

## **001. Barry Firkin Oration: The man behind the oration**

**Salem H**

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I was fortunate to have experienced firsthand what it was like to work and train with Barry Firkin. He was a strong believer in Australian science and had enormous passion for clinical medicine and clinical research. My presentation will focus on the man, what drove him, his successes and lessons learnt en route.

## **002. Ruth Sanger Oration: Will individualised medicine mark the end of randomised controlled trials? 50 years curing childhood acute lymphoblastic leukaemia.**

**Cole C**

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It is 50 years since the first published study from Berlin showed 20% continuous complete remission in children with acute lymphoblastic leukaemia (ALL). Since then, the cooperative groups have shown a slow, step-wise improvement in outcome through a series of randomised controlled trials (RCTs) each building on the last, such that in 2014, 80-90% of children will be cured of the disease with few long term side effects.

The size of the trials, only possible through international cooperation, has allowed the disease to be subclassified into prognostic groups and treatment is risk adapted. These prognostic groups are based on patient characteristics such as age, initial white cell count (WCC) (1960s), and disease characteristics-metaphase cytogenetics (1970s), FISH (1980s), minimal residual disease testing (1990s), and the game-changing addition of imatinib to Ph+ ALL (2000s) such that this is no longer considered of high enough risk to justify transplant in first remission.

As we identify new prognostic markers, each treatment group becomes smaller. Despite international cooperation, randomised comparisons are more difficult, just at the time that new therapeutic agents-small molecules and monoclonal antibodies, bring hope of replacing traditional cytotoxic therapies with fewer long term toxicities.

The challenge for the next 50 years, is to reshape RCTs for patients, and the paradigm for testing new medicines in the pursuit of curing childhood leukaemia.

### **003. Carl de Gruchy Oration: A Cytogeneticists Tale**

**Campbell L**

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It was one hundred years ago that Theodor Boveri theorized that chromosome changes could cause cancer but it was not until 1960 that significant progress was made towards proving this theory. And when Peter Nowell and David Hungerford reported their identification of a small marker in the bone marrow chromosomes of patients with chronic myeloid leukaemia, they cannot have envisaged the explosion of knowledge that the following 50 years would bring to our understanding of the genetic basis of malignancy.

When I first started working in the field of cancer cytogenetics, G-banding was the mainstay of chromosome analysis and in situ hybridization required the use of probes labelled with tritiated thymidine. Now, G-banding has been augmented by fluorescence in situ hybridization, quantitative PCR, comparative genomic hybridization and SNP arrays, and next generation sequencing. How to incorporate these new technologies into the testing algorithm for patients with haematological malignancies is a major challenge for haematologists and cytogeneticists in the coming years

## **004. Generating CD19-specific chimeric antigen receptor expressing T lymphocytes using PiggyBac transposon/transposase gene modification system to treat B-cell malignancies**

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### **Aim**

To produce a simple protocol for the clinical production of CD19-specific chimeric antigen receptor (CAR19) T-cells using the non-viral PiggyBac transposon/transposase gene modification system.

### **Methods**

CAR19 T-cells were produced from peripheral blood mononuclear cells (PBMCs) by electroporation with PiggyBac transposase and CAR19 transposon plasmids and expanded over 3 weeks by stimulation with autologous PBMCs. CAR19 T-cell yields with varying culture conditions were assessed. Applicability of conditions was confirmed with CAR constructs containing the CD28 (CAR19.28z) or 4-1BB (CAR19.4-1BBz) co-stimulatory domains and in patients with B-cell malignancies.

### **Results**

Optimal conditions included an effector:stimulator ratio of 1:2 in gas-permeable rapid expansion flasks supplemented with IL-15. In cultures from 3 healthy donors, expansion of CD19.28z CAR T-cells was 710 (range 680-765) and of CD19.4-1BBz CAR T-cells was 437 (range 79-711). Final CAR expression of CD19.28z was 62.6% (range 57.0-67.5%) and of CD19.4-1BBz was 75.2% (range 64.5-89.7%). CAR19 T-cells produced interferon-gamma and lysed the CD19+ Nalm-6 cell line. Mean percentage specific lysis at E:T ratio 20:1 was 94.9% & 83.1% for CD19.28z and CAR19.4-1BBz respectively.

Expansion and CAR expression in patient cultures were as follows (n=2 for each tumour): B-chronic lymphocytic leukaemia (CLL) - 121 (62 and 180) and 85.6% (80.8% and 90.4%); B-acute lymphoblastic leukaemia (ALL) - 99 (89 and 109) and 82.9% (77.2% and 88.5%); and diffuse large B-cell lymphoma (DLBCL) - 112 (120 and 105) and 68.1% (48.0% and 88.1%). Mean percentage specific lysis of Nalm-6 cells and autologous PBMCs at 20:1 was as follows: CLL (75.3% and 53.8%), ALL (79.7% and 67.0%), DLBCL (70.0%).

### **Conclusion**

We have developed a simple and efficient protocol for expanding CAR19 T-cells from healthy donors and patients. Based on these results, a phase I clinical trial protocol has been developed, testing the safety of PiggyBac generated CAR19 T-cells in patients with relapsed B-cell malignancies.

## **005. The addition of Interleukin-6 inhibition to standard GVHD prophylaxis to prevent acute GVHD**

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<sup>1</sup> QIMR, <sup>2</sup> RBWH

### **Background**

IL-6 mediates graft-versus-host disease (GVHD) in experimental allogeneic stem cell transplantation (alloSCT) and is an attractive therapeutic target.

### **Methods**

A registered phase I/II study (ACTRN12612000726853) of IL-6 receptor (IL-6R) neutralizing antibody administration on day -1 to patients receiving full or reduced-intensity conditioning (RIC) and alloSCT from HLA-matched sibling or unrelated donors with standard cyclosporin and methotrexate GVHD prophylaxis. The primary endpoint was incidence of grade II–IV acute GVHD. Outcomes were compared to a non-randomized but contemporaneous group of study patients receiving the same alloSCT in the absence of IL-6R mAb. The final results are presented here.

### **Results**

Cytokine and pharmacokinetic analysis confirmed transient IL-6 dysregulation in the first month after alloSCT with complete inhibition following IL-6R mAb administration. With median follow up of 497 days, the incidence of grade II-IV GVHD was 12.5% in recipients of IL-6R inhibition (n = 48) versus 41.5% in the (n = 53) control cohort ( $P = 0.001$ ). Protection was noted in patients receiving both myeloablative (12.5% vs. 46.4%,  $P = 0.03$ ) and RIC (12.5% vs. 36.0%,  $P = 0.04$ ). The incidence of grade III/IV acute GVHD was 4.2% in recipients of IL-6R inhibition versus 20.8% in the control cohort ( $P = 0.012$ ). Relapse and chronic GVHD were unchanged. Immune reconstitution was preserved in recipients of IL-6R inhibition, but qualitatively modified with suppression of known pathogenic STAT3–dependent pathways.

### **Conclusions**

IL-6 is the principal cytokine dysregulated after alloSCT and its inhibition protects from acute GVHD despite robust immune reconstitution, without compromise of the graft-versus-leukaemia effect.

## **006. GRP78 (78-kDa glucose-regulated protein) as a biomarker for clinical outcome and as a potential therapeutic target in multiple myeloma**

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### **Background**

Induction of molecular chaperone GRP78 (78-kDa glucose-regulated protein) occurs in stress conditions and is associated with chemoresistance in solid tumours. We have shown that GRP78 is overexpressed in multiple myeloma (MM) cell lines compared to other cell lines. We investigate the association of GRP78 expression on clinical outcome in patients with MM to see whether this correlates with the pattern of in vitro chemosensitivity of MM cell lines based on their level of GRP78 expression.

### **Method**

Clinical data was abstracted for 243 patients with newly diagnosed MM at St.Vincent's Hospital Melbourne, who were treated with front-line autologous stem cell transplant (ASCT) between 2000-2014. Immunohistochemistry(IHC) was done to examine GRP78 expression in CD138+ myeloma cells on patients' baseline bone marrow trephine. The association of the level of GRP78 expression to depth of response, progression free survival(PFS), time-to-next-treatment(TTNT) and overall survival(OS) was assessed using Kaplan-Meier product limit method and the Mantel-Cox logrank test. GRP78 expression was also quantified in various MM cell lines by RT-PCR and western blot. The association of GRP78 expression to chemosensitivity was assessed.

### **Result**

Low GRP78 expression by IHC was associated with a shorter PFS (HR 2.4, p=0.0006) and shorter TTNT (HR 2.5, p=0.008) compared to intermediate or high GRP78 expression. No significant difference was seen in OS. High GRP78 correlated with a higher probability of achieving CR (p=0.03). In vitro, the RPMI8226 MM cell line had the highest level of GRP78 expression compared to U266 and H292 MM cell-line. Preliminary data showed no differences in chemosensitivity between these cell lines. The effect of selectively inhibiting GRP78 in MM cell line is pending.

### **Conclusions**

GRP78 is a useful biomarker in predicting response and survival outcome in patients with MM. GRP78 overexpression in myeloma cells renders it a potential therapeutic target in MM that warrants further investigation.

## 007. The Bcl-2 inhibitor ABT-199 co-operates with chemotherapy to overcome drug resistance mechanisms in acute myeloid leukemia

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### Aim

Drug resistance remains a major obstacle to improved outcomes in acute myeloid leukaemia (AML). Directly targeting pro-survival proteins in AML may synergize with chemotherapy, improving the sensitivity of drug resistant AML. This work assesses which Bcl-2 proteins mediate pro-survival activity in AML and the benefit of combining drugs targeting Bcl-2 with chemotherapy.

### Method

Primary AML samples were treated with ABT-737 (targeting Bcl-2, -xL, -w), ABT-199 (targeting Bcl-2), A-5463 (courtesy of Prof Huang, WEHI, targeting Bcl-xL), SNS-032 (downregulating Mcl-1) alone, and in combination with idarubicin or cytarabine. Colony assays were performed in agar supplemented with cytokines. NOD SCID gamma (NSG) mice were transplanted with MV4;11 cells transduced with lentiviral vectors expressing tet-inducible Bim<sub>s</sub>2A (targeting Mcl-1, courtesy of Dr Glaser, WEHI).

### Results

*In vitro*, 5/12 of AML samples were killed by ABT-199 (LC50 of <1µM). A strong correlation between ABT-737 and ABT-199 sensitivity was observed, suggesting apoptosis was mediated by Bcl-2 and not Bcl-xL. This was confirmed by lack of AML sensitivity to A-5463. *In vivo*, mice xenografted with MV4;11 treated with ABT-199 75mg/kg daily for 5 days delayed, but did not prevent leukaemic death. However, combined targeting of Bcl-2 (ABT-199) and Mcl-1 (Bim2A) cured mice xenografted with leukaemia. Examination of the effect of cytotoxic drugs on Mcl-1 revealed that high concentrations of idarubicin caused Mcl-1 downregulation and p53/Noxa/PUMA-independent cell death; low concentrations induced p53/Noxa/PUMA-dependent cell death but was ineffective at downregulating Mcl-1. Combining ABT-199 with high-dose idarubicin was able to overcome resistance conferred by p53 and Bak deficiency. Furthermore, the ABT-199/idarubicin combination synergistically killed primary AML cells. Importantly, the combination selectively inhibited CFU activity of AML cells while relatively sparing normal CD34+ haemopoietic stem cells.

### Conclusion:

1. Neutralizing Bcl-2 targets a subset of primary AML cells *in vitro*.
2. Targeting Bcl-2 and Mcl-1 simultaneously is more effective at suppressing AML survival *in vivo*.
3. High-dose idarubicin is capable of downregulating Mcl-1.
4. The combination of ABT-199 and idarubicin synergistically targets AML survival *in vitro*.

## 008. Tractopods: Novel platelet membrane anchors promoting platelet-endothelial interactions and thrombo-inflammation

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### Background

Platelets have an important proinflammatory function linked to a broad range of human diseases, including ischemia-reperfusion (I/R) injury. However, the mechanisms by which platelets promote leukocyte recruitment to the ischemic microvasculature remains ill-defined. Utilising a mouse model of I/R injury in conjunction with a novel high-resolution intravital imaging system and in vivo thrombosis models, we sought to investigate the thromboinflammatory role of platelets.

