).

Results
20 patients have completed induction, HSCT and commenced maintenance. Median age 57.5 range:female ratio is 3:1. IgG=10, IgA=6, light chain=4. Stage: ISS1=9, ISS2=9, ISS3=2. Median CD34+cells collected = 10.8×10⁶(4.9 – 40.6) over a median of 3(2-6) days. Median number of CD34+cells infused was 4.8×10⁶(2.9-11.5) with a median time to recovery of neutrophils and platelets of 12(9-18) and 11(8-25) days respectively. Post induction overall response(OR)=85%, partial response(PR)=65%, very good PR(VGPR)=20%, stable disease(SD)=5% and refractory disease(RD)=10%. Improvement post autograft to PR=35%, VGPR=40% and complete response (CR)=10%. At a median follow-up of 17(5-32) months, the best response achieved is PR=30%, VGPR=25% and CR=35% with median PFS and OS not reached. The best response was achieved post induction in 33%, post autograft in 33% and during maintenance in 33%. Presently 15(75%) patients continue on maintenance having received a median of 9.5(0-22) cycles.

Conclusion
Low dose lenalidomide and dexamethasone induction followed by HSCT in untreated myeloma patients is associated with high response rates. Depth of response is improved by HSCT and maintenance treatment with lenalidomide.

This research was supported by Celgene Corporation, 86 Morris Ave, Summit, NJ, 07901, USA. The company had no role in analysing the data or preparing the abstract.
**P265**

*Thoracic Extramedullary Plasmacytoma Causing Near Fatal Haemorrhage*

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**Case Report**

We report the case of a 51 year-old male with known multiple myeloma (MM) who presented in hypovolaemic shock after being found unconscious at home. Chest x-ray revealed a left sided haemothorax, which was confirmed on computerised tomography as a large complex heterogeneous mass in the left hemithorax with active extravasation of contrast from the eighth intercostal artery. An intercostal catheter (ICC) was inserted, draining 2800mL of blood. This was followed by a left exploratory thoracotomy that identified a 5cm x 7cm mass invasive to the chest wall and lung. This mass, identified as a plasmacytoma on pathological examination, was subsequently resected and required ligation of the three intercostal arteries (T4-7) adjacent to the tumour, as well as thoracic angiography for embolisation of T4 to T7 intercostal arteries. A further 2 ICCs were inserted intra-operatively, draining an additional 3000mLs. The patient was then transferred to intensive care where he received aggressive blood product replacement and inotropic support with noradrenaline. Within 24 hours our patient had improved, with minimal drainage from the ICCs, weaning of his noradrenaline and extubation. He was transferred to the ward 48 hours post surgery where he continued to improve.

**Discussion**

Extramedullary plasmacytoma (EMP) is a rare plasma cell neoplasm of soft tissue without bone marrow involvement and constitutes 3 percent of all plasma cell tumours. Of the patients diagnosed with MM, 7 percent have an EMP at the time of diagnosis, and a further 6 percent of patients will go on to develop EMP at a later stage in their disease. 80 percent of all EMPS occur in the upper respiratory tract (oronasopharynx and paranasal sinuses); however less common sites of involvement include the gastrointestinal tract, liver, lymph nodes, testes, skin, and central nervous system. Only 2 cases of pleural EMPS have been reported in the literature and there have been no reports of plasmacytomas infiltrating the surrounding vascular supply.

**Conclusion**

Although invasion into the vessel walls by a plasmacytoma is an extremely rare occurrence it is a possibility, as highlighted by our case. As such, possible haemorrhage as a complication of a plasmacytoma warrants consideration.

**References**


No conflict of interest to disclose
Gemcitabine and Vinorelbine: An Effective Salvage Regimen In Primary Refractory/Relapsed Aggressive Lymphoma – PeterMac Experience

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In the modern era, we are faced with individuals with DLBCL who relapse after standard frontline immunochemotherapy (R-CHOP). Except for those who have chemoresponsive disease and candidates for autologous stem cell transplant (ASCT), salvage therapy remains poor, with 2-yr PFS around 20-40%. Similarly, with Hodgkin lymphoma (HL) particularly in advanced and poor risk disease, relapse occurs in 30-40% within 5-10 years – with the standard approach being salvage chemotherapy followed by ASCT. Conventional salvage regimens, however, have excess toxicity, particularly in pre-treated patients, limiting their utility and rendering late toxicity. Equally patients, due to morbidity, are often ineligible for intensified therapy. New active regimens with acceptable toxicity profiles are needed. We present our experience utilising gemcitabine-vinorelbine.

Methods
Retrospective analysis of all patients with relapsed/refractory lymphoma who have received Gemcitabine-Vinorelbine at PeterMac from 2001-2011.

Results
30 patients (14 DLBCL, 6 HL, 1 MCL, 8 TCL, 1 FL) are included in the cohort, 16 male, median age 52 years (range: 25-82). Median number of prior lines of therapy 2 (range 1-6), 12 were post-ASCT. ORR was 39 %, CR 23 % and 43% had SD. Importantly, 5 patients were bridged to ASCT and 4 to allogeneic SCT, with 6 patients remaining in CR. Episodes of Grade III/IV neutropenia and thrombocytopenia were 25% and 33% respectively of a total 95 cycles of therapy, with 50% patients not experiencing either. 10 patients required platelet transfusions (total units 55) and 7 patients packed red cells (total units 64). The rate of febrile neutropenia was low (4%) and there was no documented Grade III/IV neurotoxicity or pneumonitis. The regimen was administered in the ambulatory setting for all cycles.

Discussion
These preliminary data demonstrate good activity of Gemcitabine-vinorelbine with relatively safe toxicity profile in relapsed/refractory aggressive lymphoma, providing an option for transplant ineligible and multiple relapsed/refractory patients.

No conflict of interest to declare
P267
The Role of ATF6 in the Mechanism of Action of Proteasome Inhibitor in Multiple Myeloma

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Background
The proteasome inhibitor, bortezomib is effective in some myeloma patients. However it is difficult to predict patient sensitivity as the subcellular mechanisms governing these processes are not well understood. Bortezomib inhibits the 26S proteasome and disrupts the unfolded protein response (UPR) which is regulated by XBP-1, ATF6 and PERK. Our preliminary results suggest that XBP-1, is predictive of response¹. At present there is little data on the role of ATF6.

Aim
To analyse ATF6 expression in vitro and patients and relate to response to bortezomib.

Method
1. Western blot of ATF6 and other regulators was performed on myeloma cell lines.
2. Proliferation inhibition assay.
3. Adaptation to bortezomib was associated with downregulation of ATF6.

Result
1. High ATF6 expression in cell lines is associated with bortezomib sensitivity
2. Adaptation to bortezomib was associated with downregulation of ATF6.
3. ATF6 mRNA levels are not predictive of clinical response.

Conclusion
ATF6 seems to be associated with response to bortezomib in cell lines. However, in the small cohort, ATF6 is not predictive of clinical response, suggesting that its role is modest relative to other UPR regulators such as XBP-1.

Reference


No conflict of interest to disclose
P268
Myeloma – Karyotype v’s iFISH and the Future

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Multiple myeloma (MM) is difficult to assess by routine karyotype due to low proliferation rate of plasma cells (PCs) in culture. PCs uniquely express CD138 surface antigen allowing for selection of CD138+ cells from bone marrow (BM) for targeted analysis.

Aims
- Validate new genomic profiling techniques for stratification of MM;
- Identify new, recurrent regions of genetic aberrations in MM PCs;
- Optimise FISH panels for MM diagnosis;
- Ultimately, revise prognostic indices for genetic changes in conjunction with other clinical parameters.

Methods
BM from 10 patients was selected on morphology for MM and sufficient PC content. Conventional karyotype was performed on unselected cultured BM cells. In addition, BM samples were enriched for CD138+ PCS and then: (1) A panel of MM-specific FISH probes were applied to investigate gain, loss or rearrangement of clinically relevant genomic regions. (2) Comparative genomic hybridisation array (aCGH) was performed on DNA using the 135k OncoChip™ to detect genome wide copy number changes. (3) aCGH was performed using MM-specific translocation array to detect recurrent, disease specific translocations.

Results
Abnormal karyotype was found in 1/10 patients, confirmed by FISH and aCGH. 5/10 patients showed concordance between FISH and aCGH, 5/10 patients showed an increase in sensitivity for FISH to detect specific abnormalities compared to aCGH.

Conclusion
CD138 enrichment is essential for study of MM. As expected, aCGH reveals more complex patterns of genomic aberrations; possibly indicating novel, recurrent genomic abnormalities. Utility of conventional karyotype may prove to be inferior compared to molecular cytogenetic techniques with higher resolution and sensitivity. These initial findings of copy number changes and future studies using translocation arrays are required for detection of known and novel translocations associated with MM.

This research was supported by SignatureGenomics. The company designed the OncoChip™ arrays and performed aCGH testing on the samples provided.
P269
High Cut-Off Extended-Hours Haemodialysis for the Management of Acute Kidney Injury From Myeloma Cast Nephropathy: Results From a Prospective Victorian Study

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Background
An early reduction in serum free light chains (FLC) may improve the renal outcome for patients with AKI from cast nephropathy due to multiple myeloma. A two-year prospective study of extended hours dialysis was undertaken using high cut-off (HCO) dialysers to improve FLC clearance in conjunction with chemotherapy.

Methods
A 2 year Victorian State Government New Technology grant was obtained by collaboration of 7 renal units in July 2009. Patients with multiple myeloma presenting with dialysis-requiring AKI (eGFR<10ml/min), biopsy proven cast nephropathy and high circulating FLCs were eligible for HCO dialysis. Initially, chemotherapy regimens were not mandated but later a standardised regimen containing bortezomib, dexamethasone and cyclophosphamide was recommended.

Results
27 patients newly diagnosed (n=26) or previously diagnosed (n=1) multiple myeloma presented with dialysis-requiring acute renal failure underwent extended hours HCO dialysis daily for 5 days then second daily for a total of three weeks of therapy. Mean age was 64.3 years (range 49-82), with a mean of 11.9 dialyses. Anti-myeloma therapy included various combinations of lenalidomide, thalidomide, cyclophosphamide and bortezomib in conjunction with steroids. Dialysis independence was achieved in 63% of patients (n=17) at a mean 35.5 days post treatment. There were two early deaths, and 8 who remained dialysis dependant. 17 out of 18 patients who achieved a more than 90% reduction in pre-dialysis abnormal FLC by end of HCO dialysis became dialysis independent. In contrast none of the remaining patients were able to cease dialysis. Most patients treated with a Bortezomib containing regimen achieved >90% reduction in pre-dialysis FLC by the end of the period of HCO dialysis. Of patients who did not receive any Bortezomib, only 3 patients achieved >90% reduction in FLC in the same time frame and there were 3 patients who failed to achieve a 50% reduction in FLC

Conclusions
94% of patients who achieved a greater than 90% reduction in serum FLC in the first 3 weeks of therapy became dialysis independent. Early use of highly efficacious anti-myeloma therapy appears to be essential. Further studies will help to elucidate the contribution of the HCO membrane dialysis in this approach.

No conflict of interest to disclose
P270
Treatment of Acute Renal Failure Secondary to Light Chain Secreting Disease with High Cut-Off Haemodialysis and Chemotherapy

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Background
Acute renal failure requiring renal replacement therapy is a frequent complication of myeloma, and approximately 85% of cases subsequently require long term haemodialysis. High concentration of free light chains (FLCs) in the patient’s sera causing cast nephropathy is the principle cause. Plasma exchange and conventional dialysis do not increase the likelihood of renal recovery. A new technique, high cut-off dialysis (HCO-HD), has been shown to remove significant quantities of light chains and data is emerging that when combined with effective chemotherapy, rates of renal recovery are increased.

Aim
To report a single centre’s experience of HCO-HD and chemotherapy in patients with acute renal failure due to light chain secreting disease.

Method
Retrospective analysis of the first four cases utilising HCO-HD at the Royal Perth Hospital. Data on serum light chain concentrations, renal function, disease response and survival were recorded.

Results
The four patients (52-74 years old) all had renal biopsy proven light chain nephropathy. Three patients had myeloma (two newly diagnosed, one relapsed) and one had a FLC secreting lymphoplasmacytic lymphoma. They received between 11 and 17 sessions of HCO-HD. Three were concurrently treated with Bortezomib based regimens, and one with VAD. Mean peak FLC levels were 14105 mg/l. Each session of HCO-HD caused a 20-92% fall in serum FLC, although they often rebounded to near previous levels by the next dialysis. Prior to the last HCO-HD, FLCs had fallen to a mean 34% of presenting levels. Following HCO-HD no patient became dialysis independent and none had achieved a complete remission. Two have died, 9 and 21 months after presentation.

Conclusion
Despite reduced FLC levels, HCO-HD and chemotherapy did not result in renal recovery in any patient in this small single centre sample. Factors accounting for this response rate include late presentation, high risk disease and poor response to drug therapy.

No conflict of interest to disclose
P271
Discordant Response to Novel Therapies in Multiple Myeloma: A Case of Multiple Clones?

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Introduction
Emerging data suggest that multiple myeloma (MM) is a polyclonal disease. Recently, whole-genome analysis of high risk-patients with MM has shown clonal diversity that is subject to the selective pressure of therapeutics, as well as the expected longitudinal evolution (Egan IMW 2011). Consequent implications include the requirement for multiple drugs to eradicate disease and re-emergence of drug-sensitive clones, even after previous treatment failures.

Case Report
We report the case of a 50-year-old man diagnosed with ISS stage I IgG lambda MM on a background of MGUS diagnosed 2 years prior. The patient achieved a partial response (PR) following CID induction and a MEL200 autograft. Progressive disease (PD) (paraprotein 8 to 14g/L) occurred after 1 month of thalidomide consolidation therapy with documented extramedullary (EM) disease (pleural plasmacytoma). The patient was treated with radiotherapy (20Gy in 5 fractions) and lenalidomide/dexamethasone (Rd) achieving a PR (paraprotein of 4g/L and lambda free light chains (LFLC) of 57.9mg/L) after 3 months (Figure 1).

Figure 1. Response of patient (Lambda FLC and Paraprotein) to therapy

By 4 months PD recurred with a rise in LFLC to 937.5mg/L (“light-chain escape”), bone pain and new EM disease (gum infiltration). Bortezomib, cyclophosphamide, dexamethasone and thalidomide – VCD-T was commenced. A rapid response occurred within 1 month with the LFLC falling to 33.3mg/L and resolution of symptoms. However, there was disease progression at 4 months (rise in both paraprotein and LFLC) and the patient was entered into a clinical trial (lenalidomide, dexamethasone and dasatinib) but died shortly afterwards.

Discussion
This case is illustrative of the emerging concept of polyclonal disease, in that a discordant response to therapy was observed. “Light chain escape” and EM disease occurred during treatment with Rd and responded to VCD-T, whereas the intact immunoglobulin component responded to prior Rd but recurred on VCD-T.

No conflict of interest to disclose
P272
Whole Body MRI Versus Technetium-99-sestamibi Bone Marrow Scan in Estimation of Bone Disease in Multiple Myeloma

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Background
Multiple myeloma (MM) is the most common cancer to involve bone, with up to 90% of patients developing bone lesions. The bone lesions are purely osteolytic in nature and do not heal in the vast majority of patients. Bone destruction in MM can involve any bone. Technetium-99-sestamibi was found to be more sensitive than skeletal survey in myeloma patients (77% vs 45%) and was highly specific for staging myeloma patients. Whole-body MRI (WB-MRI) is considered a method for high-resolution screening of the whole skeleton in patients with multiple myeloma. The aim of this study is to detect the rate and extent of skeletal events in myeloma patients on WB-MRI, Technetium-99-sestamibi compared to conventional x-rays for the staging and disease outcome of patients with newly diagnosed and existing multiple myeloma.

Methods
A cohort of 62 patients with confirmed multiple myeloma who presented to a single institution between January 2010-January 2011. The median age was 62 years (range 37.5-88) with male to female ratio of 33:29. All patients underwent a whole body MRI scan as well as sestamibi bone marrow scan

Results
Preliminary results showed that, in the skull, there appears to be no significant difference between the two scans, while in the cervical, thoracic and lumbar vertebrae, there appears to be a greater amount of tumour detected by the MRI scan compared to the sestamibi scan. In the pelvis and long bones, the differences are less clear, but the MRI may be detecting more tumour burden than the sestamibi scan. In the rib cage, the MRI scan was routinely uninterpretable in the ribs (but not the sternum), with more sensitivity to the sestamibi scan in this case.

Conclusion
Although overall, whole body MRI performed better than Technetium-99m-sestaMIBI bone marrow scan, combination between both scans increased the sensitivity in the detection of bone disease activity in multiple myeloma and should be considered in the staging system for myeloma.

No conflict of interest to disclose.
P273
A Case of Second Primary Malignancy Associated with Prolonged Thalidomide Maintenance Therapy following Autologous Stem Cell Transplantation for Multiple Myeloma.

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Case Report
We describe a 71 year-old woman who presents with symptomatic Stage IIIA IgA Lambda Myeloma. IgA paraprotein was 73 g/L with 65-80% CD 138 positive Plasma Cells on bone marrow examination. Initial therapy consisted of spinal radiotherapy with six cycles of VAD chemotherapy. Six months later the patient relapsed with rising IgA paraprotein (15 g/L to 45 g/L) and Thalidomide, 200mg nocte, was commenced with good effect. She subsequently proceeded to high dose Melphalan and Autologous Stem Cell Transplantation and achieved a near complete remission. Post transplantation, thalidomide continued, as the patient was unwilling to withdraw therapy.

Four and a half years later she developed progressive neutropenia without an increasing IgA paraprotein. At which time bone marrow examination demonstrated more than 90% CD 34 and myeloperoxidase positive blasts, consistent with Acute Myeloid Leukemia (AML). Thalidomide was ceased.

Her clinical course was further complicated by a fractured neck of femur, neutropenic sepsis and recurrent episodes of decreased levels of consciousness with focal myotonic twitches which were treated Sodium Valproate. Subsequently, the patient’s neutrophil counts recovered with bone marrow examination confirming a morphological remission of the AML with a normocellular marrow and normal haemopoeisis.

One year later, the bone marrow examination performed to assess an increasing IgA paraprotein demonstrated a relapse of Acute Myeloid Leukemia and a population of CD 138 positive Plasma Cells and died several months later.

Discussion
This patient developed a secondary primary malignancy following prolonged, high dose Thalidomide maintenance which spontaneously resolved with its withdrawal raising concerns about a potential causal relationship as raised with Lenalidomide. Furthermore, this case also questions the therapeutic role of Sodium Valproate in second primary malignancies.

No conflict of interest to disclose.
Relapsed Multiple Myeloma: Who Benefits from Salvage Autografts?

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Aim
Despite novel agents, multiple myeloma (MM) remains incurable, and the role of salvage autologous stem cell transplant (ASCT) is undefined. We aimed to evaluate the role of salvage ASCT in relapsed MM patients.

Methods
We performed a retrospective analysis of patients who underwent salvage ASCT for relapsed MM at our centre between 1992 and 2011.

Results
During this period, 292 patients received ASCT for newly diagnosed MM. Thirty patients subsequently underwent salvage ASCT for relapsed MM, using melphalan conditioning chemotherapy. The median age of patients at salvage ASCT was 59 (34-73) years and most patients (87%) were in ISS I and II at diagnosis. The median overall survival (OS) from diagnosis was 110 months (95% CI: 36-184), with the median progression-free survival (PFS) and OS following salvage ASCT being 22 (95% CI: 8-35) and 45 months (95% CI: 17-72) respectively. Non-relapsed mortality at 2 years was 7%.

Following salvage ASCT, the median PFS (31 vs. 8 months, p = 0.002) and OS (83 vs.15 months, p = 0.009) were significantly longer in patients who relapsed > 24 months following initial ASCT. Maintenance therapy following salvage ASCT and the use of novel agents in salvage induction did not influence outcome.

Conclusion
Salvage ASCT is a feasible therapeutic option in the setting of relapsed MM and may result in durable responses, particularly in patients with longer PFS following initial ASCT (> 24 months). This could be included as selection criteria. Our results suggest that even in the era of novel agents, salvage ASCT has a role in augmenting the benefits of these agents.

No conflict of interest to disclose
Iron Overload in Myelodysplastic Syndromes: An Audit of Management in a Sydney Hospital

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Aim
Patients receiving chronic red blood cell (RBC) transfusions for myelodysplastic syndromes are at risk of iron overload and subsequent complications including increased mortality. Non-randomised studies suggest that iron chelation therapy (ICT) may lead to increased survival in these patients. Uptake of iron chelation has been variable in other countries. An audit was conducted to see whether the haematology department of a metropolitan Sydney hospital was following current guidelines and how performance compared to overseas data.

Method
A retrospective audit was conducted at Royal North Shore Hospital in Sydney, collecting data from patients followed up at the hospital from December 2009 to December 2010. Inclusion criteria were those patients with a diagnosis of myelodysplasia or other severe anaemia receiving at least one RBC transfusion. Eligibility for ICT was considered as 20 or more units of RBCs transfused, or serial serum ferritin levels exceeding 1000 µg/L. Primary endpoints were the proportion of patients adequately assessed for iron overload and the proportion of eligible patients who received ICT. Secondary endpoints were the efficacy of ICT in maintaining serum ferritin below 1000 µg/L, and documentation as to why eligible patients were not chelated. The groups were compared using paired-t tests, Fisher’s exact test and Kaplan-Meier survival curves.

Results
Among the 51 patients meeting enrolment criteria, 25 (49%) were eligible for ICT. Of these, 12 (48%) received ICT and 24 (96%) were evaluated for iron overload with serum ferritin levels. From the patients receiving ICT, only 2 (17%) met current guidelines for efficacy. Of the 13 eligible patients not receiving ICT 6 (42%) had no documentation as to why chelation was not commenced. There was no significant difference in survival or transformation to acute myeloid leukaemia between chelated and non-chelated patients.

Conclusion
This audit finds that the uptake of chelation in this hospital is similar to that seen in overseas studies, though most eligible patients are not receiving ICT. Further studies are required to determine the survival benefit of iron chelation as well as how to best increase chelation utilisation.

No conflict of interest to disclose
P276
Towards Individualised Therapy for Myelodysplastic Syndromes

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Myelodysplastic syndromes (MDS) are characterised by ineffective haemopoiesis and increased leukaemia risk. Hypomethylating agents improve survival in high-risk MDS but responses vary. Patients likely to benefit from therapy are difficult to identify as factors that govern response are poorly understood. Novel therapies include histone deacetylase inhibitors (HDACi) and mTOR inhibitors (mTORi). The karyotype determined by metaphase cytogenetics (MC) is a powerful predictor of outcome in MDS. Single nucleotide polymorphism arrays (SNP-A) identify genomic changes that go undetected by MC, thus SNP-A plus MC improves prognostication. We hypothesised that combination therapy would deepen response over single agent therapy and drug sensitivity would correlate with the MC+SNP-A karyotype.

Aims
(1) To determine sensitivity to decitabine (hypomethylating agent), sodium valproate (HDACi), rapamycin (mTORi), combination decitabine/rapamycin and combination sodium valproate/rapamycin (2) to perform SNP-A (3) to identify factors that correlate with drug response.

Methods
Bone marrow samples from 19 patients with MDS were identified. Cells were cultured in methylcellulose containing vehicle, decitabine, sodium valproate, rapamycin, decitabine/rapamycin or sodium valproate/rapamycin. SNP-A testing was performed using the CytoSNP-12 Beadchip from Illumina.

Results
Diagnoses were: RA (n=5), RCMD (n=12), RAEB-2 (n=1) and MDS with 5q- (n=1). 8/19 patients (42%) had an abnormal karyotype by MC. MC and the SNP-A karyotype were discordant in 5 cases. Colonies had dysplastic morphology and were derived from the MDS clone in cases where an informative karyotype was present. Decitabine suppressed colony formation in a patient with a karyotypic abnormality predicting sensitivity (monosomy 7) and failed to suppress growth in a patient demonstrating clinical resistance to azacytidine. Responses to single agent therapy were heterogeneous and improved by drug combination.