### Results

Here we demonstrate the existence of two distinct leukocyte recruitment mechanisms mediated by platelets. The first involves leukocyte recruitment by elongated, discoid platelets that adhere to ischemic endothelial cells through a previously unknown adhesion structure that we have termed tractopods (Latin: *tractus* – to pull; Greek; *pod* – foot; tractopod = pulling foot). Tractopods exhibit a complex, branched morphology induced by localized cytoskeletal remodeling. Tractopod formation is initiated by integrin  $\alpha_{IIb}\beta_3$  by outside-in signaling events that are sufficient to promote stable platelet adhesion and low level P-selectin expression, independent of thrombin, adenosine diphosphate (ADP) and thromboxane  $A_2$  (TXA<sub>2</sub>). Tractopod-adherent platelets mediate the slow, progressive accumulation of leukocytes to sites of endothelial perturbation over several hours. The second leukocyte recruitment mechanism involves the conversion of tractopod-adherent platelets to fully activated platelets through the generation of thrombin. This process leads to microvascular thrombus formation, and the rapid (over 5-10 mins), dramatic increase (>10 fold) in the number of leukocytes adherent to sites of vascular injury.

### Conclusion

These studies define a key role for platelet tractopods in initiating two distinct mechanisms of leukocyte recruitment; one that is likely to be relevant to chronic inflammatory changes initiated by platelet-endothelial interactions, and a second, that mediates rapid and profound leukocyte recruitment relevant to acute thrombo-inflammatory diseases.

## **009. GFI1B mutation causes a novel human platelet defect with heterogeneous deficiency of alpha-granules and altered expression of platelet proteins**

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**Aim:** The study aimed to identify and characterise an autosomal dominant bleeding disorder present in a four-generation Australian family.

**Method:** Genetic linkage analysis and massively parallel sequencing were used to localise the mutation causing the disease phenotype on chromosome 9. Functional studies were then performed on platelets and megakaryocytic cell lines to determine the biological effects of the mutation.

**Result:** Bleeding scores for affected individuals were increased with affected patients experiencing both mucosal bleeding and excessive bleeding after surgery. Blood film examination demonstrated macrothrombocytopenia and red cell anisopoikilocytosis. PFA100 closure times were prolonged in affected family members and all affected individuals demonstrated markedly impaired platelet aggregation responses to collagen. Genotyping with a SNP array followed by massively parallel sequencing on telomeric chromosome 9 identified a single nucleotide insertion in exon 7 of GFI1B leading to a frameshift mutation. GFI1B is a transcription factor important for haematopoiesis but previously unknown to be associated with human disease. This mutation disrupts the DNA-binding region of the fifth zinc finger domain. The identified mutation in GFI1B alters the transcriptional function of the protein in a dominant negative manner with the introduction of the c.880-881insC mutant transcript de-repressing the promoter of validated GFI1B target gene TGFBR3 and GFI1B itself in megakaryocytic cell lines as measured by a luciferase assay (TGFBR3 16.6 vs 23.8,  $P=0.03$ ; GFI1B 0.73 vs 2.24,  $P<0.01$ ). The number of platelet alpha-granules was reduced in affected individuals (1.3 vs 3.1,  $P<0.001$ ) and this was associated with marked reductions in alpha granule-related proteins P-selectin and fibrinogen (84% vs 21%,  $P<0.001$  and 75% vs 26%,  $P<0.001$ ). Introduction of the mutant transcript into megakaryocytic cell lines recapitulated this phenotype with significant reductions in P-selectin (1.06 vs 0.69,  $P<0.001$ ).

**Conclusion:** GFI1B mutation causes a novel human bleeding disorder with variable alpha-granule deficiency and red cell shape change.

**010. 50 years of ANZSBT**

**Benson S**

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**ABSTRACT NOT SUBMITTED**

## 011. Horses for courses? Clinical management and outcomes in massively transfused patients across clinical specialties: An update from the ANZ Massive Transfusion Registry (ANZ-MTR)

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### Background/Aim

Critical bleeding (CB) requiring massive transfusion (MT) occurs across clinical specialties however there is a paucity of information regarding CB/MT management in the non-trauma setting. The ANZ-MTR was established to generate observational data on the types & frequency of conditions associated with CB/MT, ratios & quantities of blood component therapy used, and patient outcomes across all clinical specialties.

### Method

All adult patients receiving a MT ( $\geq 5$  units of red blood cells [RBC] in 4h) were identified at 16 Australian & NZ hospitals between April 2011-Dec 2013. Patient data, including transfusion history, laboratory results & hospital admission data, were extracted.

### Results

A total of 2280 MT patients were identified. Median age was 63y [IQR46-74]; 62.4% were male. Differences in presence of comorbidities, coagulopathy, non-RBC component use and mortality were observed between clinical groups (Table 1).

Table 1	All	GI Haem	Trauma	CT Surg	Liver Surg	Vasc Surg	Obstet
MT patients (% total)	<b>2280 (100)</b>	317 (13.9)	357 (15.7)	307 (13.5)	140 (6.1)	181 (7.9)	144 (6.3)
% comorbidity present	<b>66.8</b>	81.1	32.5	73.0	94.3	92.8	4.2
Median [IQR] RBC units (24h)	<b>8 [6-11]</b>	7 [6-10]	8 [6-12]	8 [6-11]	10 [7-15]	9 [6-13]	7 [6-10]
FFP:RBC (4h)	<b>0.5</b>	0.4	0.5	0.4	0.7	0.5	0.6
Median [IQR] Cryo units (24h)	<b>1 [0-8]</b>	0 [0-4]	2 [0-8]	4 [0-10]	10 [4-20]	2 [0-8]	0 [0-4]
% PTX use	<b>8.7</b>	13.3	5.6	11.4	5.7	12.2	0
Mean INR pre-MT onset	<b>1.5</b>	1.7	1.5	1.4	2.0	1.4	1.1
% in-hospital mortality	<b>21.1</b>	27.4	25.2	24.7	8.6	38.7	2.1

### Conclusion

Preliminary results from the ANZ-MTR show variation in the management and outcomes of MT across clinical groups and generally high mortality associated with CB/MT. Further analysis of contributing factors is underway. More research into management of MT in different clinical contexts is needed.

## 012. Carbohydrates on red blood cells: More than just blood group antigens

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### Background and Aims

Posttranslational glycosylation, in the Golgi apparatus, addresses proteins for transport to the cell surface and reduced activity in enzymes which catalyse these glycosylation steps in developing red blood cells (RBC) is reported to result in haematological disease (Khoriaty *et al.*, 2012 Blood, 120(1):31-38). Acetylation in sialic acid of gangliosides is anti-apoptotic for tumour cells (Birks *et al.*, 2011, Neuro-Oncology 13(9):950-60), however for RBC 9-O-acetylation has pro-apoptotic effect (Mukherjee *et al.*, 2007, BBRC 362:651-7). In this study a model of *in vitro* RBC differentiation was used to examine associations between carbohydrate modification and the RBC lifecycle.

### Methods

Erythroid leukaemic cell lines K562 (human) and BB88 (murine) were exposed to cytosine  $\beta$ -D-arabinofuranoside, mitomycin C, butyric acid, dimethyl sulfoxide (DMSO), *N,N'*-Hexamethylene bis(acetamide) (HMBA) and all-trans retinoic acid (RA). Erythroid differentiation was assessed by the expression of glycophorin A (K562) and TER119 (BB88) via flow cytometry. Haemoglobin expression was determined via flow cytometry, qPCR and Western blot. Changes in surface carbohydrates were examined using a panel of lectins (*Maackia amurensis*, *Erythrina cristagalli*, *Sambucus nigra*, *Triticum vulgare* and *Cancer antennarius*, EY).

### Results

Following 4 days exposure to 0.72  $\mu$ M cytosine  $\beta$ -D-arabinofuranoside (for K562) or 1.92  $\mu$ M DMSO (for BB88) differentiation and haemoglobin expression were observed. The array of cell surface carbohydrates changed significantly during differentiation as evidenced by changes in the lectin binding profile. Of particular interest, changes reflecting disruption of acetylation of RBC surface carbohydrates were evident.

### Conclusions

We have utilised *in vitro* models of RBC differentiation to examine the role of carbohydrate modification in RBC differentiation. These data suggest that modification of carbohydrates is important, not only in terms of blood group antigens, but also in regulation of the RBC lifecycle.

## **013. Neonatal alloimmune thrombocytopenia (NAIT): Initial data from the Australian Registry**

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### **Aim/Background**

Neonatal alloimmune thrombocytopenia (NAIT) is rare but important. Presentation is variable (ranging from incidental thrombocytopenia to severe intracranial haemorrhage) and often unexpected. The national NAIT Registry aims to better define the incidence, natural history and clinical outcomes of NAIT in Australia, and comprises cases of pregnant women who develop or have a history of NAIT, and their children.

### **Methods/Results**

The NAIT registry commenced in 2009 with 8 hospitals; 29 hospitals now participate nationally and 50 cases have been accrued. 32 of 50 (64%) cases were not anticipated and identified following delivery. Two cases were identified during pregnancy and 16 were anticipated in the setting of past history of a neonate with thrombocytopenia, confirmed NAIT or a family history. The most common clinical presentation was incidental thrombocytopenia found in 16/50 cases (32%), whilst intracranial haemorrhage was seen in 3/50 neonates (6%). Two deaths were reported, one definite and one likely from NAIT. Twenty-nine cases (58%) were confirmed as definite NAIT due to anti-HPA-1a or anti-HPA-5b antibodies. Ten cases (20%) were classified as probable or possible NAIT and included pregnancies where mothers received expectant antenatal treatment with IVIG/steroids. The most commonly identified antibody in confirmed cases was anti-HPA-1a (80% of cases), in keeping with international studies.

### **Conclusions**

Experience from the national NAIT registry has highlighted the difficulties capturing confirmed cases of this rare condition. Whilst diagnosis of anti-HPA-1a NAIT may be fairly straight-forward, rarer platelet antibodies, concurrent HLA antibodies and/or incomplete investigations can hinder a complete workup and diagnosis. Neonates with unexpected thrombocytopenia should undergo thorough laboratory investigation for NAIT. The implications for future pregnancies are significant, including the risk of neonatal intracranial haemorrhage and the intensity of treatment required to manage an at risk pregnancy. The registry is ongoing and welcomes new sites and new cases.

## **014. Remote Release by e-Blood Management System: Results of Long Term Safety and Reliability**

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### **Aim**

A safe and reliable method for release of blood remotely after an electronic crossmatch can significantly reduce wastage and improve efficiency in transfusion practice. This study evaluated the long-term safety and reliability of remote release practice utilizing 'e-Blood', an electronic cross match system developed in-house.

### **Method**

Remote release is defined as release of blood product at a site remote from the main transfusion laboratory by a non-laboratory end-user trained in use of the software. A retrospective analysis of 'e-Blood' data evaluating remote release sites in the network and total number of users was undertaken. The total number of products released, cross-match ratio and average time to release was analyzed. Software failures, outages, errors in cross-match releases or reported near misses were analyzed. Logistical hurdles and sites where remote release was discontinued were specifically evaluated.

### **Results**

The 'e-Blood' data was analyzed over the period from 1999 to 2014 (total of 15 years). A total of 10 remote sites and 945 users were recorded. Compliance with software requirements as per Australian and international standards was noted. A total of 20078 red cell units, 628 emergency group 'O' and 11382 batch products were released safely and accurately in this period. There were no episodes of misidentification or error in release by the software. Remote release was discontinued at two sites during this period for reasons unrelated to software function. Outages had a minimal impact on the electronic remote release.

### **Conclusion**

This study reports the long-term results of networked electronic remote release of blood products by non-laboratory staff. An electronic software system that has been locally developed in Australia known as 'e-Blood' enables remote release of blood products in a safe and reliable manner.

## **015. Transfusion information for patients with intellectual disabilities**

**Thrift L**

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### **Introduction**

Clinical Transfusionists are good at providing information leaflets regarding blood transfusions in a variety of formats and languages. However, there is one group of patients that have not been considered in New Zealand - those with an intellectual disability.

### **Aim**

In MidCentral District Health Board (DHB) the Transfusion Nurse Specialist (TNS) and the Clinical Nurse Specialist (CNS) for patients with intellectual disabilities felt developing an information leaflet for those with intellectual disabilities receiving blood transfusions should be addressed, as it was linked to the consent process.

### **Method**

A trial document was developed and assessed prior to publishing. Both words and pictures were used. Care was given with the pictures as they could not use too many pictures of people and the same pictures had to be used for the same purpose.

Each point of receiving a blood transfusion was identified, separated and a picture and word format utilised.

The leaflet was then piloted by the CNS and given to people with intellectual disabilities in a variety of age groups to establish their thoughts and opinions.

These were all taken into consideration, the general response was positive and the leaflet was found to be easily understood.

### **Conclusion**

When the document is given full approval, the information leaflet will be printed in to a booklet, for use throughout the DHB.

This will allow more of our patients the opportunity to receive informed consent prior to their blood transfusion.