Conclusions
MC and SNP-A testing provide complementary information and suppression of colony growth is a valid drug sensitivity assay in MDS. Preliminary data suggest a potential role for hypomethylating agent/mTORi and HDACi/mTORi combinations.

No conflict of interest to disclose
P277
Antithymocyte Globulin in Patients with Aplastic Anaemia and Hypoplastic MDS

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Aims
To analyze the profile and outcomes of patients treated with antithymocyte globulin (ATG) for aplastic anaemia (AA) or hypoplastic myelodysplastic syndrome (MDS) in Singapore General Hospital (SGH) over the last thirteen years.

Method
A retrospective analysis of patients who received ATG for AA or hypoplastic MDS. Definitions of severity of AA and response criteria were as per International Study Group criteria. Main outcomes measured were overall survival, overall response and response to ATG at 3 and 6 months.

Results
From 1998 a total of 22 patients (12 females and 10 males) received 26 courses of ATG. Median age was 47.5 (16-70) years with a median follow up of 62 (range 1 - 150) months. Diagnoses of patients were as follows: non-severe AA 2 patients, SAA in 13, VSAA in 4, and hypoplastic MDS in 7 patients. Thymoglobulin® was used for 18 courses, Lymphoglobulin® 4 courses and ATGAM® 2 courses. Records of the type of ATG used in 2 patients were not available. 6 patients achieved a complete remission after a median of 27.5 months (range 9 – 44.5) from the last ATG dose. One of these patients achieved CR only after a second course of ATG (Thymoglobulin) was given. Seven patients achieved partial remission while 11 patients did not respond. Two patients passed away within 6 weeks of treatment from sepsis. The response rates at 3-months and 6-months were similar in all patients except for one patient who responded only after 6 months. Overall survival (OS) at 5 years was 77%. Four patients have undergone bone marrow transplant due to poor response to ATG.

Conclusion
In patients with AA and hypoplastic MDS without a transplant option, ATG remains an important treatment with potential for long-term remission. Overall response rate in our patient cohort was 59% with a 5-year OS of 77%.

No conflict of interest to disclose
P278
Experiences of Decitabine in Elderly Patients with MDS

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Background
Patients with myelodysplastic syndromes (MDS) are challenging to treat, given the advanced median age and comorbidities of the population. Decitabine, a hypomethylating agent that allows for the re-expression of tumor suppressor genes, represents a treatment option for MDS patients. In phase 2 and 3 studies, decitabine has been associated with durable responses in MDS patients and delayed time to acute myeloid leukemia (AML) transformation or death compared with supportive care. Decitabine has been shown to be well tolerated with a toxicity profile expected for this class of agent. However, the treatment of older patients ≥70 years seemed to be associated with increased toxicity in the first two courses. The delayed cycles of subsequent treatment might be caused by prolonged myelosuppression owing to the continuing presence of underlying disease and the adverse effect of decitabine. Fever-neutropenia occurred frequently (around one third) in patients who received decitabine.

Aims
Although decitabine has been associated with durable responses in MDS patients, the treatment of older patients >70 years seemed to be associated with increased toxicity in the early courses. There is a need to confirm the safety of decitabine in elderly patients.

Methods
We analyzed clinical data of 26 MDS patients age >70 years who received at least 3 cycles of decitabine.

Results
There were no significant differences in clinical outcomes of patients >70 years comparing with those of younger patients. Fever-neutropenia was observed in 15 patients (57.6%) and the majority of infectious complications occurred during the first two cycles.

Conclusions
Decitabine is effective to treat elderly patients with MDS, however, it is associated with an increased risk of infectious complications in the early period of treatment.

No conflict of interest to disclose
P279
Are Adverse Events Manageable in Patients with Myelodysplastic Syndromes Treated with Hypomethylating Agents?

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Aim
DNA hypomethylating agents (azacitidine and decitabine) are commonly used for the treatment of patients with myelodysplastic syndrome (MDS). In several studies, the adverse events were mainly hematologic abnormalities, most commonly neutropenia or thrombocytopenia. However, in large randomized trials hematologic adverse events, most frequently observed during early treatment cycles, decreased during subsequent cycles and were usually managed with dosing delays. We retrospectively analyzed the patients who discontinued the treatment due to adverse events.

Methods
We retrospectively reviewed the medical records of MDS patients with received hypomethylating agents. Between 2006 and 2011 clinical data were collected from Soonchunhyang university hospitals.

Results
Forty nine patients were included in this study and with data on all adequate for analysis. Twenty nine of 49 patients received azacitidine and 20 patients decitabine. The median number of cycles administered per patient was four (range, 1-14). A total of 23 (46.9%) patients received less than 4 cycles of treatment; 7 (14%) patients received more than six cycles. Fourteen patients (28.6%) discontinued treatment due to adverse events and 12 (12/14, 85.7%) patients received less than 4 cycles. Twelve of 14 patients died and 7 of 12 patients died during the treatment (within 20 d of the last treatment). Causes of death were infection with neutropenia (n=5) and bleeding associated thrombocytopenia (n=2).

Conclusion
Although the published experience suggested that cytopenias were manageable, we observed more severe hematological toxicites in our patients. We suggest that clinicians need to be vigilant for the onset, duration and management of adverse events.

No conflict of interest to disclose
P280
Purpura Fulminans in a Patient with Secondary Paroxysmal Nocturnal Haemoglobinuria (PNH)

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Introduction
Paroxysmal nocturnal haemoglobinuria (PNH) is associated with a range of both arterial and venous thrombotic complications. However, purpura fulminans (PF) has only rarely been reported in association with this disorder.

Case report
A 22 year-old male with prior severe aplastic anaemia (SAA) had known 2nd PNH. His 2nd PNH was characterized by moderate-severe thrombocytopenia (40-60x10^9/L) though normal Hb and WCC / differential. His 2nd PNH had never been associated with haemolysis, with normal serum haptoglobin / LDH. Multiple PB flow cytometry for PNH-associated surface markers demonstrated a stable clone affecting 100% of granulocytes / monocytes, ~30-40% of platelets, and variably 0-30% of erythrocytes (Type 2 cells only). The patient had never suffered any thrombotic complication prior to his presentation with a widespread palpable purpuric rash affecting his face and trunk ~2wks after suffering a non-specific viral URTI. He had also been commenced on the SSRI Lexapro at the time of his URTI. Investigations at presentation revealed (stable) thrombocytopenia, mildly elevated PT / APTT (18sec and 51 sec respectively), with mixing studies suggesting the presence of a factor deficiency. Protein C levels were mildly reduced (0.62 U/ml), and skin biopsy was histologically diagnostic of PF. Blood cultures, LFTs and vasculitic screen were all normal. The patient was treated with Vitamin K, daily FFP, platelet transfusions, Tazosin, prednisone (1mg/kg/day) as well as clexane 40mg BD SC (dose adjusted for platelet count). Over the next 7-10 days, his purpuric rash dramatically improved, and repeat protein C level increased to normal. Prednisolone was weaned rapidly on discharge, though Clexane continued for 4mths, 2mths post complete resolution of rash. 5mths post cessation of clexane the patient remains well, with stable (unchanged) FBC parameters and stable PNH clone on repeat flow cytometry.

Conclusion
Dermal venous thrombosis is a rare thrombotic complication of PNH. The precipitating factors of PF in this case are unclear but may have been the SSRI or the URTI.

No conflict of interest to disclose
P281
Utility of Flow Cytometry In Myelodysplastic Syndromes

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Introduction
Myelodysplastic syndrome is a heterogenous stem cell disorder associated with cytopenia and dysplastic features. Diagnosis can be difficult sometimes with equivocal morphology findings, absence of ring sideroblasts and normal cytogenetics.

Aim
To determine correlation of abnormal granulocyte maturation by flow cytometry with morphological findings

Methods
BM sample of patients with myelodysplastic syndrome (MDS) and control patients (non-myeloid disorder) are used. A panel of 9 antibodies are used and run using a 10 color flow cytometer (Beckman Coulter –Gallios). Below are the antibodies and dot plots used:

1) SS vs FS
2) SS vs CD45
3) CD13-PE vs CD34-PC7
4) CD117-PC5.5 vs CD71 FITC
5) CD11b-APC vs CD34-PC7
6) CD16-PB vs HLA-DR-APC H7
7) CD11b-APC vs CD13-PE
8) CD16-PB vs CD13-PE
9) CD16-PB vs CD11b-APC
10) HLA-DR-APC H7 vs CD11b-APC
11) CD13-PE vs CD45
12) HLA-DR – APC H7 vs CD34-PC7
13) CD11b-APC vs CD34-PC7
14) HLA-DR-APC H7 vs CD13-PE

Samples are then compared with template (performed on normal bone marrow donor) by pattern recognition and then classified as normal, borderline and abnormal.

Results
20 patients sample were run with a total of 17 MDS patient with 13 abnormal results, 2 borderline results and 2 normal results. Sensitivity 75%. All 3 controls have normal flow phenotype with a specificity of 100%. The two MDS sample with normal flow phenotype can be explained by the absence of the dysgranulopoiesis morphologically.

Conclusion
Flow cytometry may be useful as an additional diagnostic and supportive tool for the diagnosis of MDS.
No conflict of interest to disclose
P282
Efficacy of Antithymocyte Globulin and Cyclosporine Regimen for Aplastic Anemia

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Aim
Allogeneic bone marrow transplantation (BMT) is curative for the majority of transplanted patients with aplastic anemia, however it is difficult to apply this procedure because most of patients have no histocompatible sibling donor. Combined immunosuppressive regimen of Antithymocyte Globulin (ATG) and Cyclosporine (CSA) can achieve similar response rates to BMT. Since 2005, ATG has been available in Vietnam. We conduct this study to assess efficacy of this combined immunosuppressive regimen in Vietnamese patients.

Method
We performed a prospective non-randomized study at the Blood transfusion and Hematology Hospital in Ho Chi Minh City. Fifty two patients with any grade of severity aplastic anemia were treated with ATG and CSA. Criteria of evaluation were adapted from Camitta and Bacigalupo. We used the Fisher Exact test to compare categoric variables and the Student t test to compare continuous variables. The probability of survival was analysed using the Kaplan-Meier method. All statistical analyses were performed using Stata 10.0 software.

Result
The median age was 12.5 years (range, 1-57 years). At baseline, the median neutrophil count was 0.68 x 10^9/L (range, 0.05-5.3 x 10^9/L), median hemoglobin count was 83 g/L (range, 35-118g/L) and median platelet count was 12 x 10^9/L (range, 2-86 x 10^9/L). The response rate after six months from treatment is 47%. Trilineage hematologic recovery was seen in 66% patients after eighteen months from treatment, consisting of 38% complete response and 28% partial response. There were 34% patients who had no response. Among them, there were 12 patients died during treatment due to disease complications. The median follow-up duration of surviving patients was 31 months (range, 6-60 months). Overall probability of survival at sixty months is 74%. Up until now, there have been 5 relapses.

Conclusion
This study shows a high probability of aplastic anemia patients becoming transfusion independent and surviving with a good quality of life after treatment with ATG and CSA.

No conflict of interest to disclose.
The Combination Therapy of Azacitidine and the Oral Deacetylase Inhibitor (DACi) Panobinostat (LBH589) in Untreated MDS/AML: Planned Interim Analysis of a Phase Ib/II Study

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Background
The management options for patients with high-risk MDS or AML who are not eligible for intensive chemotherapy remain limited. The combination of hypomethylating agent and deacetylase inhibitor (DACi) has been shown to be synergistic, both in terms of leukaemia cell killing and gene reactivation in vitro.

Aim
To investigate the safety, tolerability and preliminary efficacy of combining the oral pan-DACi panobinostat (LBH589) with azacitidine in previously untreated MDS/AML patients, not fit for standard induction therapy.

Methods
Phase Ib/II multi-centre open label dose escalation and expansion study. Inclusion criteria: IPSS intermediate-2 or high risk MDS, or untreated AML not eligible for standard induction therapy. Patients received azacitidine 75 mg/m² SC on days 1-5 of each 28-day cycle with either 10, 20, 30 or 40mg panobinostat orally 3 days per week (M/W/F) for 7 doses per cycle commencing on day 5. The safety and tolerability of the combination was assessed.