## **016. Interrogating the architecture of cancer genomes**

***Campbell P***

*Wellcome Trust Sanger Institute, Cambridge, UK*

Cancer is driven by mutation. Using massively parallel sequencing technology, we can now sequence the entire genome of cancer samples, allowing the generation of comprehensive catalogues of somatic mutations of all classes. Bespoke algorithms have been developed to identify somatically acquired point mutations, copy number changes and genomic rearrangements, which require extensive validation by confirmatory testing. The findings from our first handful of genomes illustrate the potential for next-generation sequencing to provide unprecedented insights into mutational processes, cellular repair pathways and gene networks associated with cancer development. I will also review possible applications of these technologies in a diagnostic and clinical setting, and the potential routes for translation.

## **017. Defining high risk myeloma**

**Lonial S**

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The improvement in outcomes for myeloma patients all over the world, has been a consequence of the development of new agents and new treatment strategies. However, one group of patients that appears to not have gained the same benefit are patients with high risk myeloma. These patients often rapidly develop drug resistance, particularly when single agents or lack of intensive treatments are utilized to manage their disease, and can be associated with early death as a consequence of rapidly acquired drug resistance. Defining high risk myeloma has incorporated the use of simple testing such as B2M, CRP, or LDH from the peripheral blood, or can involve the use of more modern testing such as conventional cytogenetics, FISH testing, gene expression profiling (GEP), and most recently genome sequencing. Identification of these high risk patients early in the disease course allows for the initiation of appropriately aggressive therapy and maintenance therapy as well. Such aggressive approaches with minimal exposure to alkylating agents, has allowed our group to demonstrate unprecedented improvements in PFS and OS even among genetically identified high risk patients and warrants further study.

## 018. Prognostic factors in Waldenstroms

Leleu X

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Waldenstrom's macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by bone marrow infiltration of lymphoplasmacytic cells and monoclonal IgM gammopathy in the serum. The median age is approximately 70 years old. The estimated median survival is of 5 to 7 years, although 11 years if considering waldenstrom-related death; which confirms that although systematically mortal, WM does not carry the same adverse prognosis as to Myeloma for example. WM is usually diagnosed as indolent or symptomatic, and no prognostic scoring system has been developed for indolent WM to determine the risk of developing symptomatic disease. Regarding symptomatic WM, the IPSS WM scoring system was published recently, based on age, hemoglobin and platelet counts, and serum IgM M spike, beta 2 microglobulin levels. This score is quite complicated and was not developed for patients treated in the era of novel agents and as such should be ideally reframed. Starting in the early 2000, the physiopathology of WM started to be better unravelled, with the first reports of the deletion 6q, the most frequent cytogenetic aberration in WM; it was suspected that this region harbours a tumour suppressor gene of pathogenic significance for WM. However, no clear and definite prognostic relationship was linked to del6q in WM. More recently, a groundbreaking discovery was made on WM based on whole exome sequencing, confirmed thereafter using various sequencing techniques with various sensitivity that was the discovery of an alteration of the MYD88 gene locus in approximately 90% of WM, essentially via the L265P mutation. This alteration appears to be present in IgM MGUS or WM with a very limited tumour burden, which tends to propose MYD88 mutation as a possible first genetic hit in WM that promotes NF- $\kappa$ B and JAK-STAT3 signalling, and subsequently initiates alteration of major pathways, such as apoptotic pathways. WM cells may acquire additional genetic hits over time, mediated through loss of heterozygosity, gene amplification or epigenetic changes that may potentially contribute to further deregulation of the WM clone and promote tumour progression. In that regards, alteration of the MYD88 gene locus does not seem to carry any prognostic impact, confirmed on various studies that have confirmed the lack of survival impact; MYD88 L265P mutation appears to be more of a molecular signature of WM. Finally, CXCR4 C1013G was recently identified in approximately 20% of the WM, alongside MYD88 L265P mutation, being therefore the second most frequent mutation described in WM. However, it was recently reported that CXCR4 C1013G mutation was related to significant tumor proliferation and dissemination to extramedullary organs, leading to disease progression and decreased survival in WM. The presence of the mutation was also associated to drug resistance in WM cells exposed to Bruton's tyrosine kinase, mammalian target of rapamycin, and phosphatidylinositol 3-kinase inhibitors, but not proteasome inhibitors.

In conclusion, in the era of molecular medicine, 2 key molecular markers were identified in a one year time, MYD88 L265P and CXCR4 C1013G mutations, the former more likely will be used a molecular signature, while the latter might become the first ever described molecular prognostic marker in WM.

## **019. CAR T cells**

**Campana D**

*National University of Singapore*

Despite advances in the treatment of hematologic and non-hematologic malignancies, a substantial proportion of patients do not attain durable complete remissions. Even when treatment is successful, it may have serious toxicities and long-term sequelae. Immunotherapy as a cancer treatment is attractive because it can potentially bypass the resistance of cancer cells to standard therapy while sparing normal tissues. In addition to therapeutic antibodies and vaccine strategies, infusion of immune cells that can directly lyse tumor cells is being increasingly implemented in the clinic. Results of recent clinical trials have demonstrated the tremendous potential of infusing autologous T cells redirected against tumor cells via the expression of chimeric antigen receptors ("CAR"), with the most impressive responses being achieved by targeting CD19 in patients with B-cell malignancies. These results have spurred great interest in this area of translational research and encouraged efforts to further improve T cell therapy of cancer. To overcome the need of developing an individual CAR for each target and allow targeting of multiple cancer antigens simultaneously, we recently developed a CD16V-41BB-CD3z receptor which endows T cells with antibody-dependent cell cytotoxic capacity. This receptor has shown promise in preclinical studies and is about to be tested clinically. Current research is directed towards developing ways to enhance T cell anti-tumor activity and curb its possible acute and chronic side effects. Simplifying ex vivo cell processing, widening the range of targetable antigens and generating a safe allogeneic T cell product are also important objectives to move this promising field forward.

## **020. Mesenchymal stromal cells: Applications in haematology and beyond**

**Keating A**

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There has been extraordinary interest over the past decade in the clinical translation of mesenchymal stromal cells (MSCs) or mesenchymal stem cells as they are also termed.

This was related in part to the notion that MSCs had stem cell-like properties with the capability of differentiating along numerous lineages, including those of non-mesodermal origin, thereby enabling them to replace the injured cells of many different tissues. Fortunately, this concept has given way to the current thinking that MSC-mediated tissue regeneration is related to the paracrine release of bioactive molecules, especially those promoting anti-inflammatory effects. Despite considerable advances, with few exceptions the outcomes of numerous clinical trials with MSCs have not lived up to early promise. Several explanations can be provided for these shortcomings but a reappraisal of the field is underway and there is cause for optimism. In that context, pre-clinical studies and clinical trials will be evaluated and future directions for the clinical translation of MSCs will be discussed.

## 021. Treatment of "the older patient" with Chronic Lymphocytic Leukaemia

Leblond V

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Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia, and mainly affects older patients. The age threshold used to define elderly patients varies greatly, with chronological age not being a reliable indicator for defining the aged patients. Moreover, the health status of the elderly depends on several factors: reduced homeostasis; altered drug pharmacokinetics, comorbidities that may increase drug toxicity; unstable functional status; interaction of psychosomatic, functional, and social factors. It is thus necessary to decide whether the patient's "medical condition" is compatible with the administration of treatment schemes considered to be optimal for the disease. The management of an elderly patient must therefore begin by identifying frailty, geriatric syndromes (dementia, incontinence, falls, undernutrition, etc.), and comorbidities. First-line treatments for 'very fit' and 'very unfit' CLL patients are well defined in the form of FCR combination chemoimmunotherapy and chlorambucil monotherapy, respectively. However, the majority of CLL patients fall between these two extremes and the standard-of-care for these patients is not well defined. In elderly patients eligible to fludarabine-based regimen, the higher rates of adverse events in this population have led to debate as to the tolerability and feasibility of this standard treatment in the elderly, with an adaptation modification of FCR doses or the reduction of the number of cycles (Mulligan SP, E Vandeneste 2014). In elderly patients who are ineligible to fludarabine, several trials have been conducted, evaluating novel cytostatic agents, combination chemotherapy and chemoimmunotherapy regimens. The CLL 11 trial showed a longer progression-free survival and overall survival time in patients treated with Obinituzumab and Chlorambucil as compared with chlorambucil (Goede 2014). The combination of Ofatumumab and chlorambucil was more effective than chlorambucil in the Complement-1 trial (Hillmen 2013). The results of the MaBLE trial comparing the efficacy of Rituximab + chlorambucil to Rituximab + bendamustine are pending. Chemo-free regimen could be also an option in frail patients: lenalidomide and more recently BCR signaling inhibitors have been evaluated in elderly patients used alone or in combination with anti-CD20 monoclonal antibodies (Badoux 2011). Ibrutinib gave a high overall response rate with 24 month progression-free survival of 96.3% and was well-tolerated in elderly patients (O'Brien 2014).

In conclusion it is necessary to avoid overtreating a frail patient with poor prognostic factors, but also undertreating a patient when effective and relatively well-tolerated treatments may be proposed. A well-defined, easy-to-use scale to determine comorbidity and fitness among CLL patients would be a useful tool to help improve clinical decision-making and tailor the most appropriate treatments to defined CLL patient subsets.

## 022. Novel therapies in CLL

### Mulligan S

*Royal North Shore Hospital, Sydney, NSW*

The therapy of CLL is on the cusp of another revolutionary change with the introduction of B-cell receptor signal pathway receptor inhibitors (BCRi), and bcl-2 inhibitors. Novel antibodies, particularly obinutuzumab, have also contributed to significant progress in CLL, especially patients with co-morbidities as shown with the CLL11 study. Drugs that block molecules within the BCR pathway such as Bruton's tyrosine kinase, particularly ibrutinib, and PI3Kinase, particularly idelalisib have both shown dramatic effectiveness in patients with relapsed and refractory (R/R) CLL. These agents have a highly characteristic clinical feature with the occurrence of a significant lymphocytosis in a high proportion of patients concurrent with dramatically beneficial effects with major reduction in lymphadenopathy and splenomegaly, together with improvement in bone marrow function. These agents appear to function primarily by blocking BCR signalling within the lymph node microenvironment proliferation centre, the principal location for maintenance of CLL cell survival and proliferation. Btk is a member of the Tec-kinase family which also includes Inducible T-cell kinase (ITK) and Tec-kinase and off (Btk) target Tec-kinase inhibition may be responsible for some of the adverse events seen with ibrutinib such as bruising and atrial fibrillation. A number of other inhibitors of Btk have also been developed and are under investigation. BCRi's may cause diarrhoea, and with idelalisib, a small proportion develop colitis. The activity of these agents appears to be effective across all CLL adverse risk groups although follow-up is still relatively short. Three large clinical trials comparing ibrutinib plus rituximab versus the fludarabine, cyclophosphamide, rituximab (FCR) combination are in progress. A very small number of patients develop secondary resistance after two years or more and most of these have acquired mutations in either Btk or PLC $\beta$ 2. A number of small molecule inhibitors of other BCR pathway molecules are under investigation. The bcl-2 anti-apoptotic protein is over-expressed in CLL and several attempts to block this machinery have been made with oblimersen and novatoclax. Abt-199 is the only agent in ongoing development in this class but also appears extremely promising. Prior problems with tumour lysis syndrome (TLS) appear to have been resolved with an incremental dosage schedule over 5 weeks. Clinical trials with this agent in 17p-deleted and R/R CLL remain in progress. The BCRi and bcl-2 inhibitors are already crucial in the management of R/R CLL, and will no doubt change the way in which we treat CLL over the coming years.

## **023. PET in low grade B NHL**

**Hutchings M**

*Copenhagen University Hospital, Copenhagen, Denmark*

Multiple studies have shown high diagnostic and prognostic value of PET and PET/CT in patients with high-grade B-cell lymphomas. As a consequence PET/CT is considered standard of care for the staging and restaging of these patients. Fewer studies have looked at low-grade B-cell lymphomas, but recent data indicate that PET/CT increases the staging accuracy and results in clinically important stage migration in follicular lymphoma, mantle cell lymphoma, and nodal marginal zone lymphomas. For all indolent lymphomas the impact on patient outcome of the changes in risk stratification and treatment approach caused by PET/CT is yet largely unclear. Nevertheless, PET/CT is recommended for staging of all FDG-avid indolent lymphomas, while CT remains the recommended imaging tool for non-FDG-avid lymphomas, including extranodal marginal zone lymphoma and small lymphocytic lymphoma. PET/CT may be a useful investigation in CLL with suspected Richter's transformation, particularly as a guide to selection of biopsy site.

There seems to be a good prognostic value of interim PET/CT in follicular lymphoma, but the clinical implications are unclear, since it is uncertain if early treatment intensification to poorly responding patients will positively affect outcome. This is in contrast to high-grade B-NHL, where PET response-adapted therapy is being investigated in several trials.

A number of studies have demonstrated a good prognostic value of post-treatment PET/CT in follicular lymphoma. Even though this could impact the selection of patients for maintenance therapy, the clinical implications are still unclear.

There are no data to support the use of PET/CT or other imaging modalities in the routine follow-up of low-grade B-NHL patients.

## **024. Minimal residual disease monitoring of acute myeloid leukaemia**

**Coustan-Smith E**

*National University of Singapore, Singapore*

The classification of acute myeloid leukaemia (AML) is defined by a combination of clinical, morphologic, immunophenotypic and genetic features. This combined approach has been used to improve risk assignment and predict response to therapy. However, it remains difficult to estimate prognosis with precision on the basis of presenting features. To this end, minimal residual disease (MRD) monitoring can provide additional useful information. Flow cytometry allows monitoring of MRD in the majority of patients with AML. It can distinguish leukemic from normal immature myeloid cells by detecting multiple immunophenotypic abnormalities. Contemporary flow cytometers can detect 8 or more markers simultaneously, a feature that increases the reliability and sensitivity of the assay. MRD levels measured by flow cytometry after remission induction chemotherapy are an important indicator of outcome. When measured at subsequent time-points, tracking of MRD may allow timely changes in treatment strategies. MRD is increasingly being used as an inclusion criteria and a response parameter in clinical trials of novel agents. Factors that can impact quality such as sample preparation, choice of fluorochromes and instrument stability will be discussed. Details of antibody panels will be described including markers that can clearly distinguish normal myeloid progenitors from leukemic blast cells.

## **025. Minimal residual disease in paediatrics acute lymphoblastic leukaemia**

**Campana D**

*National University of Singapore, Singapore*

In children with acute leukaemia, peripheral blood and bone marrow samples are periodically examined to monitor response to therapy and recovery of normal hematopoiesis. This is traditionally done by assessing cell morphology, a practice that lacks sensitivity and is prone to errors. Assays that can detect minimal residual disease (MRD), referring to leukemic cells undetectable by standard methods, have, by definition, a higher sensitivity than morphology and are generally much more precise. The most reliable methods to study MRD in acute leukaemia are flow cytometric detection of aberrant immunophenotypes and polymerase chain reaction amplification of rearranged immunoglobulin and T-cell receptor genes. These methods can detect 1 leukemic cell among 10,000 or more normal bone marrow or peripheral blood cells and are applicable to most patients. The results obtained with the two methods in childhood acute lymphoblastic leukaemia (ALL) are highly concordant. Newer methods relying on deep sequencing of antigen receptor genes promise to improve the sensitivity of molecular analysis of MRD in ALL. With this approach, it is also possible to monitor clonal evolution during treatment. MRD studies can have a great impact on the clinical management of patients with acute leukaemia. For example, it has been known for more than 4 decades that early response to therapy is a strong prognostic factor in ALL; MRD methods can considerably refine assessment of early treatment response and provide powerful prognostic parameters for risk-classification algorithms. Prospective studies of MRD in patients with newly diagnosed ALL have shown that presence of MRD in bone marrow is strongly and independently associated with a higher risk of relapse. There is strong evidence that MRD levels before hematopoietic stem cell transplantation in patients with ALL are closely related to the risk of relapse post-transplant. Recent methodological advances and novel clinical applications of MRD will be discussed.

**026. Chronic graft versus host disease**

**Apperley J**

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**ABSTRACT NOT SUBMITTED**

## **027. Posttransplant Lymphoproliferative disorders**

**Leblond, V**

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Prevention of organ rejection requires long-term immunosuppression, which places recipients at an increased risk of both infections and neoplastic diseases such as Kaposi's sarcoma and posttransplant lymphoproliferative disorders (PTLDs). Patients who have received solid-organ transplants have a 20- to 120-fold higher incidence of non Hodgkin's lymphoma, depending on the degree and duration of immunosuppression. PTLDs have been reported after transplantation of kidneys, bone marrow, heart, heart and lung and liver. They are mostly of B-cell origin and are associated with active infection by Epstein-Barr virus (EBV) in 50% of cases. EBV-negative PTLD are observed later after transplantation and met the criteria of B-DLCL, mimicking the morphology and clonality found in immunocompetent hosts (Leblond 2008). These lymphomas characteristically have a rapid onset, aggressive behavior and a tropism for extranodal sites, and show partial or complete regression after reduction or withdrawal of immunosuppressive therapy. The risk of developing PTLD is influenced by the immunosuppressive regimen, the organ and the EBV status of the recipient, EBV negative recipient receiving EBV positive graft having a 10 to 20 fold higher incidence of PTLD. The EBV viral load can be an early predictive factor of developing PTLD after transplantation, allowing a pre-emptive intervention

(Choquet 2014). At the time of the disease, decrease of immunosuppressive drugs, anti-CD 20 monoclonal antibodies used alone (Choquet 2006) or in combination with chemotherapy (Trappe 2012), T-cell therapy with autologous or allogeneic EBV specific T-cells (Gallot 2014) could be therapeutic options. The management of CNS-PTLDs is still a challenge (Evens 2013).

In conclusion, since 2000, the outcome of PTLDs has been improved, with the use of immunotherapy. A pre-emptive intervention could be useful in high-risk patients after organ transplantation.

## **028. Sexuality: An important quality of life issue**

**Tierney K**

*Stanford School of Medicine, Stanford, CA, USA*

The diagnosis and treatment of cancer can result in significant changes in sexuality affecting the quality of life not only of the hematopoietic cell transplant survivor but also his/her sexual partner(s). This presentation will review the physiologic, psychological and social dimensions of altered sexuality following hematopoietic cell transplant. Barriers to assessing and treating alterations in sexual health will be explored and intervention strategies to address sexual dysfunctions will be provided.

## **029. The addition of lenalidomide to azacitidine in higher risk MDS is deliverable with promising response rates: First analysis of the ALLG MDS4 study**

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### **Background**

Although azacitidine (AZA) has improved treatment of MDS, virtually all patients (pts) progress with poor prognosis and improved therapies are needed. We present a first analysis of ALLG MDS4 – randomised phase II study comparing AZA +/- lenalidomide (LEN) in higher risk MDS.

### **Methods**

Thirty centres participated; eligible pts had low blast AML, MDS (RCUD and RARS with at least one clinically significant cytopenia) or non-proliferative CMML. Treatment for all pts was AZA 75mg/m<sup>2</sup>/d sc 5-2-2 schedule until progression or intolerance; those randomised to combination began LEN at cycle3, 10mg D1-21 each 28d cycle for total 10 cycles with AZA reduced to 5d. Primary endpoint of the study (to be analysed) is rate of clinical benefit (alive with absence PD) at 12mths.

### **Results**

March 2011 to March 2013 160 pts randomized. Arms were balanced for all baseline variables; median age 70.6y (42.5-87.2), 69% male. IPSS risk low/int1 60%; 14% patients carried 5q-. Median follow up 12mths (0.7-26.7), median number cycles AZA =11 in both arms; median cycles LEN=8. ORR (CR to HI) 54% (AZA) v 68% (AZA+LEN) (p=0.08). No difference median time to first response or best response. Median PFS 21.6mths (AZA) v 17.4mths (AZA+LEN).

Overall rate Gr3+ nonhaem AEs (excluding infection) 42% (AZA) v 47% (AZA+LEN); only significant difference raised GGT in AZA+LEN 14%. Gr3+ infections 42% (AZA) v 43% (AZA+LEN) predominantly respiratory and febrile neutropenia in both arms. No difference in emerging Gr3+ haematologic toxicity: new Hb <80g/L in 39%/39%, neutrophils<1x10<sup>9</sup>/L 43%/48%, platelets<50x10<sup>9</sup>/L in 35%/42% AZA/AZA+LEN.

### **Conclusion**

The regimen of concurrent AZA+LEN in pts with higher risk MDS/low blast AML/CMML is deliverable with numerically higher response rates. Toxicity is not excessive, with similar rates of emerging haematologic toxicity and infections. We await main analysis for assessment of primary endpoint, OS, quality of life and biomarker studies.

## **030. Confirmatory open-label, single-arm, multicentre phase 2 study of the BiTE® antibody blinatumomab in study subjects with relapsed/refractory B-precursor acute lymphoblastic leukaemia (r/r ALL).**

**Topp MS<sup>1</sup>**, Goekbuget N<sup>2</sup>, Stein AS<sup>3</sup>, Bargou RC<sup>4</sup>, Dombret H<sup>5</sup>, Fielding AK<sup>6</sup>, Ribera JM<sup>7</sup>, Foà R<sup>8</sup>, Zugmaier G<sup>9</sup>, Holland C<sup>10</sup>, Maniar T<sup>11</sup>, Huber B<sup>9</sup>, Nagorsen D<sup>11</sup>, Kantarjian HM<sup>12</sup>

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Research (Munich) GmbH, Germany; <sup>10</sup>Amgen Rockville, Inc., Rockville, MD, USA; <sup>11</sup>Amgen Inc,

Thousand Oaks, CA, USA; <sup>12</sup>Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

### **Aim**

Blinatumomab is an investigational bispecific T-cell engaging (BiTE®) antibody that directs cytotoxic T-cells towards CD19-expressing target cells. Blinatumomab has shown anti-leukaemia activity in an exploratory study in adult r/r B-precursor ALL. Here we report on the efficacy and toxicity of blinatumomab from a large confirmatory phase 2 study.

### **Methods**

Study subjects (≥18 years) with Ph-negative r/r ALL (refractory; 1st relapse <12 months; relapse post HSCT <12 months; ≥2nd salvage) were eligible. Blinatumomab was given by continuous IV infusion (4 weeks on/2 weeks off) for up to 5 cycles (cycle 1 only: 9 µg/d days 1-7; then 28 µg/d). The primary endpoint was complete remission (CR) or CR with partial haematological recovery (CRh\*) within the first 2 cycles.

### **Results**

A total of 189 study subjects were enrolled and received blinatumomab for a median (range) of 2 (1–5) cycles. The median age was 39 (18–79) years. As of January 2014 (primary analysis in February 2014), 43% of study subjects achieved CR/CRh\* with 80% of responses occurring within cycle 1. CRs/CRh\* were seen in all subgroups (Table). Regardless of causality, the most frequent adverse events (AEs) were pyrexia (59%), headache (35%) and febrile neutropenia (29%). The most frequent grade ≥3 AEs were febrile neutropenia (26%), anaemia (15%) and neutropenia (15%); 2% had grade ≥3 cytokine release syndrome. The most common grade ≥3 nervous system disorders were headache (4%), encephalopathy (3%) and ataxia (2%). 3 (2%) study subjects had grade 5 AEs considered treatment-related (sepsis, n=2; candida infection, n=1).

Endpoint	All Patients N=189
<b>Primary</b>	
CR/CRh*, n (%) <sup>a</sup>	82 (43) 95% CI, 36%–51%
Prior aHSCT (n=64)	30 (47)
No prior aHSCT:	
No prior salvage (n=25)	10 (40)
1 prior salvage (n=47)	24 (51)
≥2 prior salvages or primary refractory (n=53)	18 (34)
<b>Secondary</b>	
CR, n (%) <sup>†</sup>	64 (34)
CRh*, n (%) <sup>a</sup>	18 (10)
Median relapse-free survival, mo (95% CI)	5.9 (5.0–8.4)
Median OS, mo (95% CI)	6.1 (4.2–7.5)
<b>Exploratory</b>	
Minimal residual disease (MRD) response, n (%) <sup>a</sup>	Responders n=82 61 (74)

<sup>a</sup>First 2 cycles (central review). aHSCT allogeneic stem cell transplantation

## Conclusions

This large phase 2 study confirmed the anti-leukaemia activity of single-agent blinatumomab in a difficult-to-treat population with r/r ALL.

## **031. Effects of the Bite® Antibody Blinatumomab on Molecular Response in a Phase 2 Open-Label, Multicentre Confirmatory Study in Relapsed/Refractory B-Precursor Acute Lymphoblastic Leukemia (R/R ALL)**

Goekbuget N<sup>1</sup>, Brüggemann M<sup>2</sup>, Topp MS<sup>3</sup>, Stein AS<sup>4</sup>, Bargou RC<sup>5</sup>, Dombret H<sup>6</sup>, Fielding AK<sup>7</sup>, Ribera JM<sup>8</sup>, Foà R<sup>9</sup>, Zugmaier G<sup>10</sup>, Holland C<sup>11</sup>, Maniar T<sup>12</sup>, Huber B<sup>10</sup>, Nagorsen D<sup>12</sup>, Kantarjian HM<sup>13</sup>

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### **Aim**

To evaluate the efficacy of blinatumomab in terms of molecular response, determined by exploratory analysis of minimal residual disease (MRD) in adults with r/r ALL.