Results
This preliminary analysis includes 23 patients (Male 16, Female 7), median age 70 years (36-81). 15 AML patients had intermediate (10/15) or poor cytogenetic risk (5/15); 8 MDS patients with intermediate-2 (7/8) or high risk (1/8) IPSS. Patients were enrolled into panobinostat cohorts of 10mg (4 patients), 20mg (7), 30mg (6) or 40mg (6). The principal dose limiting toxicity (DLT) was fatigue, as haematological toxicity was not considered dose-limiting. Grade 3/4 non-haematologic toxicities of cycle 1 are: 20 mg panobinostat cohort: fatigue (1 patient); 30mg panobinostat cohort: fatigue (1); 40mg panobinostat cohort: fatigue (3), syncope (2), hyponatraemia (1), and somnolence/decreased level of consciousness (1). There were no unexpected adverse events or drug reactions. Therefore, in combination with azacitidine, the maximum tolerated dose (MTD) of panobinostat was defined at 30mg; this dose level has been selected for expanded accrual. After a median follow-up of 247 days, the median OS is 239 days (22-472). The median number of treatment cycles initiated was 4 (1-16).

Conclusion
In previously untreated MDS/AML, azacitidine in combination with the pan-DACi panobinostat is tolerated and preliminary assessments demonstrate some clinical activity. Further evaluation of this combination is ongoing.
This research was supported by Novartis and Celgene.
P284
The Diagnostic Utility of Bone Marrow Biopsies Performed for the Investigation of Fever and/or Cytopenias in HIV-infected Adults at Groote Schuur Hospital, Western Cape, South Africa

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Aim
To determine the diagnostic utility of bone marrow biopsies performed for the investigation of fever and/or cytopenias in HIV-infected adults at Groote Schuur Hospital, Western Cape, South Africa.

Method
A retrospective review was conducted on consecutive bone marrow biopsies performed over a three year period on HIV-positive adults being investigated for fever and/or cytopenias at our institution. Clinical data, haematological parameters, morphological features, Ziehl-Neelsen staining and microbiological culture results were analyzed. Descriptive statistics were employed to define the study population. Overall diagnostic yield, yield according to indication, diagnostic yield for bone marrow histological examination and diagnostic yields for bone marrow and blood cultures were determined. Univariate analysis was used to assess the relationship between each predictor and diagnosis. Non parametric statistics were applied to determine a possible relationship between a unique diagnosis (yes, no), a diagnosis of tuberculosis (yes, no) and clinical parameters. Analysis was performed to establish the predictive factors for a diagnosis.

Results
Sixty-three males and 84 female patients were included. The bone marrow biopsy gave a high diagnostic yield of 47% (70 patients) and a unique diagnosis in 33% (49 patients). Immune thrombocytopenic purpura and disseminated mycobacterial infections were the most common unique diagnoses made (14% respectively), followed by malignancies (4%). In this cohort, 4 cases of primary bone marrow involvement by Hodgkin lymphoma and 1 case of involvement by non-Hodgkin lymphoma were diagnosed.

Conclusions
In our study group, a bone marrow biopsy was a useful investigation with a high diagnostic yield.

No conflict of interest to disclose
P285
Evaluation of the Effectiveness of a Single Dose of Rasburicase in the Management of Tumour Lysis Syndrome

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Aims
To evaluate the efficacy of a single 3mg dose of rasburicase in the management of tumour lysis syndrome (TLS).

Methods
A retrospective review of all adults receiving rasburicase between November 2007 and January 2011 was conducted. Medical files and pharmacy dispensing records were used to identify patients. Patient demographics, biochemistry, treatment and response to therapy were documented. Adherence to the local treatment guideline was also assessed.

Results
Forty-two patients were administered rasburicase 3mg, between November 2007 and January 2011. Adherence to the guideline was 95%.

The majority of patients were diagnosed with Acute Lymphocytic Leukaemia (32%), Acute Myeloid Leukaemia (29%) and Burkitt’s Lymphoma (24%).

19 patients (45.2%) were hyperuricemic, defined by uric acid above 0.46mmol/L, at baseline (range 0.47-2.0mmol/L). Of these, 14 required one dose and five required multiple doses. Twenty 23 (54.7%) patients had a normal uric acid (range 0.13-0.46mmol/L) but high risk factors for TLS. Of these, 20 patients required one dose and three patients required multiple doses.

In the 34 patients who required a single dose only, there was a decline in mean uric acid levels from 0.43mmol/L to 0.21mmol/L at 24 hours, with all patients having a normal uric acid level at 72 hours. No hypersensitivity reactions were noted due to the administration of rasburicase, and no patients required haemodialysis.

Conclusion
A single 3mg dose of rasburicase, in the setting of institutional guidelines, is efficacious in the management of TLS. A single dose provided an initial and sustained response as measured by uric acid levels. The appropriateness of the institutional guideline was reflected in the high adherence rate to this guideline.

No conflict of interest to disclose
Parvovirus Induced Pure Red Cell Aplasia in a Heart Transplant Recipient

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We report the case of a 29yo female who presented with unexplained anaemia to The Alfred Hospital 5 years post orthoptic heart transplantation for familial dilated cardiomyopathy. Over the preceding two weeks she described progressive lethargy, decreased exercise tolerance, and recurrent syncopal episodes. Clinical examination revealed no evidence of lymphadenopathy, hepatosplenomegaly or decompensated heart failure. Her immunosuppression regimen included tacrolimus and mycophenolate mofetil.

Her haemoglobin at presentation was 77g/L. The blood film demonstrated a normochromic normocytic anaemia with no evidence of haemolysis and a normal white cell differential. Her prior haematological parameters were entirely normal. Further investigation revealed normal iron, B12 and folate studies, normal haemolytic markers, markedly elevated erythropoietin (356 U/mL, normal range 2.5-18.5U/mL) and inappropriately marked reticulocytopenia (1x10⁹/L, 0.04%). Parvovirus serology for both IgG and IgM were negative. No clonal B- or T-cell population on flow cytometry and no radiological evidence of thymoma or lymphoma were identified.

A diagnostic bone marrow aspirate and trephine was performed following initial red cell transfusion support to further investigate the marked anaemia and reticulocytopenia. Bone marrow aspiration demonstrated markedly reduced erythroid precursors with atypical giant pronormoblasts. Immunohistochemistry for parvovirus B19 was positive on the trephine biopsy. Parvovirus DNA was subsequently detected in peripheral blood by PCR.

Following confirmation of the diagnosis of parvovirus induced pure red cell aplasia she was treated with intravenous immunoglobulin resulting in resolution of the anaemia.

Parvovirus B19 infection is a well-established cause of severe pure red cell aplasia in immunocompromised hosts. However, the utility of parvovirus serology (IgG and IgM) testing in this setting may be limited and care must be taken in result interpretation. We therefore recommend testing for parvovirus B19 DNA by PCR to confirm the diagnosis. Additionally, specific immunohistochemistry of bone marrow trephine specimens may aid in clarifying the diagnosis.

No conflict of interest to disclose
P287
Clinical Case Report: Successful Intravenous Immunoglobulin Therapy for Reactive Haemophagocytic Lymphohistiocytosis Precipitated by Plasmodium Falciparum Infection
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Case summary
A 33 year-old male presented with fevers, splenomegaly and pancytopenia following adequately treated falciparum malaria infection. A bone marrow examination revealed prominent haemophagocytosis and a diagnosis of reactive haemophagocytic lymphohistiocytosis (RHLH) secondary to falciparum malarial infection was made. The patient was treated with 9 days of intravenous Immunoglobulin (IVIG) monotherapy, and made a full clinical and haematological recovery.

Discussion
Our case illustrates effective and sustained remission following intravenous immunoglobulin monotherapy for Reactive Haemophagocytic lymphohistiocytosis following adequately treated falciparum malaria in an adult patient. RHLH is rare in adults and there is no consensus regarding diagnosis and treatment in adults. Paediatric data has often been extrapolated to adults, but there are clear differences between adult and paediatric disease in terms of precipitants, immune response, underlying genetic defects and clinical manifestation. Less aggressive treatment strategies have been successful in adults, particularly in non-EBV infection associated RHLH. Malaria as a precipitant for RHLH has been reported only rarely in the literature. The sub-population who might benefit from IVIG monotherapy is still to be clearly defined and further prospective studies are needed to identify this group.

No conflict of interest to disclose
Haemopoietic Improvement following Iron Chelation for Transfusional Haemosiderosis in patients with Haematopoietic Neoplasia and Aplastic Anaemia: An Observational Study

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Background
The benefit of iron chelation therapy (ICT) in patients with thalassaemia major is well established; however its role in adults with haematopoietic neoplasia (HN) and aplastic anaemia (AA) is less certain. Post hoc analysis of the EPIC study revealed ICT therapy was associated with improved haemopoietic parameters in approximately 20% of patients with myelodysplasia. Deferasirox has been reported to inhibit NF-κB, a key transcription factor involved in the inflammatory response. We speculate that deferasirox may have an additional action as an immunosuppressive agent.

Aim
To identify cases of HN and AA demonstrating haematopoietic improvement following ICT and the factors associated with response.

Method
Multicentre observational study; Physician recall of HN and AA cases showing haemopoietic improvement with ICT.

Results
Eight cases of haemopoietic improvement following ICT were identified: median age 60 (25-68), Male 5. Disorders included: hypoplastic MDS (3), AA (2) RARS (1) RCMD (1) and IMF (1). All patients were transfusion dependent requiring 3.4 units/month (2.6-6.1) with a median pre-transfusion Hb of 82g/l (66-95) prior to commencement of ICT, and baseline ferritin 1896 mcg/L (1017-5480). Two patients had raised ESR. All patients received deferasirox: median dose 12 mg/kg/day (5.6-20.6). Seven patients became transfusion independent but all had an erythroid response with median Hb following ICT of 120g/L (85-135). Median time to erythroid response: 96 days (61-450). Four patients received other therapies that may have contributed to their improvement. Six patients had platelet and four neutrophil responses.

Conclusion
Haematological responses occurred prior to significant falls in ferritin and at deferasirox doses considered inadequate for ICT. The over-representation of patients with AA/hypoplastic MDS (5) and presence of raised inflammatory markers in two of three cases without marrow hypoplasia supports the hypothesis that deferasirox may have beneficial immunosuppressive activity and may explain the observed responses in this series. Prospective studies are needed.

Novartis Australia provided support to facilitate discussion of this topic. The company had no role in analysing the data or preparing the abstract.
**P289**

**Vancomycin-Resistant Enterococci Bloodstream Infections (VRE BSI) in Haematology Inpatients**

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**Aim**

VRE BSI occurs in 4% of patients colonised with VRE. Alarmingly, 9 new VRE BSI cases were detected amongst haematology inpatients in early 2010, with only 2 in the previous 6 months. VRE BSI is associated with longer hospital stays (10.5 to 46 days) and an estimated increased cost of $27,190 per patient [US dollars]. A review of factors contributing to the development of VRE BSI was performed and the impact of interventions to reduce transmission studied.

**Methods**

Haematology admission details and microbiology results between 1/6/2009-30/11/2010 were reviewed. Interventions to reduce VRE BSI were introduced in July 2010: improved hand hygiene education, additional staffing allocations and cleaning services, antimicrobial stewardship, improved patient education and staff awareness, monthly census VRE screening of all patients on the 4 East Oncology/Haematology ward and contact tracing.

**Results**

Haematology inpatient admissions were grouped into three 6 month cohorts based on admission dates: Group #1 (1/6/2009-30/11/2009), #2 (1/12/2009-31/5/2010) and #3 (1/6/2010-30/11/2010). The three groups were similar: admission number [289-350], patient number [190-198], median age [62-64 years] and median length of stay (LOS) [6-7 days]. In contrast, VRE BSI numbers varied: 2 patients in Group #1, 9 in Group #2 and 5 in Group #3. VRE was isolated in 29 patients from 1/6/2009-31/5/2010. Average LOS was significantly longer in patients with VRE than for all haematology inpatients [19.2±4.8 vs 10.8±1.4 days, p=0.01]. 24% of patients with acute leukaemia were VRE positive as compared with only 7% of all non-acute leukaemia inpatients. 16 patients with VRE died. Median time-to-death from VRE detection was 30 days [0-236].