### **Methods**

Patients with Ph-negative r/r ALL (refractory; 1st relapse <12 months; relapse post-HSCT <12 months; ≥2nd salvage) were eligible. Blinatumomab (continuous IV infusion, 4 weeks on/2 weeks off) given for ≤5 cycles (cycle 1: 9 µg/d days 1-7; then 28 µg/d). The primary endpoint was complete remission (CR) or CR with partial haematological recovery (CRh\*) within the first 2 cycles. MRD was assessed using allele-specific real-time quantitative PCR for clonally-rearranged Ig and/or TCR genes. MRD response (fewer than 10<sup>-4</sup> detectable blasts) and complete MRD response (no detectable blasts) within the first 2 treatment cycles were exploratory endpoints.

### **Results**

189 patients (median age 39 [18–79] years) were treated; 64 patients (34%) had prior SCT. 81 patients had a CR (n=63) or CRh\* (n=18) during the first 2 treatment cycles. 17 patients had a morphologically blast-free or aplastic bone marrow. 73 of 81 patients (90%) with CR/CRh\* and 10 of 17 patients (59%) with blast-free bone marrow had evaluable MRD results. Of those, 60 patients (82%) with CR/CRh\* had an MRD response; 51 (70%) had a complete MRD response. MRD and complete MRD responses occurred in patients with CR and CRh\*. 5 of the 10 patients (50%) with blast-free marrow and CR/CRh\* had an MRD response (Table). Among patients who achieved CR/CRh\*, the rate of MRD response was 81% in patients without and 85% in patients with prior SCT.

Most frequent grade ≥3 adverse events included febrile neutropenia (26%), anaemia (15%), and neutropenia (15%).

### **Conclusion**

High MRD response rates (82%) were observed in patients with CR or CRh\*, and some patients with blast-free bone marrow showed MRD response. Further analyses of MRD outcomes are ongoing.

<b>Response</b>	<b>Pts with best hematologic response and MRD data n</b>	<b>Pts with MRD response n (%)<sup>a,b</sup></b>	<b>Pts with MRD complete response n (%)<sup>a,b</sup></b>
CR/CRh*	73	60 (82)	51 (70)
CR	58	50 (86)	43 (74)
CRh*	15	10 (67)	8 (53)
Blast-free hypoplastic or aplastic bone marrow	10	5 (50)	2 (20)

<sup>a</sup>During first 2 cycles; <sup>b</sup>Percentage based on number of pts with MRD data

## 032. Higher CD3 T cell numbers is a favourable prognostic factor in acute myeloid leukaemia with normal cytogenetics

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### Aims

Acute myeloid leukaemia with normal cytogenetics (CN-AML) is biologically and clinically heterogeneous. We sought to determine whether higher T cell numbers in CN-AML at diagnosis portend improved survival.

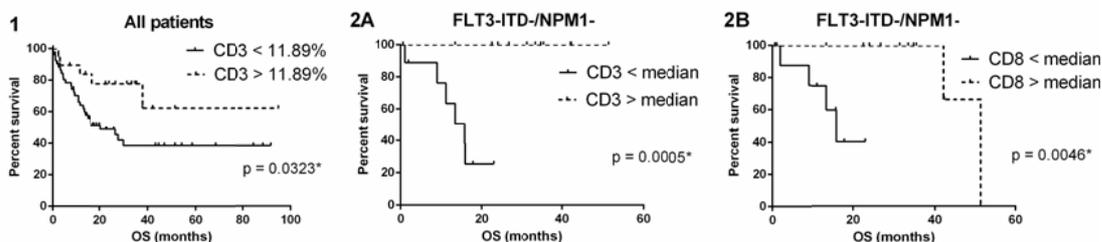
### Methods

Diagnostic trephine sections of patients with CN-AML diagnosed between 2006 and 2013 were immunohistochemically stained for CD3, CD8 and Granzyme B (GB). Positive cells, enumerated using Fiji© image analysis software (v1.48o), were expressed as a percentage of total cells. The primary outcome was overall survival (OS). Cox regression was used for univariate and multivariate analyses. Survival was estimated by Kaplan-Meier analyses and categories compared using the log-rank test.

### Results

75 patients (52% male, median age 61 years) were analysed. Median follow-up was 15.9 months. Of the 33 (44%) patients who died, 17 never achieved complete remission and 15 relapsed. 21 patients (28%) were allografted. Patients with CD3% above the 75th centile (>11.89%) had significantly better OS than those below ( $p = 0.0323$ ) (Figure 1). Factors significantly associated with OS on univariate analyses were age, preceding myelodysplastic syndrome, primary refractory disease, allograft and FLT3-ITD+. CD3 ( $p=0.096$ ), CD8, GB, NPM1, relapse and initial blast% were not significant. In a multivariate analysis of the significant variables, however, higher CD3 was an independent predictor of OS (Hazard ratio 0.922 for death, 95% CI 0.851-0.998,  $p=0.045$ ).

Within molecular subgroups, FLT3-ITD+ ( $n=20$ ) and NPM1+/FLT3-ITD- ( $n=11$ ), there was no survival difference between groups split by the median for CD3, CD8 or GB. In FLT3-ITD-/NPM1- patients ( $n=22$ ), CD3 > median (11.89%) (Figure 2A) and CD8 > median (10.66%) (Figure 2B) was associated with significantly superior OS but GB > median (1.17%) was not ( $p=0.2330$ ).



### Conclusion

In CN-AML, especially the FLT3-ITD-/NPM1- subgroup, higher CD3 T cell numbers are associated with improved survival. These findings suggest baseline immune status may have prognostic value and provide a foundation to study immune therapies in CN-AML.

### **033. Methylation of the phosphatase PTPRK is associated with poor survival in acute lymphoblastic leukemia and is reversible with epigenetic therapy**

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#### **Aim**

Aberrant DNA promoter methylation with associated gene silencing is a common epigenetic abnormality in ALL and is associated with poor survival. This study aimed to characterise methylation changes in a family of phosphatase genes identified in a genome wide methylation study performed on chemotherapy refractory ALL.

#### **Method**

DNA promoter methylation was measured by pyrosequencing after bisulfite treatment in primary leukemia samples and survival analysis performed. The effect of *PTPRK* silencing was examined in ALL cell lines by cell proliferation and study of intracellular kinase signalling.

#### **Result**

Promoter methylation of membrane bound phosphatase genes was common in adult ALL and Burkitt lymphoma in leukemic phase and was associated with transcriptional repression. *PTPRG* was methylated in 63% of ALL samples, *PTPRK* in 47%, *PTPRM* in 64% and *PTPRO* in 54% of cases, with most ALL samples containing methylation at multiple phosphatase loci. In a multivariate model, *PTPRK* promoter methylation was associated with decreased overall survival in this adult ALL cohort (n=57 with age range 17-79 years) treated with the Hyper CVAD chemotherapy protocol ( $P<0.05$ ). Biological study of *PTPRK* indicates that this gene modulates the phosphorylation status of intracellular signalling proteins and demonstrates tumor suppressor function. In leukemia cells where *PTPRK* has been silenced by promoter methylation, restoration of *PTPRK* transcript to normal levels by lentiviral transduction reduced cell proliferation, inhibited colony formation and increased sensitivity to cytotoxic chemotherapy. These biological changes were associated with a reduction in levels of phosphorylated Erk1/2, Akt, STAT3 and STAT5 as measured by Western blotting and intracellular flow cytometry suggesting functional phosphatase activity after transcript re-expression. Methylation of the phosphatase promoters was reversible with decitabine and the histone deacetylase inhibitor vorinostat.

#### **Conclusion**

DNA promoter methylation of membrane bound phosphatases is common in adult ALL and may be targeted with epigenetic therapies including decitabine and vorinostat.

### 034. Persistent mutations affecting DNMT3A and IDH1/2 are frequently found post-induction chemotherapy despite morphologic remission in acute myeloid leukaemia

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#### Aim

Recently, mutations affecting DNMT3A and IDH1/2 have been linked to pre-leukaemic founder clones. The frequency and prognostic significance of AML mutations persisting in remission after induction chemotherapy is uncertain.

#### Methods

86 patients who underwent frontline induction chemotherapy for AML at the Alfred Hospital, Melbourne (2007-2013), were screened using a customised multiplexed PCR assay resolved by mass spectrometry (MassArray; Sequenom™). Of these, 35 patients had intermediate risk karyotype. Clinical outcome and molecular mutations at diagnosis and post-chemotherapy were analysed.

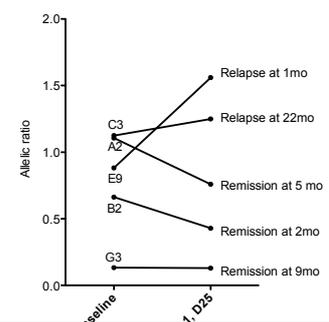
#### Results

The type and frequency of mutations detected by Sequenom analysis are shown in the table. 28 patients had paired pre- and post-chemotherapy samples available.

Mutations involving	Total patients (n=86)	Intermediate cytogenetics (N=35)	Persisting mutations in available paired samples in complete remission (CR)(positive/number tested)
IDH1	13 (15%)	9 (25%)	2/6 (33%)
IDH2	18 (20%)	8 (23%)	1/8 (12%)
DNMT3A	21 (24%)	13 (37%)	9/12 (75%)
NPM1	26 (30%)	21 (60%)	0/10 (0%)
FLT3-ITD	Not available	11 (31%)	1/5 (20%)

The 9 patients with persistent DNMT3A mutations post-chemotherapy in CR1 were further analysed.

Of these, 5 patients received maintenance therapy with lenalidomide-azacitidine in a phase 1B clinical study (unpublished). Stable remission in 3 of these patients was associated with either low level or reducing allelic burden on maintenance therapy, whilst rising levels in 2 patients predicted disease relapse (Fig 1). Four other patients with persistent DNMT3A after induction received an allograft; 2 remain in CR and 2 have relapsed.



#### Conclusion

In our preliminary analysis, persistent DNMT3A and IDH1/2 mutations are frequently found in post chemotherapy bone marrow samples. These patients may benefit from further post-remission therapy such as maintenance immunotherapy or stem cell transplantation to eliminate residual leukaemic clones. 2

Fig 1. Mutant:wild-type levels of mutant DNMT3A R882C/H at study screening and after cycle 1 of lenalidomide-azacitidine and patient outcome in terms of time to relapse or continuing remission (in months)

## 035. Gene mutation screening by Next Generation Sequencing (NGS) identifies frequent occurrence of NOTCH1 and TP53 Mutations in patients with Chemorefractory Chronic Lymphocytic Leukemia

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### Background

Established prognostic factors in CLL include cytogenetics, FISH analysis and IgHV mutation status. Although select markers are associated with chemoresistance and poor survival, molecular mechanisms underlying therapy resistance remain unexplained by current testing techniques. To rapidly and efficiently test for novel mutations including those important in CLL (NOTCH1, SF3B1, MYD88, TP53, BIRC3), we developed a 17-gene, NGS-based lymphoid panel using a targeted amplicon assay. In this study, we evaluated the performance of our NGS panel in material collected from patients with chemoresistant CLL or Richter transformation. Where sequential samples were available, we correlated molecular evolution with clinical outcomes.

### Method

Library preparation was done on Fluidigm® Access Array (multiplexed PCR library enrichment) and sequencing was performed on Illumina MiSeq®. The data was curated by two independent molecular scientists. To date, samples have been analysed for TP53, NOTCH1, SF3B1, MYD88, BIRC3 mutations.

### Results

Fourteen patients were analysed (12 male, 2 female) with 7 of these at ≥ 2 timepoints. The median age was 64 years (range 33 to 75).

Patient	Age	Sex	No. Prior Rx	Cytogenetics(CG)/FISH status	IgHV Mutatio	NGS: Variant gene (%)	Timepoint
1	57	M	1	Trisomy 12 on CG/FISH	Unmutated	NOTCH1 (32%)	FCR-Refractory CLL (Marrow sample)
						NOTCH1 (52%)	FCR-Refractory CLL (Blood sample)
2	33	M	0	Nil mutation	Mutated	No mutation detected	Baseline, pre-FCR
				6	7 Complex CGs incl 17p-; FISH negative for 17p13-		Failed OC
						TP53 (75%)	Further clonal evolution 3 years after allograft
3	71	M	3	11q-, 13q- on CG/FISH	Unmutated	NOTCH1 (34%)	Early Relapse after FCR
4	51	M	2	Complex CGs; 13q-, 17p- on FISH	Unmutated	TP53 (39%)	Early Relapse after FCR
5	64	M	1	Complex CG.; 17p13- on FISH	Unmutated	TP53 (57%), NOTCH1 (46%)	FCR-Refractory CLL
6	67	M	0	17p- on CG/FISH	Unmutated	TP53 (48%)	Baseline, pre-FCR
				1		TP53 (80%)	FCR-Refractory CLL
				2		TP53 (88%)	Progressive CLL after steroids/rituximab
7	69	M	0	Trisomy 12 on CG/FISH	Unknown	NOTCH1 (22%)	Baseline, pre-FCR
				1		No mutation detected	Early Relapse after FCR
8	64	M	0	13q- on CG/FISH	Unmutated	NOTCH1 (46%)	Baseline, pre-FCR
				2		NOTCH1 (23%)	Early Relapse after FCR
				2		NOTCH1 (46%)	Richter transformation (uninvolved marrow)
				2		Low DNA conc - no result	Richter's transformation (uninvolved marrow)
9	62	F	1	Trisomy 12, 17p-, 13q- on FISH/CG	Equivocal	Low DNA conc - no result	Early Relapse after FCR, with Richter's
				3		TP53 (4%), NOTCH1 (8%)	Persistent CLL after salvage for Richter's
10	75	M	2	Monosomy 13 & 17 on CG, FISH negative for 17p-	Unmutated	NOTCH1 (31%)	FCR-Refractory CLL
11	60	M	0	Nil mutation	Unmutated	No mutation detected	Baseline, pre-FCR
12	64	F	2	Trisomy 12 on CG/FISH	Unmutated	No mutation detected	FCR-Refractory CLL
				4		Low DNA conc - no result	Richter transformation (tissue sample)
13	74	M	6	Trisomy 12 on CG/FISH	Unmutated	TP53 (5%)	FCR-Refractory CLL
14	64	M	4	17p- on CG/FISH	Unknown	TP53 (60%)	FCR-Refractory CLL, Richter's (tissue sample)

Table 1. Case Series and Sequential Gene Mutation Analysis

Patient	Age	Sex	No. Prior Rx	Cytogenetics(CG)/FISH status	IgHV Mutatio	RGS: Variant gene (%)	Timepoint
1	57	M	1	Trisomy 12 on CG/FISH	Unmutated	NOTCH1 (32%)	FCR-Refractory CLL (Marrow sample)
			1			NOTCH1 (52%)	FCR-Refractory CLL (Blood sample)
2	33	M	0	Nil mutation	Mutated	No mutation detected	Baseline, pre-FCR
			6			Failed OC	Persistent disease after allograft for chemorefractory CLL
			7	Complex CGs incl 17p-, FISH negative for 17p13-		TP53 (75%)	Further clonal evolution 3 years after allograft
3	71	M	3	11q-, 13q- on CG/FISH	Unmutated	NOTCH1 (34%)	Early Relapse after FCR
4	51	M	2	Complex CGs; 13q-, 17p- on FISH	Unmutated	TP53 (39%)	Early Relapse after FCR
5	64	M	1	Complex CG; 17p13- on FISH	Unmutated	TP53 (57%), NOTCH1 (46%)	FCR-Refractory CLL
6	67	M	0	17p- on CG/FISH	Unmutated	TP53 (48%)	Baseline, pre-FCR
			1			TP53 (80%)	FCR-Refractory CLL
			2			TP53 (88 %)	Progressive CLL after steroids/rituximab
7	69	M	0	Trisomy 12 on CG/FISH	Unknown	NOTCH1 (22%)	Baseline, pre-FCR
			1			No mutation detected	Early Relapse after FCR
8	64	M	0	13q- on CG/FISH	Unmutated	NOTCH1 (46%)	Baseline, pre-FCR
			2			NOTCH1 (23%)	Early Relapse after FCR
			2			NOTCH1 (46%)	Richter transformation (uninvolved marrow)
			2			Low DNA conc - no result	Richter's transformation (uninvolved marrow)
9	62	F	1	Trisomy 12, 17p-, 13q- on FISH/CG	Equivocal	Low DNA conc - no result	Early Relapse after FCR, with Richter's
			3			TP53 (4%), NOTCH1 (8%)	Persistent CLL after salvage for Richter's
10	75	M	2	Monosomy 13 & 17 on CG; FISH negative for 17p-	Unmutated	NOTCH1 (31%)	FCR-Refractory CLL
11	60	M	0	Nil mutation	Unmutated	No mutation detected	Baseline, pre-FCR
12	64	F	2	Trisomy 12 on CG/FISH	Unmutated	No mutation detected	FCR-Refractory CLL
			4			Low DNA conc - no result	Richter transformation (tissue sample)
13	74	M	6	Trisomy 12 on CG/FISH	Unmutated	TP53 (5%)	FCR-Refractory CLL
14	64	M	4	17p- on CG/FISH	Unknown	TP53 (60%)	FCR-Refractory CLL, Richter's (tissue sample)

7/14 patients had NOTCH1 mutations; only 3 of these had trisomy 12. 6/14 patients had TP53 mutations,

including 2 who had normal 17p FISH. 2 patients had both NOTCH1 and TP53 mutations. Importantly, TP53 and/or NOTCH1 mutations were identified in 11 of 12 (92%) patients with poor response to FCR (primary refractory or short response of  $\leq 3$  years). 3 paraffin fixed specimens yielded poor quality DNA inadequate for mutational analysis. Patient 2 alone demonstrated molecular clonal evolution. Testing is ongoing on 16 further cases.

### Conclusion

Mutations of NOTCH1 and TP53 are commonly detected in patients with FCR resistant CLL. A priori knowledge of a patient's mutational profile may add important information to guide clinical decision-making.

## **036. Second interim analysis of a phase 3 study evaluating idelalisib and rituximab for relapsed CLL**

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### **Aims**

Idelalisib (IDELA), an oral inhibitor of PI3K $\delta$ , is highly active in heavily pretreated patients with CLL as a single agent or combined with rituximab (R) as demonstrated in Phase 1 trials. This report presents the results from the second interim analysis of a Phase 3, randomized, double-blind, placebo-controlled study of IDELA+R vs. placebo (PBO)+R.

### **Methods**

Patients with CLL requiring therapy after progression <24 mos since completion of last therapy and considered unfit to receive cytotoxic therapy were enrolled. Primary endpoint PFS was assessed by IRC and standard criteria (Hallek 2008, Hallek 2012, Cheson 2012). After progression, patients could enroll into a blinded extension study to receive IDELA at 150 mg BID (prior PBO+R) or 300 mg BID (prior IDELA+R). The first interim analysis (Furman et al, NEJM 2014) led to the decision of early termination due to overwhelming efficacy.

### **Results**

A total of 220 patients (110 patients on each arm) with a median age of 71 yrs (78%  $\geq$ 65 yrs), a median time since diagnosis of 8.5 yrs, and a median number of 3 prior therapies (range: 1-12)

were randomized. 44% of patients had del(17p)/TP53 mutation, 84% had unmutated IGHV. The

EFFICACY		GROUP		SIGNIFICANCE
		IDE LA+R	PBO+R	
Progression-free survival, all	Median	NR	5.5 mos	HR=0.18 95% CI: 0.10-0.32 p<0.0001
	At 24 wks	90%	50%	-
Overall response rate, all		75%	15%	OR=17.3 p<0.0001
Lymph node response rate, all		92%	6%	OR=165.5 p<0.0001
Overall survival (incl. extension study)	Median	NR	NR	HR=0.28 95% CI: 0.11-0.69 p=0.003
	At 24 wks	96%	86%	-
SAFETY		GROUP (ANY GRADE/GRADE ≥3, %)		
CATEGORY	TERM	IDE LA+R	PBO+R	
Adverse Events (AEs) in ≥20% of patients	Any AE	96/64	98/52	
	Pyrexia	35/3	17/1	
	Fatigue	26/5	28/3	
	Nausea	26/0	21/0	
	Chills	21/2	16/0	
	Infusion-rel. reaction	19/0	30/4	
	Cough	17/1	28/2	
Select AEs	Diarrhea*	19/4	15/0	
	Bleeding**	14/1	19/1	
	Pneumonia	10/8	13/9	
	Rash	10/1	5/0	
	Pneumonitis	6/4	1/1	
	Colitis*	5/3	1/0	
Select laboratory abnormalities	ALT elevation	35/8	10/1	
	Creatinine increased	13/0	9/1	
	Neutropenia	60/37	51/27	
	Anemia	29/7	32/17	
	Thrombocytopenia	19/11	32/18	

\*3/5 patients with colitis on IDE LA+R were also reported with diarrhea

table summarizes efficacy and safety. \*\*Includes 16 PTs according to a standardized MedDRA query (SMQ, narrow scope)

### Conclusion

Similar to the first interim analysis, IDE LA+R demonstrated significant improvement in progression-free survival, overall response rate, and lymph node response rate, compared to control, with acceptable safety. The overall survival of patients on IDE LA+R remained superior, including patients that crossed over into the extension study.

## **037. Local experience with Ibrutinib for the treatment of Chronic Lymphocytic Leukaemia and Mantle Cell Lymphoma**

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### **Aim**

Ibrutinib is an irreversible Bruton's tyrosine kinase inhibitor that was granted accelerated approval for CLL and MCL treatment in the US. We review our local experience with patients receiving ibrutinib monotherapy.

### **Method**

We undertook a retrospective analysis of 19 patients from 2 centres receiving ibrutinib for treatment of relapsed/refractory CLL (n=15), de novo CLL (n=3) or relapsed MCL (n=1).

### **Results**

Median age was 68 years (48-83) and 79% were male. The median number of prior therapies for those with pre-treated disease was two. CLL patients had a preponderance of adverse risk features including 17p deletion in 67%, 11q deletion in 17%, and fludarabine-refractory disease in 78%. Median starting WCC was 61x10<sup>9</sup>/L. Following initiation of therapy, all patients developed a lymphocytosis (an on-target effect due to inhibition of adhesion and migration; median rise 147%; 39%-325%); lymphocytosis paralleled improvement in functional state and reduction in nodes, and resolved to baseline at a median of 12 weeks. Nodal mass reduction: 94% achieved >50% reduction. The reduction in tumour nodal volume was substantial with a mean reduction of 91% (43%-100%) versus baseline. Overall response: 90% responded including 16% CR, 42% PR and 32% PR with lymphocytosis. The patient with MCL experienced nodal shrinkage but died from perforation of a bowel tumour deposit prior to reaching PR. Two CLL patients relapsed with plasmablastic Richter's transformation; the remainder remain relapse-free at a median 4.5 (2-17) months of follow-up. Adverse events included diarrhoea (15.8%), rash (15.8%), atrial fibrillation (10.5%), arthralgias (10.5%) and increased bruising or bleeding (from platelet dysfunction) (26.3%).

### **Conclusion**

Ibrutinib is well tolerated and effective in patients with advanced CLL/MCL. Toxicities including platelet dysfunction, atrial fibrillation and arthralgias complicate therapy, with potential consequences for long-term use.

## **038. Peripheral blood stem cell mobilisation using G-CSF alone versus cyclophosphamide/G-CSF in multiple myeloma patients receiving novel agent-containing induction therapy : A single centre review of safety and efficacy**

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### **Aim**

Induction chemotherapy followed by high dose melphalan (HDM) and autologous stem cell transplantation is considered standard therapy in medically fit multiple myeloma (MM) patients up to the age of 70. The mobilising regimen of high dose cyclophosphamide followed by G-CSF has historically been deployed based on data and experience in an era when novel agents were not routinely used. A G-CSF (G) alone regimen has a number of potential advantages including convenience, minimal resource utilization and lower toxicity when compared with cyclophosphamide/G-CSF (CG). We performed a retrospective review of these 2 mobilisation approaches.

### **Method**

We retrospectively reviewed data for 45 consecutive transplant-eligible MM patients undergoing induction therapy (including thalidomide, bortezomib or lenalidomide) followed by peripheral blood stem cell (PBSC) mobilization and then ASCT. Patients were treated at Geelong Hospital between April 2009 and December 2013. PBSC mobilisation was performed using G (n=25) or CG (n=20). Evaluated data included patient characteristics, PBSC yield, number of apheresis episodes required, hospitalization, infection, transfusion rates, engraftment and patient outcomes.

### **Results**

Patients undergoing G or CG mobilisation were well balanced with respect to patient and MM-specific characteristics (age, sex, ISS, cytogenetic/FISH status, number of treatment cycles and response status prior to induction). Median first PBSC yield was  $4.00 \times 10^6$  (range 1.1 – 11.4) versus  $4.65 \times 10^6$  (range 1.3-38.7) CD34+ cells/kg for G and CG mobilised patients respectively ( $p=0.227$ ). A median of 2 apheresis episodes were required for each group. Not included in the study is one G patient who failed but subsequently successfully mobilised with the addition of Plerixafor. Additional data on rates of infection, complications, hospitalization, transfusion, engraftment and disease-related outcomes will be presented.

### **Conclusion**

In this small retrospective review, PBSC mobilisation with G was non-inferior to CG in terms of total PBSC yield and number of apheresis episodes required to achieve the target CD34 yield. In the current era of routine novel agent-containing induction therapy for MM patients, G alone represents an acceptable alternative mobilisation regimen to CG.