**Conclusions**

Early results are encouraging with only one new VRE BSI since July 2010. Increased mortality and health care costs are associated with uncontrolled VRE transmission in high-risk populations.

No conflict of interest to disclose
P290
Enterovirus Menigoencephalitis after Therapy with Rituximab

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Introduction
We report two cases of enterovirus meningoencephalitis from our institution from the past two years following treatment with rituximab.

Cases
Patient 1: 59 year-old male with follicular lymphoma was initially treated with chlorambucil and prednisolone. On the third relapse he was treated with rituximab, fludarabine and cyclophosphamide (RFC) with good response. 2 months later after a holiday in Thailand he developed a febrile illness with decreased conscious level requiring intubation. A diagnosis of Enterovirus 71 meningoencephalitis was made based on enterovirus RNA detection in CSF and consistent MRI, EEG and brain biopsy findings. IgG level was 4.76g/L. One month following initial presentation, 2g/kg IVIg was administered. There was no neurological improvement. The patient died following withdrawal of supportive care.

Patient 2: 71 year-old woman diagnosed with follicular lymphoma, four years following treatment with R-CHOP for diffuse large B-cell lymphoma. She commenced treatment with RFC which was ceased because of bone marrow failure. Syngeneic stem cell transplantation without conditioning resulted in complete tri-lineage engraftment. Nine months after her last treatment with RFC she developed a febrile diarrhoeal illness with deterioration in neurological function necessitating intubation for decreased conscious level. A diagnosis of Enterovirus 71 encephalitis was made by RNA detection on CSF, with supportive MRI and EEG findings. IgG level was 5.92 g/L (N 6.39-15.6 g/L). IVIg was administered aiming to maintain IgG level greater than 10g/L. The patient made a slow recovery and returned to almost baseline function.

Conclusion
Two patients in two years at our institution had enteroviral encephalitis within months of rituximab therapy. Both were due to Enterovirus strain 71. Risk factors include non-Hodgkin’s lymphoma, concurrent immunosuppressive/chemotherapy treatment and co-existing hypogammaglobulinaemia. IVIg seems to improve outcome with delay in initiating IVIg possibly related to poor outcome. Enteroviral infection should be considered in patients with appropriate symptoms and risk factors who may benefit from early diagnosis and intervention.

No conflict of interest to disclose
P291
Large Mycotic Aneurysm of the Right Coronary Artery (RCA)

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Aim
Mycotic aneurysms involving coronary arteries are extremely rare, usually a complication of infective endocarditis and predominantly a post-mortem diagnosis. We report the case of a 64 year-old male, who developed a 3cm RCA mycotic aneurysm following induction chemotherapy for secondary AML. Diagnosis was made using helical CT coronary angiogram with cardiac gating.

Case
The patient developed transfusion dependent sAML in June 2010 after a 40 year history of essential thrombocythaemia treated with hydroxyurea and subsequently interferon therapy, complicated by drug-induced lupus requiring prednisolone immunosuppression. Daunorubicin and cytarabine induction was uncomplicated but he had residual blasts of 7%. Re-induction was complicated by pseudomonal sepsis responsive to IV antibiotics. Shortly after neutrophil recovery he developed 3 cutaneous nodules and small pulmonary nodules. A skin lesion cultured Fusarium solani.. He received liposomal amphotericin after developing severe neurotoxicity with voriconazole. The cutaneous lesions rapidly resolved and bone marrow biopsy confirmed haematological remission.

Despite being in remission he developed further fevers, refractory rapid atrial fibrillation and cardiac failure with bilateral pleural effusions. Urine cultures grew a multi-resistant pseudomonas aeruginosa but blood cultures were initially negative. CT chest showed a 3cm soft-tissue epicardial mass, not evident on echocardiogram. TOE showed no evidence of infective endocarditis. Cardiac gated CT scan illustrated a large RCA aneurysm. This was treated with a prolonged course of Meropenem and Polymyxin B for presumptive mycotic pseudomonal infection, however antifungal therapy was also continued. Surgical treatment was not possible. Despite prolonged antimicrobial therapy, the patient had recurrent but intermittent febrile episodes complicated by septic shock with progressive cardiac failure and he died on Christmas Eve.

Conclusion
Our novel means of diagnosing this RCA mycotic aneurysm allowed appropriate prolonged therapy but did not prevent a fatal outcome in an immunocompromised host.

No conflict of interest to disclose.
Heterogeneous Use of Oral Iron Chelation in Patients Receiving Chronic Transfusion Support for Acquired Anaemia. An Overview of Patients Receiving Multiple Blood Transfusions in a Tertiary Centre in 2010

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Many patients with chronic anaemia benefit from ongoing transfusion support, but this treatment may result in iron overload. The oral iron chelator deferasirox is approved for use in patients with iron overload associated with disorders of erythropoiesis. Differing guidelines have been proposed for the use of iron chelation in myelodysplasia (MDS), the commonest acquired cause of chronic transfusion-dependent anaemia. We undertook a review of patients receiving maintenance transfusions in our institution with the aim of assessing what criteria are currently being used to guide therapy. Patients were identified from the transfusion database if they had received at least one unit of packed red cells every 2 months in the period from January 2010 to January 2011. Patients were deemed potentially eligible for chelation therapy if their serum ferritin was >1000 ng/mL and life expectancy was estimated in excess of 12 months. Patients with ongoing blood loss and receiving chemotherapy were excluded. Thirty-four patients received an average of 22 units per year (range 6-72 units). The median age of patients was 82 (range 70-91) years. The primary diagnosis was MDS n=27 (other n=9). Life expectancy was based on conventional disease-specific prognostic markers. Eighteen patients were potentially eligible for iron chelation. Ten patients were not evaluable due to missing data. Nine patients had received deferasirox, but only 2 of these remained on continuing therapy. Forty-four percent of patients discontinued deferasirox due to intolerance. There is significant variation both in the proposed guidelines for iron chelation and clinical practice. Several trends in what appeared to influence the prescribing of deferasirox were evident, for example patients who were chelated had a higher median ferritin than those who were not (4300 ng/mL versus 2500 ng/mL). These findings illustrate a need for a coordinated approach in managing patients receiving chronic transfusion support for acquired anaemia.

No conflict of interest to disclose
P293
Feasibility of Double Lumen Hickman Line as a Sole Venous Access for Stem Cell Harvest in Children with Solid Tumours

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Aim
Autologous peripheral blood stem cell harvest requires one venous access for inflow and one for out flow. Use of a pre-existing double lumen Hickman line for stem cell harvest in children with solid tumours as a sole venous access is rarely reported, although it has several advantages. We report here our successful experience of stem cell harvest with double lumen Hickman line.

Method
We retrospectively reviewed our experience. Between 2000 and 2011, 60 children with high risk solid tumours underwent Autologous Peripheral Blood Stem Cell (PBSC) harvest using double lumen Hickman line as sole venous access.

Results
There were 35 boys and 25 girls. The median age at the time of harvesting was 9.2 years (range, 1.1-16.1 years) and the median weight was 28.6 kg (range 9.2-56.7 kg). Clinical diagnosis were PNET (1), Medulloblastoma(7), Ewing's Sarcoma(20), Neuroblastoma (19), Hodgkin’s Disease(3), B cell Lymphoma(3), Wilm’s Tumour(2), Rhabdoid Tumour (1), Sacrococcygeal Teratoma (1). All patients had priming chemotherapy plus G-CSF for mobilising stem cells. We noticed only few minor complications at the time of procedures. Out of these, 45 were successful. In 35 cases, CD 34+ cells yield was >2*10^6/KG by one apheresis. In 9 cases, 2 apheresis were required on consecutive days to get yield more than 2*10^6/KG. The CD 34+ count in peripheral blood in these patients on the day of apheresis ranged between 9 And 336 with median value of 49. In 15 cases, harvest was failed due to multiple reasons.

Conclusion
Our experience supports that pre-existing double lumen Hickman line can be used safely, conveniently and cost-effectively. Our harvest results are comparable to results reported by other groups using different central venous catheters. It avoids the need of another central venous access in children with solid tumours at the time of stem cell harvest.

No conflict of interest to disclose
P294
Effect of CD34+ Cells Infused and G-CSF on Erythroid and Myeloid Engraftment at Time of Autologous Peripheral Blood Stem Cell Transplantation (PBSCT)

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Aim
Autologous peripheral blood stem cell transplantation is standard therapy for some relapsed haematological disorders and consolidation therapy for others. We retrospectively assessed the number of CD34+ cells infused, and other factors, to determine what are predictors for erythroid and myeloid engraftment at the time of PBSCT.

Patients and Methods
Patients with Hodgkins Disease, Myeloma and Non-Hodgkins Lymphoma having a PBSCT from 1995 to 2008 were included. G-CSF was administered from day +1 (period 1, 1995-2001) or from day +5 (period 2, 2002-2008). Packed red blood cells (PRBC) were transfused at the discretion of the haematologist usually when the Hb was <90g/l, and were summed over the patients entire admission. Neutrophil engraftment was when neutrophils reached 0.5x10^9/L and remained above this level for 3 consecutive days.

Standard statistical analysis used Stata 10. A nonstandard model was developed amongst those patients who required transfusion, to model the number of units of PRBC’s transfused (which used R).

Results
207 transplants were assessed. Neutrophil engraftment had a mean of 10.1 days and a median of 10 days. More CD34+ cells were infused during period 2 (p=0.005), although time to engraftment was less (mean 9.58 days versus 10.28 days, p<0.001). A logistic regression model for the occurrence of transfusion, showed that the admission Hb (p<0.001) and log(CD34+ cells infused) (p=0.0498) were predictors of the need for transfusion. A novel model for those patients who required transfusion demonstrated that the initial admission Hb (p<0.001) and diagnosis (p=0.047) predicted PRBC transfused, however CD34+ cells infused was not predictive.

It has been demonstrated that the number of CD34+ cells infused can predict the need for transfusion, although not of the number of units of PRBC transfused in those patients. G-CSF commencing on D+1, compared to G-CSF commencing Day +5, resulted in more rapid myeloid engraftment although the clinical significance of this is doubtful.

No conflict of interest to disclose
P295
Inferior Viable CD34 Recovery in Cryopreserved Allogeneic compared to Autologous HPC, Apheresis Products

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Allogeneic haemopoietic progenitor cells collected by apheresis (HPC, Apheresis) are cryopreserved less often than autologous harvests. In recent years, cryopreservation of allogeneic HPC has been performed more frequently to store excess HPC or T-cells for donor lymphocyte infusions or to circumvent issues of donor availability / product transport, particularly in light of recent worldwide events (Sept 11, swine flu and flight disruptions due to volcanic ash).

During 2006 to 2009, 30 allogeneic and 349 autologous HPC, Apheresis products were cryopreserved in this laboratory. It was noted that the post-thaw viable CD34⁺ recovery was lower in the allogeneic (median = 63%, range 16-92) than autologous products (72%, 13-151; p <0.0004). Hence this study aimed to determine factors that influence post-thaw CD34 recovery.

Univariate analysis demonstrated weak inverse correlations between viable CD34 recovery and the collection to freeze time interval (Spearman r=-0.10, p=0.048), frozen nucleated cell concentration (NCC; r=-0.17, p<0.0001) and neutrophil content (r=-0.19, p=0.0008). Multiple regression analysis demonstrated that collection to freeze interval (p=0.006), neutrophil content (p<0.0001) and allogeneic donors (p<0.001) significantly affected viable CD34 recovery, but NCC did not (p=0.14).

Nine of the cryopreserved allogeneic products have been infused, with patients attaining an absolute neutrophil count of >0.5x10⁹/L in a median of 18 days (range 11-31) and platelets >20x10⁹/L in 27 days (14-58), which is not significantly different to that observed following infusion of 133 fresh HPC (neutrophil median 17 (9-45) days, p=0.9; platelets median 18 (10-101) days, p=0.2).

This data indicates that the lower post-thaw viable CD34 recovery in allogeneic HPC, Apheresis may be due to intrinsic properties of allogeneic donors in addition to the higher neutrophil content and more prolonged pre-cryopreservation storage periods for these products. Post-thaw analysis of viable CD34⁺ content is recommended to ensure sufficient viable CD34+ to facilitate engraftment.

No conflict of interest to disclose
An Intensified Conditioning Regiment With Intravenous (IV) Busulphan-Melphalan (Bu-Mel) and Pharmacokinetic Monitoring Prior to Autografting For Poor Prognosis Non-Hodgkin Lymphoma (NHL)

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2. Department of Pharmacy, Austin Hospital, Heidelberg, VIC, Australia
3. Department of Haematology, Westmead Hospital, Sydney, VIC, Australia

Background
Oral-busulphan and IV-melphalan (bu-mel) conditioning prior to autografting has been used for many years but 10-fold variability in busulphan gut absorption results in efficacy and toxicity (particularly mucositis) which is unpredictable in individual patients. IV busulphan has more predictable PK and drug levels can be monitored in real-time allowing individualised dose-adjustments to achieve an optimal drug exposure. Overseas experience suggests that this regime improves the outcome of chemorefractory lymphoma or myeloma compared with conventional regimens.

Aims
To determine engraftment, transplant-related mortality/morbidity, treatment outcome and effect of PK-monitoring in high-risk NHL patients receiving IV bu-mel. Relevant outcomes were compared with a concurrent cohort of BEAM recipients since 2010.

Method
Eligibility consisted of patients with high-risk NHL: failure to achieve complete remission with induction; early, chemoresistant or PET-positive post-salvage for relapse; >1 relapse. PK was done on the initial d-7 busulphan dose (50% dose i.e. 1.6mg/kg) with results available to make dose adjustments (from 3.2mg/kg) for doses d-5 to d-3 as required to achieve an AUC of 4500-5000. Repeat PK was performed after the d-5 dose.

Results
7 patients received bu-mel (M:F 6:1; median age=57) while 14 patients received BEAM (M:F:4:10;median age=40). No patient died within 100 days of transplant. Engraftment was similar in both groups. Morbidities seen more frequently with IV bu-mel were mucositis, culture-positive sepsis and requirement for prolonged opioid infusions, additional anti-emetics, paraenteral nutrition, and intensive-care unit admissions. 6 of 7 bu-mel patients remain alive without disease progression (median of 204 days follow-up), including 3 of 4 with > 1 year follow-up. The estimated weight-based AUC on d-7 PK was less than expected in 6 of 7 patients (median of 9%). Dose adjustment was required in 5 pts and resulted in a higher than expected d-5 AUC in 7 pts (median of 16%).

Conclusion
While IV bu-mel increases risk of transplant morbidity, early outcomes appear promising in patients with otherwise poor prognosis lymphoma. PK-monitoring may be useful in ensuring appropriate busulphan levels but further refinement of dose-adjustment algorithms may be required. No conflict of interest to disclose
**P297**

**Comparison of Filgrastim versus Pegfilgrastim in the Mobilization of Autologous Stem Cells in Non-Hodgkin Lymphomas**

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**Aim**
To compare Filgrastim against Pegfilgrastim in the ability to mobilize Autologous stem cells in non-Hodgkin lymphoma (NHL).

**Method**
Between January 2006 and January 2011 ninety five consecutive patients with NHL underwent stem cell mobilization with chemotherapy and granulocyte colony stimulating factor. Following the completion of chemotherapy, patients received Filgrastim 5mcg/kg bd or, from June 2008, a single dose of Pegfilgrastim 6mg. The primary endpoint was mobilization failure, defined as a CD34 collection <2 x 10^6 cells/kg. A retrospective analysis was conducted with data collected from the SCGH stem cell mobilization register, patient notes and computerized hospital information systems.

**Results**
45 patients were mobilized with Filgrastim and 50 patients with Pegfilgrastim. Median age at mobilization was 57 years [17-74] with a male predominance (67%) The most common subtypes of NHL mobilized were Diffuse Large B cell Lymphoma (42%); T-cell Lymphoma (17%); Follicular Lymphoma (15%); Mantle Cell Lymphoma (9%). There was no significant difference between the two groups for age, sex, reason for mobilization and number of lines of therapy. The median time from commencement of mobilization agent to apheresis was 9.5 days [4-14] with Pegfilgrastim and 8 days [4-18] with Filgrastim. Mobilization failure rates were greater in the Pegfilgrastim group (10/50; 20%) as opposed to Filgrastim (4/45; 9%) (p=0.089). There was a significant increase in the number of days on apheresis with Pegfilgrastim as opposed to Filgrastim (p=0.036). 35% of Pegfilgrastim mobilized patients required 3 days of apheresis as opposed to 15% with Filgrastim. Median CD34 collection per day with Pegfilgrastim was 2 x 10^6 cells /kg and 4.4 x 10^6 cells/kg with Filgrastim. Median CD34 counts in the peripheral blood were higher with Filgrastim.

**Conclusion**
Pegfilgrastim 6mg was found to have increased mobilization failure rates, longer time on apheresis, lower CD34 peripheral blood counts and less CD34 cells/kg/day collected as opposed to Filgrastim 5 mcg/kg bd.

*No conflict of interest to disclose*
Evidence for Survivorship Care: Quality of Life of Long Term Survivors of Stem Cell Transplantation Is Improved After Attendance at a Dedicated Late Effects Clinic

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Aim
The Alfred Late Effects Clinic (LEC) is dedicated to providing survivorship care for long term survivors of haemopoietic stem cell transplantation (SCT). This study aims to assess the impact of SCT and LEC attendance on long term quality of life (QoL).

Method
Since May 2008, LEC attendees (disease free>2yrs) prospectively completed the FACT-BMT QoL survey. The FACT-BMT has 50 questions covering physical, social, emotional and functional well-being, plus SCT specific concerns.

Results
143 surveys were evaluated from 70 attendees. 63% had allogeneic and 37% autologous SCT. Median age 49yrs (range 19 - 67) with 57% male. Median time post SCT was 4.8yrs (range 1.5 - 17.5).
Overall, QoL was good with a mean FACT-BMT score of 117.9 (possible 0-148). Consistent with this, 65% were very content with current QoL.

Table 1: FACT-BMT QoL survey scores for LEC attendees (n = 143)

<table>
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<tr>
<th>Well-being domains</th>
<th>Min – max possible score</th>
<th>Mean score (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>0-28</td>
<td>23.3 (8-28)</td>
</tr>
<tr>
<td>Social</td>
<td>0-28</td>
<td>22.5 (8-28)</td>
</tr>
<tr>
<td>Emotional</td>
<td>0-24</td>
<td>20.2 (10-24)</td>
</tr>
<tr>
<td>Functional</td>
<td>0-28</td>
<td>21.4 (6-28)</td>
</tr>
<tr>
<td>BMT specific</td>
<td>0-40</td>
<td>30.1 (17-40)</td>
</tr>
<tr>
<td>Overall FACT-BMT</td>
<td>0-148</td>
<td>117.6 (63-146)</td>
</tr>
</tbody>
</table>

For recipients who attended more than once, serial attendance was associated with better FACT General (p=0.02), BMT specific (p=0.04) and overall FACT-BMT (p=0.02) scores.
Scores were not significantly impacted by gender, age, time since SCT or type of SCT. For allogeneic recipients, GvHD (limited in 25% & extensive in 46%) was associated with a trend to inferior QoL. Common problems included fatigue 33%, infections 18%, poor sleep 17%, skin 16%, bowel 12%, respiratory 13%, poor memory/concentration 11%. Very low satisfaction with sex life was indicated by 36% and 17% very worried about fertility.

Conclusions
While overall QoL is good for long term survivors of SCT, some areas are negatively impacted. A quality of life tool can highlight areas of need. In this study, age, gender, type of SCT and GvHD did not significantly impact late QoL. Thus, all long term survivors of SCT should be given the opportunity to benefit from specialist survivorship care. Serial attendance at a dedicated LEC clinic may improve SCT survivor’s QoL, and utilisation of such clinics should be encouraged.

No conflict of interest to declare
P299  
Aryl Hydrocarbon Receptor Antagonists for Expansion of Human CD34+ HSCs in vitro

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Aim
Expansion of Haemopoietic Stem Cells (HSCs) in vitro would potentially be a strategy for improving transplant outcomes, but culture tends to promote differentiation and loss of stemness. In this study we tested the effectiveness of two Aryl hydrocarbon Receptor Antagonists (AhRAs) on maintenance of stemness during in vitro proliferation of HSCs from human adult mobilized blood and Cord Blood (CB).

Method
This work was done at St Vincent’s AMR. Four adult GCSF-mobilized peripheral blood samples and four CB units (Sydney Cord Blood Bank) were obtained with written consent, and purified by MACS separation to CD34+ cells. Cells were grown for 2 weeks in aMEM medium supplemented with Flt3L, SCF, TPO and II-6 (all 100 ng/mL), 20% FBS, and 1mM AhRA (Merck) or Dimethoxyflavone (DMF, Sigma). Expanded cells were evaluated by flow cytometry for CD34 content, and re-selected for CD34+ cells by MACS. Cells were added to OP9 co-cultures (aMEM, 20% FBS, 5 ng/mL Flt3L, SCF, II-7) and grown for up to 91 days to assess their differentiation potential. Cells were assayed at weekly passage by flow cytometry for CD1a, CD3, CD4, CD8, CD14 & CD19. Population differences were compared by t-test.

Result
Both AhRAs promoted retention of CD34 expression during proliferation. CB CD34+ cells expanded on average 138-fold (SEM 36) in 14 days. Adult CD34+ expansion was low (mean 6 fold, SEM 1.7). Expanded CB cells differentiated to T-cell lineage CD4+ CD8+ DP cells in a normal pattern, similar to untreated controls. Expanded Adult cells generated significantly less DP cells than untreated cells. Differentiation to monocyte and B cell lineage was not affected.

Conclusion
AhRA incubation is an effective method for protecting stemness in CB cells during in vitro proliferation. Adult cells do not respond as well, and T-cell yields were at best only marginally increased.

No conflict of interest to disclose
P316
Aplastic Anemia in the Orient

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Aim
To find evidence in favour of hypotheses that aplastic anemia in the Orient differs from that in the West epidemiologically and etiologically, has a different pathophysiology and should be treated differently.

Method
Systematic review of published/presented studies from 1986 to-date from Pakistan and other countries in the East and their comparison with studies from the West.

Results
Success rates of up-to 80% have been reported for immunosuppressive therapy of aplastic anemia from Europe and North America. On the basis of this success, together with “directed” laboratory studies a consensus has emerged that immunological mechanisms underlie pathophysiology in vast majority of patients with idiopathic aplastic anemia, no matter what ever is the aetiology. On the other hand studies from Pakistan, India and other countries of Orient (except China) mostly report success rate with immunosuppressive therapy between 25% and 40%. Same countries report success rate with Bone Marrow Transplant at 80% and above. This indicates that in these countries immune mechanisms play a role in pathophysiology of idiopathic aplastic anemia in considerably smaller number of patients as compared to West. On the contrary one study from China has reported success in curing aplastic anemia with administration of immunologically activated autologus or allogeneic lymphocytes. Literature from the Orient still report a 2-4 times higher prevalence/incidence of aplastic anemia as compared to West. Some well planned studies have shown a highly significant correlation of the disease with farming, drinking water, poultry and chemicals. An environmental factor, either chemical or infectious, appears to be important. However, a study in immigrants from Oriental countries to the West, still report a higher prevalence of aplastic anemia in them. That infers that environmental factor alone is not causative but it plays its part on a genetic pre-disposition. This is supported by the fact that this disease seems to be comparatively un-common in Latin America and Africa where environmental conditions are more or less same as in Oriental countries. The hypothesis is further supported by higher prevalence of some HLA alleles in patients of aplastic anemia.

Conclusion
It is concluded from the systematic review that in a sizable number of cases of idiopathic aplastic anemia the pathophysiology can be better explained by direct toxicity of an environmental factor (most likely a chemical). This possibly results from defects in genetically determined metabolic pathways of these chemicals leading to accumulation of toxic metabolites in the bone marrow (being fat soluble).
P300
Manufacture of Autologous Chondrocytes for Implantation

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The implantation of autologous chondrocytes (articular cartilage cells) seeded on a matrix such as a collagen membrane is a method being widely used for the treatment of chondral defects which pose a significant challenge for treatment. Autologous chondrocytes are isolated, cultured \textit{ex vivo} to expand to the required number, and implanted into the damaged region.