## **039. Circulating cell-free DNA and RNA from peripheral blood plasma in patients with multiple myeloma: Validation studies for the Myeloma 1000 Biobank**

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### **Aim**

Myeloma 1000 is a biobank of peripheral blood (PB) samples from newly diagnosed multiple myeloma (MM) and MGUS patients, planned to link with the national Myeloma and Related Diseases Registry (MRDR). The PB of patients with malignancies contains both circulating cell-free DNA and RNA (cfRNA and cfDNA) that may act as biomarkers, potentially providing a comprehensive picture of the tumour genetic landscape. This report presents results of validation studies performed to optimise sample processing, isolation and quality assessment of cfDNA and cfRNA from MM patients.

### **Methods and Results**

Cell-free BCT (Streck) were used to prevent the release of cellular RNA and DNA during sample storage and processing. PB from normal volunteers and MM patients (each n=4) was collected and processed in parallel using the QIAGEN circulating nucleic acid kit at both 24 and 72 hours. Minimal differences in cfRNA amounts were observed between the time points. The amount of cfDNA and cfRNA obtained from 1 mL of plasma was between 5-200ng (n=4 normal and n=15 MM). Analysis of cfDNA for KRAS mutations and cfRNA for the housekeeping gene, GAPDH, by droplet digital PCR demonstrated amplifiable cfDNA and cfRNA in all cases and detection of KRAS mutations in 3/9 MM patients tested. The cfRNA was further assessed for potential use in next-generation sequencing technologies. Total RNA-sequencing of n=6 samples using 100bp-paired end sequencing demonstrated excellent (score: Q30) per base sequence quality and the data obtained could be assessed for differentially expressed mRNA, miRNA and fusion transcripts.

### **Conclusion**

These results provide proof of concept that disease-related mutations and the 'peripheral-blood' transcriptome can be evaluated utilising cfDNA and cfRNA, respectively, from MM patients. The MRDR-linked Myeloma 1000 biobank will be a valuable community resource to support research using circulating biomarkers that improves prevention, diagnosis and treatment of MM.

## **040. Baseline and treatment-related changes in thrombin generation in patients with multiple myeloma**

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### **Aim**

The early increased risk of venous thromboembolism (VTE) in multiple myeloma (MM) could be potentially assessed by measurement of thrombin generation (TG). We aim to (1) compare TG in patients with MM with normal and MGUS controls and; (2) assess the change in TG during the initial treatment phase in MM.

### **Method**

We enrolled 95 subjects across 2 hospitals in South Australia, including 24 MM, 20 MGUS, and 51 normal controls. Blood was assayed for known thrombophilic conditions and TG was performed on platelet-poor plasma using Calibrated Automated Thrombography. MM patients commencing therapy were then assessed at 1, 2 and 3 months with repeated laboratory measurements. Kruskal-Wallis H test was used to compare the 3 groups at baseline, and the Friedman test was used to assess changes over time.

### **Result**

Subjects with MM and MGUS had similar significantly higher levels of FVIII, VWF, and PC than normal controls. Evaluating baseline TG results triggered by 1pM and 5pM tissue factor, time-to-peak was significantly reduced in MM in comparison to both control groups ( $P<0.001$  for all), while velocity was significantly increased only when compared to normal controls ( $P<0.02$ ). Peak and endogenous thrombin potential were increased in MM compared with normal controls, but only in the presence of corn trypsin inhibitor and thrombomodulin ( $P<0.005$ ). Treatment was associated with increases in levels of FVIII, VWF, and the anticoagulant factors, protein S and antithrombin; as well as longer lag time and time-to-peak thrombin, but no significant changes in other TG parameters. Two patients developed VTE in this pilot study.

### **Conclusion**

Patients with MM demonstrate significantly different baseline haemostatic factors and TG parameters when compared to MGUS and normal controls, with important changes during initial treatment. This provides a rationale to further define the role of TG in assessing VTE risk in MM within a larger prospective study.

## **041. The immunobiological score: A robust 3-gene assay that segregates the international prognostic index into disparate survival categories in diffuse large B-cell lymphoma**

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Diffuse large B-cell lymphoma (DLBCL) is a common and aggressive lymphoma with approximately 30% mortality. Risk-stratification requires prognosticators to identify poor outcome patients in whom investigational therapeutic intervention is justified. Circulating lymphocyte:monocyte ratios are prognostic, implicating them as surrogate immune-effectors and monocyte/macrophage-checkpoints within the tumour microenvironment.

Diagnostic blood from 140 'R-CHOP' chemo-immunotherapy treated DLBCL patients from the Australasian Leukaemia and Lymphoma Group NHL21 trial was analysed. Detailed functional and quantitative assessment enabled identification of the optimal immune-effector and monocyte/macrophage-checkpoint molecules to interrogate within the tissue. CD163 identified a highly immunosuppressive subset of CD14<sup>+</sup>HLA-DR<sup>lo</sup> monocytoïd-myeloid-derived-suppressor cells 'moMDSC'. Ratios of various immune-effectors to CD163<sup>hi</sup>moMDSC were used as a measure of total anti-tumoural immunity: i.e. the net balance between the antagonistic forces of immune-effectors and monocyte/macrophage-checkpoints. All ratios were higher in early R-CHOP responders compared to delayed responders, with CD8:CD163<sup>hi</sup>moMDSC the most discriminatory. To test for intratumoural applicability, genes were quantified in diagnostic biopsies by digital multiplex hybridization (nanoString nCounter) in an independent cohort of 162 R-CHOP treated Australian patients, with long-term survival data. Co-clustering of CD8 with CD163 was observed, consistent with an adaptive immune-checkpoint response to immune-effector activation. CD8:CD163 ratios were prognostic independent of cell-of-origin and international prognostic index (IPI). Combining CD8:CD163 to the germinal-centre marker LMO2 resulted in a binary composite 'immunobiological' score (either high LMO2 and/or high CD8:CD163; versus dual low LMO2 and low CD8:CD163). The score had strong discriminatory predictive ability, identifying 24% at risk of very poor outcome. It separated low-risk IPI into 91% and 44%, and high-risk IPI into 76% and 26% 4-year survivals. Results were externally validated in an international cohort of 233 R-CHOP treated patients.

The immunobiological score is a powerful new 3-gene assay that segregates IPI into markedly disparate survival categories. Modulation of CD163 is a novel therapeutic strategy that warrants further investigation.

## **042. Outpatient ifosfamide, etoposide plus Rituximab (R-IE) represents an effective salvage regimen in older patients with relapsed or refractory DLBCL who are not candidates for stem cell transplantation**

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### **Aim**

To evaluate the efficacy of an outpatient-based salvage regimen consisting of ifosfamide, etoposide plus rituximab (R-IE) given every 3 weeks in patients > 60 yrs with relapsed/refractory CD20+ DLBCL And who are NOT eligible for stem cell transplantation (SCT).

### **Methods**

Patients were treated with Rituximab 375 mg/m<sup>2</sup> iv on day 1, Ifosfamide 4,000 mg/m<sup>2</sup> + mesna 4,000 mg/m<sup>2</sup> iv each in equally divided doses over 3 days, and Etoposide 80 mg/m<sup>2</sup> iv daily for days 1 to 3, and Pegfilgrastim 6 mg SC d4, followed by Rituximab 375 mg/m<sup>2</sup> for two doses. Outcome measures of overall response rate, progression free survival, and overall survival were analysed.

### **Results**

A total of 30 patients were included. The median age was 75.5 years (range, 64-85 yrs), including 63% males. The median number of prior treatment cycles was 1 (range, 1-2), while 33% of patients had not received prior rituximab. Patient characteristics at study entry included disease stage III/IV in 60%, raised LDH in 60%, BM involvement in 10%, and ECOGPS 0,1 in 83%. Secondary IPI risk categories were: IPI=4-5 in 13%, IPI=3 in 43%, IPI=2 in 30%, and IPI=1 in 13%. All six cycles of R-IE therapy were completed by 60% of patients, while an additional 13% completed 4 cycles, and 14% of patients completed only 1 or 2 cycles, mainly due to progressive disease. Dose delays were required in 7% and dose reductions in 7%. Major toxicities were confined to grade III/IV haematologic. Radiation therapy was administered to 40%. The overall response rate was 67%. The median progression-free and overall survival times were 12 months and 26 months, respectively.

### **Conclusion**

For older patients with relapsed/refractory DLBCL who are not suitable for SCT, fractionated outpatient-based R-IE immunochemotherapy represents an effective therapy with acceptable toxicity.

## **043. The predictive value of Interim Positron Emission Tomography (PET) differs by cell of origin determined by Immunohistochemistry in Diffuse Large B-Cell Lymphoma**

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Failure to achieve a favourable response on interim PET scan performed following R-CHOP chemotherapy for Diffuse large B-cell Lymphoma (DLBCL) has been associated with an inferior progression free survival (PFS). Whether this effect is generalisable by cell of origin has not been previously established.

### **Aim**

To determine the PFS and Overall Survival (OS) based on interim PET scan for patients with DLBCL receiving R-CHOP chemotherapy, and whether this differs by cell of origin determined by modified Choi criteria.

### **Methods**

Retrospective, single centre cohort study including all cases of DLBCL diagnosed and treated at Monash Health between July 2006 and December 2012 that included baseline, interim and end-of-treatment PET scans, and had biopsy tissue available to perform Cell of origin assessment by modified Choi criteria. Interim PET imaging was analysed qualitatively using the Deauville Criteria, with a positive scan defined as a score of 4 (uptake greater than the liver at any site) or above.

### **Results**

A total of 51 patients were included in this analysis (29 GCB, 22 ABC). The two groups did not differ in R-IPi characteristics. Within the GCB group, there was a trend towards inferior OS and PFS in patients who failed to achieve a favourable interim PET result. Within the ABC group, whilst there was a markedly inferior PFS (0.3 vs 3.9 years, P=0.0021), it appears that this group of patients were able to be successfully salvaged, with no effect on overall survival (5 year OS of 80% vs 64%).

### **Conclusion**

Failure to achieve a favourable response on Interim PET for patients with ABC phenotype DLBCL is associated with an inferior PFS, however this does not appear to impact on overall survival

## **044. Deliverability and efficacy of R-CHOP chemotherapy in very elderly patients with DLBCL: an Australian retrospective analysis**

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### **Aim**

Elderly people with DLBCL have a poor prognosis, due in part to advanced age and pre-existing comorbidities, with reduced tolerability and deliverability of standard R-CHOP chemotherapy. This study examines deliverability, toxicity and efficacy of R-CHOP in a truly elderly ( $\geq 75$  years) Australian cohort as well as the prevalence of the non-germinal centre (non-GCB) subtype within this cohort.

### **Methods**

This retrospective analysis identified patients aged  $\geq 75$  with DLBCL across three centres over ten years. Chemotherapy data, toxicity profiles, response and survival data were collected for all R-CHOP treated patients. Baseline patient demographics and chemotherapy characteristics were compared with PFS and OS and significant prognostic factors determined using Cox regression analysis. Immunohistochemical staining, using the Hans algorithm, identified the prevalence of the non-GCB-subtype within the cohort.

### **Results**

Of 111 patients diagnosed with DLBCL, 92 (83%) received R-CHOP for a median 6 cycles (1-8), with 26/92 (28%) receiving  $\leq 4$  cycles. Non-GCB-subtype was identified in 44/72 (61%) patients with IHC data. Median average relative total dose (ARD) was 0.80 (0.07-1.17). Median average relative dose intensity (ARDI) was 0.89 (0.33-1.18). SAEs occurred in 77% of patients with  $\geq$ Gd3 AEs in 74%. Overall response rate was 85%. Two year PFS was 63% and OS 74%. ARD and ECOG PS  $\geq 2$  were significant prognostic factors for PFS and OS but not ARDI.

### **Conclusion**

Despite high response rates, dose reductions and serious toxicity of R-CHOP therapy in this cohort highlights the need for the development of less toxic yet efficacious treatments for very elderly patients with DLBCL. The high prevalence of the poor prognosis non-GCB-subtype in the elderly underscores the need to develop better tolerated targeted biological therapies for this rapidly growing population.

## 045. A novel deep sequencing method for tracking of driver mutations in diffuse large B-cell lymphoma

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### Introduction

Diffuse large B-cell lymphoma (DLBCL) is the commonest type of non-Hodgkin lymphoma (NHL) in the western hemisphere. Currently, a significant proportion of patients fail standard treatment with chemotherapy and immunotherapy, indicating a gap in our understanding of the pathogenesis of lymphoma. Whole genome sequencing has identified a number of somatic mutations in genes involved in the B-cell receptor pathway including the myeloid differentiation primary response gene 88 (MYD88). Mutations in the TIR domain of MYD88 occur in ~40% of ABC-DLBCL, resulting in gain-of-function proteins which induce increased IRAK4 and NF-κB activity. However, it is currently not known whether this mutation is limited to the lymphoma clone indicating a late origin or whether it arises earlier in ontogeny.