To ensure quality, freedom from bacterial contamination, and safety of the final product, processing is performed using Good Manufacturing Practice (GMP) in a Class B cleanroom. Following manufacture autologous chondrocyte products may be cryopreserved for future use or dispatched for surgical implantation.

The aim of this review of manufacturing data was to develop process limits for critical processing parameters as part of process control required for GMP. These data would also provide guidelines to clinical clients for required biopsy size and time required for manufacture of requested cell dose.

Arthroscopic biopsies from a non-load-bearing edge of the patella-femoral groove were performed on ninety five patients with chondral defects. The harvested samples were transported to the laboratory, enzymatically digested and expanded in tissue culture flasks using culture medium supplemented with 10% foetal bovine serum and ascorbic acid. When chondrocytes numbers were sufficient, surgeons were notified and a second surgery was scheduled for implantation. For implantation chondrocytes were loaded onto a prepared scaffold at a seeding density of 0.5-1 x 10⁶ cells/cm².

The median patient age was 38 years (range of 19 to 59 years) with a median total defect size of 4cm² (range of 1 to 32cm²). Biopsy weight varied from 10mg to 850mg with a median weight of 230mg. There was no correlation between biopsy weight and dose requested or cell dose achieved but in all cases sufficient cells were isolated and expanded to meet clinical dose requirements.

No conflict of interest to disclose
Platelet Count Response to *H. pylori* Eradication in Iranian Patients with Immune Thrombocytopenic Purpura

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**Aim**

*Helicobacter pylori* is a gram-negative bacterium that is the causative agent of active chronic gastritis and peptic ulcer. It is a cofactor in the development of adenocarcinoma and mucosa-associated lymphoid tissue (MALT). The relationship between *H. pylori* infection and idiopathic thrombocytopenia purpura (ITP) is not certain but some studies have reported increased platelet counts subsequent to the eradication of *H. pylori* infection. The aim of this study was to investigate platelet recovery in ITP patients after *H. pylori* infection eradication.

**Method**

Between 2009 and 2011 a total of 32 patients (16 male, 16 female) diagnosed with both ITP and *H. pylori* infection. ITP was diagnosed when platelet counts were less than 120×10\(^3\)/µL. These patients were tested for *H. pylori* infection by urea breath test and serum *H. pylori* antibody. All patients received triple therapy for 14 days to eradicate *H. pylori* infection. These patients were followed for six months. Complete response (CR) was defined as a platelet count at least 150×10\(^3\)/µL, partial response (PR) was defined as a increasing in platelet count of at least 30×10\(^3\)/µL and no response (NR) was defined as a platelet count below 30×10\(^3\)/µL.

**Result**

Of all patients, 12 (37.5%) exhibited a complete response, 9 (28.1%) a partial response and 11 (34.3%) were nonresponse. There was a significant difference between the responders and non-responders (p<0.001). In this study all responders had initial platelet count more than 50×10\(^3\)/µL and other patients that were non-responders had an initial platelet count less than 50×10\(^3\)/µL.

**Conclusion**

The results of this study showed that eradication of *H. pylori* infection can lead to a significant improvement in platelet count in these *H. pylori*-positive ITP Iranian patients. Further studies are required to clarify other causative factors involved in platelet recovery.

No conflict of interest to disclose
P302
Evaluation of Freezing Bags for the Cryopreservation of Haemopoietic Progenitor Cells-Apheresis at Peter Mac

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²University of Melbourne, Parkville, Australia

Aim
Cryostore (Origen) and CryoMACS (Miltenyi Biotec) freezing bags were evaluated and compared with the widely used Cryocyte bag (Baxter Healthcare) for the cryogenic storage of Haemopoietic Progenitor Cells-Apheresis (HPC-A).

Method
The validation was performed in two parts:
1) Integrity Testing: A bag of each size (small, medium, large) from the three manufacturers was filled with extreme volumes of cryoprotectant mixture and subjected to five sequential freeze-thaw cycles. This tested the bag’s ability to withstand rate-controlled freezing, liquid nitrogen vapour phase storage and subsequent thawing in a waterbath. In addition, the plastic used, connection and ports, label adhesion, ability to purge air, compatibility with current equipment and ARTG listing were evaluated.
2) Cryopreservation & Sterility Testing: One HPC-A product was split across a bag from each manufacturer and evaluated for cellular cryopreservation efficiency (CD34 recovery and viability). Ethical considerations and product availability prevented testing on additional product.

Result
In the normal clinical setting, bags must withstand a single freeze-thaw cycle. All bags passed at least two freeze-thaw cycles at each volume tested. During the third freeze-thaw cycle, the Miltenyi Biotec overfilled ‘medium’ bag was observed to leak during thawing in a waterbath. All three manufacturers resulted in similar CD34 recovery and viability. Sterility testing was performed pre-freezing and post-freezing and no growth was found across all the manufacturers.

At Peter Mac, HPC-A products are reinfused using a Baxter Interlink solution set with male luer-lock adapter. After spiking, the bag was checked for leaks and damage to the bag wall. Only the Origen bags provided a secure, leak-free connection: the Miltenyi Biotec port leaked, thus compromising product.

Conclusion
Origen CryoStore bags are confirmed suitable for routine use at Peter Mac. Future use of the Miltenyi Biotec CryoMACSs bags will require investigation of a suitably compatible filterless infusion set.

No conflict of interest to disclose
Automated Counting of Hematopoietic Colonies Reduces Assay Variability

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The hematopoietic colony-forming-cell (CFC) assay is the standard for measuring the progenitor content of cell products for hematopoietic stem cell transplantation. To date CFC have been enumerated manually based on morphology using a microscope, a process which is time-consuming and requires skilled operators to maintain consistent scoring criteria.

We have developed an automated system (STEMvision™) for colony enumeration and classification. To assess the efficacy of this system, cord blood mononuclear cells were plated in either Methocult Express or Methocult H4034 and cultured for 7 or 14 days respectively. For each sample (well) automated counts were obtained using 3-5 STEMvision™ instruments. The colonies in the same wells were then manually enumerated by 3-5 operators using both the STEMvision™ images and the standard microscope method. For the 7-day (n=36) and 14-day assays (n=39), the average total colony counts for all three methods were highly correlated (>95%). The table below shows that automated counts had the lowest overall coefficient of variance (CV) for the normalized total colony counts.

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<tr>
<td>7-day</td>
<td>10%</td>
<td>10.2%</td>
<td>9.2%</td>
<td>4.2%</td>
</tr>
<tr>
<td>14-day</td>
<td>11%</td>
<td>7.9%</td>
<td>7.9%</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

Automated classification of myeloid and erythroid colonies in the 14-day assay also reduces variability relative to the manual method. The normalized total colony counts for 6 samples obtained by 3-5 operators on a single instrument had a CV of 4.1% in the 7-day assay and 4.3% in the 14-day assay, suggesting the reduced variability is operator-independent.

All applications of the CFC assay would benefit from the reduced variability, standardization of scoring criteria and image archiving offered by the automated system. Algorithms to enumerate and classify colonies from different tissue types and species will expand the applicability of this technology.

No conflict of interest to disclose
P304
Managing a TGA GMP Licence for HPCs Within NSW Health

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Introduction
The CMT team at Royal Prince Alfred Hospital (RPAH) obtained a GMP license from the TGA to manufacture haematopoietic progenitor cells (HPCs) in 2007. The license is to manufacture autologous and allogeneic HPCs processed at the RPAH Laboratory, collected from RPAH and Concord Hospital (CRGH).

Aim
To successfully maintain a GMP license for manufacture of HPCs for clinical use in New South Wales. We have built on a quality system that involves clinicians, scientists, nurses and NSW health infrastructure working in a co-ordinated quality environment. This supports a flexible hospital based GMP license to manufacture HPCs with future aims to progress into other cell therapy technologies.

Method
The apheresis and transplant laboratory service GMP facility was designed to meet TGA requirements. External contracted service providers are required to provide external testing requirements that conform to the TGA code of GMP and Biological framework specifications. A technical master file was submitted to TGA with the full HPC product specifications. Formal document controlled Standard Operating procedures (SOPs) & records; product validations; Training & monitoring systems; equipment, material, change & process control management; auditing and non-conformance reporting; transport and management review are all part of GMP.

Result
GMP licensure for the manufacture of HPCs was maintained since 2007. Rated a good TGA compliance level at mid-year 2011 audits. Neutrophil and platelet engraftment were reviewed every six months, according to CIBMTR definitions, to enable management to monitor ongoing success rates of the HPC engraftment procedures. Over four years, results demonstrate that the process remains under control despite a wide range of potential patient variability in disease. Internal and external audit citations, and non-conformances, have diminished. This indicates system and process improvement of both quality management and operational procedures.

Conclusion
Maintaining a cGMP TGA compliant facility for HPCs in NSW Health has provided ongoing product improvement that provides patients with beneficial outcomes. The process requires the co-operation of a multi skilled group of willing and dedicated health workers including hospital cleaners, apheresis nurses, transplants scientists and medical officers. Both RPAH and CRGH staff must recognise the need to comply with the TGA GMP requirements and participate in the service. The quality systems in place may now be used to build on the TGA license to manufacture HPCs and other cell therapies that may be developed.

No conflict of interest to disclose
P305
There Is No Place Like Home

Jo Cryer, Cassandra Hobbs
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Since October 2010 the Cancer Outreach Program (COP) has successfully kept 4 patients at home following early discharge post Peripheral Blood Stem Cell Transplant. This means that they have not required lengthy admissions in hospital and only required short presentations for outpatient platelet transfusions. This translates to a decrease in Length Of Stay (LOS)

COP provides a daily (Monday – Friday) visit to these patients who are discharged post stem cell re-infusion.
COP is run and co-ordinated by two highly experienced haematology/ oncology nurse consultants
A daily assessment is performed including a full set of observations, weight, urinalysis, review of fluid balance and blood review

This talk will focus on the early discharge program at the St George Hospital. It will discuss the pre and post transplant education given to the patient and family, patient eligibility and details of the visits.

No conflict of interest to disclose
P306
Retrospective Comparison of HPC-A Collection Data from the Fresenius Kabi Australia COM.TEC® Apheresis Blood Cell Separator from Three Collection Sites

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Aim
Retrospective comparison of Haemopoietic Progenitor Cell (HPC) collection data using the Fresenius Kabi COM.TEC® apheresis device from three independent hospitals; the Peter MacCallum Cancer Centre, The Royal Melbourne Hospital and the Princess Alexandra Hospital in Queensland.

Method
The Fresenius Kabi COM.TEC® apheresis device was recently trialled at the Peter MacCallum Cancer Centre. We collected HPCs from 20 patients with Multiple Myeloma. The Royal Melbourne Hospital provided results from 20 collections. Princess Alexandra Hospital provided data from 60 patient collections. Data was collected by each site independently to satisfy unique organisational quality management and validation requirements. The multi-site retrospective analysis required consolidation of data sets to enable direct comparison. All patients had Multiple Myeloma and were collected from variable mobilising regimens including colony stimulating agents with or without chemotherapy. We evaluated collection efficiencies; total blood volume processed and compared pre/post CD34+ results. Engraftment data was not included in this comparison.

Result
Collections for comparisons were from patients with multiple myeloma. Results have demonstrated that the overall mean collection efficiencies are comparable between all sites. There is a large mean difference in the peripheral CD34 ranging between 66.35 and 27.5/uL. The product CD34 count ranged between 3.8 and 4.51x10⁹/kg. There is a large difference between volumes processed, Peter Mac 9.4L, Princess Alexandra 15.0L and RMH 10.4L, indicating a variable operating procedure at local levels.

Conclusion
Retrospective data comparison provides its own challenges when different measures are used for evaluation by each site. The comparison demonstrates that each site has unique treatment requirements and processes with respect to operating procedures for HPC-A collection and subsequent data collection. The conclusion is that additional investigation is required to draw correlation between ranging mean differences in peripheral CD34 and product CD34 enumeration.

Conflict of interest is contribution by supportive staff from Fresenius Kabi Australia Pty Limited in the preparation of this project.
P307
Sterile or Asceptic Non-Touch Technique? Does it Prevent Catheter-Related Blood Stream Infections When Managing Central Venous Access Devices

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²Griffith University, Brisbane, Queensland, Australia

Aim
To assess the evidence to support using an aseptic non-touch technique (ANTT) compared to a sterile technique for preventing catheter-related blood stream infections (C-R BSI) in the management of central venous access devices (CVAD).

Methods
A literature search of available randomized controlled trials was undertaken searching the Cochrane Database of Systematic Reviews, The Cochrane Library Issue 3 2010; OVID Medline 1996 to July week 1 2010; CINAHL 1982 to July week 1 2010 and PubMED to July week 1 2010.

Result
Only one trial involving 111 CVADs in 79 patients was included in the review. Rates of bacteraemia were lower in the ANTT group; OR 0.71 (95% CI 0.16 to 3.15). CVAD tip colonization was also lower in the ANTT group; OR 0.36 (95% CI 0.14 to 0.94). Neither of the results was statistically significant.

Conclusion
Although one small trial showed some benefit favoring ANTT for CVAD management, there remains insufficient high quality evidence to recommend either technique. Until further trials are undertaken, either method may be used. However, ANTT requires fewer resources, in terms of materials and staff time so may be the method of choice.

No conflict of interest to disclose.
P308
Should Iron Chelation Therapy Be Routinely Used to Prevent Transfusional Iron Overload in Myelodysplastic Patients?

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Introduction
Myelodysplastic syndrome (MDS) is a group of blood disorders associated with ineffective production of the myeloid class of blood cells due to dysfunction of the bone marrow. The incidence of MDS in Australia is assumed to be between 4 and 5 people per 100,000 of the population. Of these cases, more than 90 per cent occur in people over the age of 60, with the incidence increasing to 20 to 50 per 100,000 in this same age group.

Aim
To conduct a comprehensive literature review of the benefits of the use of iron chelation in low to high risk MDS patients and assess if the literature supports current clinical practice in Australia.

Method
To correlate data highlighting the prevalence of iron overload in the MDS population in Australia and current treatments and comparison with international gold standard; to analyse the current survival rates and explore quality of life for MDS patients with or without iron chelation; and to critically analyse a case study of an MDS patient who is currently receiving chelation therapy at Prince of Wales Hospital, Randwick.

Results and Discussion
Evidence supports that red blood cell transfusions are clinically beneficial to treat symptomatic anaemia in MDS patients. However it is this common practice that has prompted the need for controlled clinical trials, and not simply consensus statements from a small group of practitioners, to investigate the real and potential risks of transfusion related consequences to MDS patients. Increasing awareness in the treatment of MDS, as well as growing evidence of the effects of iron overload on morbidity and mortality, have warranted the development of guidelines to inform clinicians on the best available treatment strategies of iron overload as current literature is often conflicting.

Conclusion
While clinicians recognise the aim of chelation therapy is to prevent or reverse established iron overload and end organ dysfunction, current practice will vary from institution to institution. International literature supports the use of iron chelation in patients diagnosed with low risk MDS, however governance over the administration of iron chelation remains a controversial topic.

No conflict of interest to disclose
P309
Utilising a National Myeloma Impact Survey To Improve Service Delivery

Kaye Hose
Leukaemia Foundation of Australia

Background
As part of National Myeloma Day – May 18th 2011, the Leukaemia Foundation undertook several initiatives to help provide better education and support for people with myeloma, and better awareness of the issues faced by people with myeloma. One of these initiatives included a national myeloma survey to identify the major themes of the impact of having a diagnosis of myeloma.

Objective
To identify the major issues faced by people living with myeloma in Australia in 2011 and to use the data help advocate to improve the provision of care and service delivery to this group.

Methods
The survey was developed with the support of haematologists, haematology nurses, researchers, social workers, leukaemia foundation coordinators and people who have had a diagnosis of myeloma. The project was led by the Foundation’s National Myeloma Coordinator, and undertaken by an external company - Sweeney Research.

The survey was available online or in hard copy, and was open for completion from mid-May to the end of July 2011.

Results
The findings will be collated and presented as part of this presentation, including some of the implications for health care practitioners. The data from the exercise component of the survey will be analysed in conjunction with Deakin University in Victoria. These findings will then be utilised as part of their research into developing myeloma- specific exercise programs

Conclusion
Myeloma is an incurable form of cancer. Disease symptoms and treatment side effects greatly impact on a patient’s life. Having an improved understanding of the issues confronting people living with myeloma, in an era of novel therapy options, can help to contribute in providing better care.

No conflict of interest to disclose
P310
Clinical Application and Nursing Attitudes towards the Early Warning Score (EWS) in Acute Haematology

Courtney McKerrow, Deborah Kidner
Canterbury District Health Board, Christchurch, New Zealand

Aim
Early warning scores have been used internationally for >10 years to identify patients at risk of clinical deterioration. Introduced at Canterbury District Health Board 2 years ago, a recent audit suggested that nurses on the Bone Marrow Transplant Unit (BMTU) are not using EWS when assessing patients. The authors wanted to identify reasons for not doing so in light of evidence suggesting that EWS enables early recognition and intervention for a deteriorating patient and can facilitate early admission to Intensive Care or avoid it, leading to better patient outcomes.

Method
A qualitative audit was used to research registered nurses’ application of and attitudes towards EWS in haematology. A questionnaire was created of 20 questions, with tick box and open answer format. These assessed how and why nurses used the EWS as well as their attitude towards the tool. Fifteen nurses responded out of 30 employed on the unit. Observer impression was used to analyse the data and identify trends.

Result
Analysis showed that nurses who were recent graduates or new to haematology found the EWS useful for identifying observation trends and assisting them to obtain prompt medical review. Senior nurses described the tool as useful yet admitted they didn’t always follow stipulated guidelines, believing instead that their clinical assessment skills were effective in identifying a deteriorating patient. Of the respondents, 10/15 described the EWS as fit for use in haematology, 5/15 did not/were unsure.

Conclusion
There is limited literature available on the use of EWS in haematology. This audit suggested that senior nurses feel their clinical skills are adequate when identifying a deteriorating patient. EWS is a tool, not a replacement for clinical judgement and when used effectively is proven to facilitate earlier intervention. Increased education and positive experience over time may help haematology nurses recognise the validity of the tool.

No conflict of interest to disclose
P311
A Single Unit’s Experience with Plerixafor - a CXCR-4 Inhibitor- in the Hard to Mobilise Patient

Victoria Milliken
Calvary Mater Newcastle, NSW, Australia

Autologous Stem Cell Transplantation (ASCT) is a well-regarded treatment strategy for patients with Multiple Myeloma and Lymphoma. There are well established mobilisation failure rates in the literature of 5-30% using the conventional mobilisation methods; colony stimulating factors with and without chemotherapy. There is also an associated rate of up to 50% of poor mobilisers in these patient groups. Poor or failure to mobilise is defined as < 2 x 10^6 cells/kg. Factors contributing to poor mobilisation may include advances in upfront therapies, patient age, underlying disease and previous treatment regimes used prior to mobilisation. Plerixafor, a CXCR-4 inhibitor, has been trialled and used on a compassionate program within Australia and internationally. This poster highlights our experience, a single unit, with Plerixafor in comparison to the available literature with the view of developing a risk stratification algorithm.

In our facility within the last 12 months we have used Plerixafor on 7 patients who all have had at least one previously failed attempt to mobilise sufficient stem cells for collection. A retrospective review of our data from this patient group has shown that 6 (85.7%) had a successful collection of >2 x 10^6 cells/kg indicating that Plerixafor has a place in our mobilisation strategy for these patient groups. We acknowledge the limitations of our analysis due to small patient group numbers and maintain that further evaluation is required to substantiate this data. However based on the outcomes reported from trial and compassionate programs both within Australia and internationally and our units' comparable data it would be appropriate to develop and trial an algorithm to incorporate the use of Plerixafor in previously failed mobilisation patient population.

No conflict of interest to disclose
A Review of the Use of the Immunosuppressed Patient's Clinical Pathway
Patient's Admitted to the Bone Marrow Transplant Unit Via the Emergency Department

Gillian Parkin, Sally Fowler
Bone Marrow Transplant Unit, Christchurch Hospital, Christchurch, New Zealand

Aim
The aim of the review was to evaluate the use of the immunosuppressed pathway in 33 patients admitted to the unit via the emergency department.

Method
Patients receiving intensive chemotherapy, bone marrow transplantation or those the consultants consider to be at risk of Immunosuppression are given an immunosuppression card. This immunosuppression card has the contact phone numbers and the process of what to do if they are unwell at home. It is presented to the emergency department and alerts emergency staff that the patient may be immunosuppressed and need to be attended to urgently and that the immunosuppression pathway should be followed. 33 patients notes were reviewed to evaluate the use of the pathway. The review included was the pathway in the notes, was the patient neutropenic, was the patient febrile and if antibiotics given.

Results
Of the 33 patients' notes, 18 patients had the pathway form in their notes, 11 of those had a temperature above 37 degrees.
14/18 were given intravenous antibiotics.
In the 18 patients 4 were found to be neutropenic.
In the remaining 13 patients, 4 were febrile and 3 were given intravenous antibiotics.
More detailed results will be included in the poster.

Conclusion
The immunosuppression card allows the patient quick access through the emergency department but staff will not know if the patient will be immunosuppressed or not when they present. Many patients present with symptoms other than febrile neutropenia although they have a card. More training of emergency department staff may be needed due to staff turnover. Education of ward staff, including a flow chart as to which patients should receive the card on discharge should be considered.

No conflict of interest to disclose
P313
Developing Evidence Based Practice for Autologous Bone Marrow Transplant Nursing

Gillian Sheldon-Collins, Rachel Prall
Royal Hobart Hospital, Hobart, Tasmania, Australia

This poster illustrates documents now in use at the Royal Hobart Hospital for nurses caring for patients undergoing autologous bone marrow transplantation. These documents consist of a clinical pathway for workup, a protocol and pathway for the reinfusion and a daily assessment form incorporating established assessment tools. These forms have been developed to guide and document practice ensuring safety and consistency for patients who transition between the outpatient and inpatient settings. These documents are also clearly identifiable in hospital records as specific to transplantation therefore facilitating current and future data collection. This data is utilised to ensure the service meets Key Performance Indicators. Over the years local protocols had been developed and reviewed according to evidence available at the time. This most recent review of practice involved a literature search and consultation with interstate hospitals. Literature regarding pre-transplant workup and nursing care during the engraftment phase is scant. The majority of articles describe the nursing care of patients undergoing allogeneic rather than autologous transplantation. Therefore these documents enable a high standard of nursing care by filling a gap in the current evidence based guidelines. At the same time they have been designed to ensure the documentation of sound data which can be used to develop future practice.

No conflict of interest to disclose
P314
Nursing Reflections of Stem Cell Collections in 1990 compared to 2011

Nicole Taylor
Nepean Cancer Care Centre, Penrith, NSW, Australia

Aim.
A nostalgic look at stem cell collections from a nursing perspective in 1990 and 2011.

Method
A review of stem cell protocols from a nursing perspective in 1990 compared to 2011. As a Registered Nurse at a major teaching hospital stem cell collections began in the 1990s and continue today. The best way to review the protocols was to create a table for each year listing all relevant sections. A table has been created to compare the differences and similarities.

Results
It appears that the clinical components of a stem cell collection remain unchanged. Machinery used, kits, setup and access remain constant. Medical advancements have increased the predictability and quality of collections.

Conclusion
When returning to Stem Cell Collection training in 2011 it was evident that minimal changes had occurred. COBE spectra was still in use and setup was the same. Access remains unchanged and visual colour recognition of blood product remains important for prediction of collection quality. The most significant medical development has been the use of G CSF. GM CSF was in its infancy in the 1990s. We had participated in clinical trials for chemotherapy patients only. Little did I realize its impact on cancer nursing in years to come. It has enabled quicker recovery post chemotherapy and accurate predictability for collection ensuring adequate staffing and transportation of product. The CD34 count has enabled accurate measurement of circulating cells for collection

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No conflict of interest to disclose
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