### Methods/Results

To identify the cell of origin of MYD88 (and other lymphoma mutations), we developed custom next-generation sequencing approaches. An initial cohort of 45 DLBCL samples has been screened for multiple lymphoma-specific somatic mutations using a customised Haloplex library preparation kit. We have identified 8 cases with *MYD88* mutations. These cases are currently being studied using a novel 'ultra-deep sequencing' assay to identify the stage in ontogeny at which these mutations arise. By the addition of nucleotide barcodes during the PCR amplification process, we have eliminated processing and sequencing errors, and have identified very low numbers of non-lymphoma cells with gain-of-function *MYD88* mutations (> 0.01%). Non-lymphoma B cells have been single cell sorted and Sanger sequencing has been performed to establish that the MYD88 mutation is present in non-lymphoma cells lacking the lymphoma-specific IgHV sequence.

### Conclusions

Novel custom sequencing approaches have demonstrated that the MYD88 mutation arises earlier in ontogeny in normal haemopoietic precursors in patients with DLBCL, potentially accounting for treatment failure and acting as a reservoir for future relapse.

## **046. Classic Hodgkin Lymphoma: Improved outcomes of allogeneic haematopoietic stem cell transplantation (SCT) in relapsed / refractory disease**

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### **Aims/Background**

To review outcomes of allogeneic SCT for classic Hodgkin's lymphoma (CHL) at our institution

### **Methods**

All allogeneic transplants performed at our institution between 2001 and 2013 for CHL were identified from an institutional data-base. Patient outcomes were then determined by review of individual medical records. All grafts were T-replete. Prior to 2008, conditioning regimen was non-standardized; since 2008, all patients underwent reduced intensity conditioning (RIC) with fludarabine (total dose 125mg/m<sup>2</sup>) + melphalan (total dose 120mg/m<sup>2</sup>). Disease free survival (DFS) and overall survival (OS) were calculated via the Kaplan-Meier method with log rank test used for comparisons between groups

### **Results**

In total of 21 patients underwent allogeneic SCT for CHL in the time period under review, including 11 prior to 2008 and 10 between 2008 and 2013. Median age was 25yrs (range 17-47yrs), with 57% male. All patients suffered relapsed CHL, with 67% chemosensitive disease (CR / PR) at time of SCT, including 5 patients (24%) in CR. Conditioning regimens used prior to 2008 included myeloablative (n=3), RIC (n=1) and non-myeloablative regimens (n=7); all patients transplanted beyond 2008 underwent RIC. At a median follow-up post SCT of 17.7mths (range 0.6-131mths), median DFS and OS is 17.7mths and 19.6mths respectively. The only factor predictive of OS was time-period of transplantation, with median DFS and OS 24.2mths and 10.4mths (range 0.6-131mths) for patients transplanted 2001-2007 versus 17.7mths and not reached at median follow-up of 19.9mths (range 1.9-53.7mths) for patients transplanted beyond 2008 (p=0.94 and 0.02 respectively). Donor type (matched sibling versus unrelated donor), sex, chemosensitivity at SCT and development of >moderate acute GVHD were not associated with post-SCT survival. Overall, only 1 of 11 patients (9%) undertaking SCT between 2001 and 2007 remains alive and disease free, compared to 4 of 10 patients (40%) transplanted 2008-2013 (p=0.31)

### **Conclusions**

Allogeneic SCT remains a viable therapy in patients with relapsed / refractory CHL. In recent years, survival outcomes post allogeneic SCT have significantly improved.

### **Keywords**

Hodgkin's Lymphoma, allogeneic transplantation

### **Conflicts of interest**

None to declare

## **047. Peri-operative haematological optimisation of surgical cancer patients: PMCC experience**

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### **Introduction**

Data suggests that both peri-operative anaemia and need for blood transfusion predicts an increased rate of post-surgical morbidity and mortality (Beattie et al. Anesthesiology 2009). Equally, whether the anaemia and/or contributing causes of the anaemia, transfusion, or a combination of these, is the major contributor to this poor surgical outcomes has not been fully elucidated. Moreover, who would benefit most from pre-operative optimisation has not been investigated in surgical oncology populations.

### **Methods**

All patients undergoing major surgery, who attended pre-admission clinic at our institution over a 2 year period were profiled with clinical and laboratory parameters (FBC, coagulation profile, thromboelastography, B12, folate, iron studies and erythropoietin). We profiled pre-operative haematological abnormalities among surgical oncology patients and correlated these with clinical characteristics, peri-operative transfusion and complication rates.

### **Results**

Sixty-three patients were included in the cohort, median age 63 (range 36-89) years, 59% male and undergoing full spectrum of major cancer surgery (75% colorectal surgery). Of these, 49% were anaemic (WHO-defined criteria), 16% had clinically significant anaemia (Hb <110g/L) and 41% were iron deplete. Post-operatively, 91% required HDU/ICU care, 32% suffered post-operative complications and 37% required a transfusion within 30-days post-surgery. Iron depletion predicted requirement for blood transfusion (OR 3.0, 95%CI 1.3-12, p=0.016). Pro-inflammatory state (defined by high platelet count and/or fibrinogen) was associated with increased likelihood of blood transfusion (OR 3.1, 95%CI 1.02-9.6, p=0.045). A pro-inflammatory-prothrombotic state (defined by high platelet count and/or fibrinogen and high TEG-maximum amplitude) correlated with complications (OR 4.1, 95%CI 1.2-14, p=0.026).

### **Conclusion**

This study identified a high incidence of abnormal pre-operative haematinics in patients undergoing major cancer surgery. This highlights the need for improved perioperative haematinic optimisation. The relationship between inflammatory and prothrombotic biomarkers, transfusion rates and complications warrants future exploration.

## **048. FibroScan for the detection of liver fibrosis in haemoglobinopathy patients requiring chronic transfusion therapy**

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### **Aim**

Iron overload from chronic transfusion therapy is a major risk factor for the development of hepatic fibrosis in haemoglobinopathy patients. Transient elastography (FibroScan) is a novel, rapid, non-invasive test which uses liver stiffness measurements (LSM) to assess hepatic fibrosis. This study aimed to examine the relationship between LSM and liver iron concentration (LIC) as measured by MRI in a cohort of haemoglobinopathy patients receiving regular transfusion therapy.

### **Method**

28 patients who receive monthly blood transfusion for their haemoglobinopathies had LSM using FibroScan. Scans were deemed successful if at least 10 valid LSM were obtained and the interquartile range/median was <30%. These results were correlated with liver iron concentration measured by MRI as part of the ongoing TIMES study.

### **Result**

Of 28 patients evaluated 24 have thalassaemia major and 4 have sickle cell disease. Four have hepatitis C. All patients are currently on iron chelation therapy and the mean ferritin was 1941 µg/l (range 344-8036). The majority of patients (n=22) had no/minimal fibrosis (LSM<7.5kPa) but 4 patients had scans consistent with advanced fibrosis/cirrhosis (all with LSM > 10.5kPa). Two of these patients have hepatitis C whilst another had a markedly elevated LIC (>43mg/g). One patient, with sickle cell disease, had elevated liver stiffness despite a low LIC and no other clear risk factors for liver disease. There was no correlation between liver iron content as assessed by MRI and LSM.

### **Conclusion**

LSM appear to be independent from LIC and significant liver fibrosis may be detected in patients without current evidence of high iron load, perhaps due to previous inadequate iron chelation. FibroScan is likely to prove useful for monitoring for the development of fibrosis in patients with iron overload from chronic transfusion therapy, particularly in those with additional risk factors such as hepatitis C, but further studies are needed.

## 049. The alpha-globin promoter: What lies beneath?

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### Aim

Alpha-globin gene expression is mainly controlled by various trans-acting regulatory factors interacting with cis-acting elements found at core, proximal and distal promoter regions. Mutations found at these regulatory elements are believed to affect the rate of transcription significantly depending on the importance of the motifs being affected. This study uses molecular and cellular tools to characterize cis-acting elements sites within the  $\alpha$ -globin core and proximal promoter region.

### Method

Using our in-house designed HBA2-WT (wild type) expression construct and mutagenesis protocol we generated the following mutant constructs carrying 10 HBA2 promoter cis-element sites including HBA2:c.-45\_-41delGGGCC, HBA2:c.-65\_-60delATAAA, HBA2:c.-85\_-81delCGTGC, HBA2:c.-98\_-93delCCGCCC, HBA2:c.-108\_-103delCCAAT, HBA2:c.-143\_-138delCTGCGG, HBA2:c.-166\_-161delCGCAGG, HBA2:c.-179\_-174delCCGCCC, HBA2:c.-203\_-199delCACCC and HBA2:c.-215\_-210delCCGCCC. In addition we studied the effect of a 2nd TATA recognition box created artificially at position -73, prior to the translation initiation site (HBA2:c.-78\_-73insATAAA) on the HBA2 isoforms' transcription levels.

### Results

Twenty-four hours post transfection human 5637 cells transfected with either the HBA2-WT or the mutant constructs were analyzed for HBA2 gene transcription levels. The Quantitative-Real-Time PCR (Q-Re-Ti PCR) analyses revealed a -78%, -99.97%, -42% and -99.98% reduction in HBA2 gene transcription levels in cells transfected with HBA2c.-45\_-41delGGGCC, HBA2:c.-65\_-60delATAAA, HBA2:c.-85\_-81delCGTGC and HBA2:c.-108\_-103delCCAAT respectively compared to the HBA2-WT. Conversely, Q-Re-Ti PCR analyses showed a +17%, +96%, +126%, +26%, +15% and +35% increase in HBA2 gene transcription levels in cells transfected with HBA2:c.-98\_-93delCCGCCC, HBA2:c.-143\_-138delCTGCGG, HBA2:c.-166\_-161delCGCAGG, HBA2:c.-179\_-174delCCGCCC, HBA2:c.-203\_-199delCACCC and HBA2c.-215\_-210delCCGCCC, respectively compared to the HBA2-WT. The results showed a 125% increase in HBA2L but a 61% reduction in HBA2S transcripts in HBA2:c.-78\_-73insATAAA group when compared with the HBA-WT. The subsequent immunofluorescence analyses of all the transfected groups confirmed the transcriptional activity variations observed above.

### Conclusions

With this work we have experimentally mapped and functionally characterized transcriptional activator and suppressor elements sites within the  $\alpha$ 2-globin core and proximal promoter region and have shown the importance of the TATA binding protein site for regulating expression of the HBA2 transcripts.

## **050. Whole exome sequencing reveals rapid acquisition of driver mutations and branching evolution in a case of NPM1 positive CMML that progressed rapidly to AML**

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Progression of CMML to AML occurs in 20-30% of cases and is thought to be driven by a clone acquiring a novel fitness conferring mutation. Mutations in NPM1 are rare in CMML. We describe a case of a 50y.o. woman who was diagnosed with NPM1 positive CMML and progressed to AML within 81 days, which unfortunately led to her demise despite intensive therapy. To understand the basis of progression from CMML to AML, we used whole exome sequencing (WES) of both leukaemias and went on to interrogate samples taken at the time of subsequent remissions and relapses.

### **Methods**

WES was performed using Agilent Sureselect Human Exon 50Mb baits and an Illumina HiSeq 2000 platform. The eight presumed driver lesions along with 13 passengers detected on WES were assessed in nine DNA samples collected during the clinical course. The target regions were amplified in samples from each time point using barcoded non-allele specific PCR followed by sequencing on a single MiSeq run.

### **Results**

WES revealed mutations in DNMT3A, TET2 and NPM1 in the dominant clone of the CMML sample. Additionally, sub-clonal mutations in CEBPA, PTPN11 and SMC3 were found. Progression to AML was associated with acquisition of sub-clonal FLT3-ITD and NRAS mutations; whilst copy number analysis showed sub-clonal, copy neutral LOH at chromosome 1p and 13. By tracking the allele frequencies over serial samples we were able to show the first relapse came from a clone carrying SMC3, TET2 and DNMT3A mutations, but not FLT3-ITD. The relapse following allograft was from a clone with TET2, DNMT3A, NPM1, CEBPA and FLT3-ITD, but not the SMC3 mutation.

### **Conclusion**

We describe a case in which progression of NPM1c positive CMML to AML involved the acquisition of additional somatic mutations, despite the strikingly large number of AML-associated mutations in the CMML clone. This suggests that the CMML was a distinct disease entity. However, the very rapid acquisition of both FLT3-ITD and NRAS mutations in separate clones can be interpreted to suggest that progression to AML was inevitable.

## 051. The aetiology of megakaryocytic hyperplasia in myeloproliferative neoplasms

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### Aims

The *BCR/ABL*-negative myeloproliferative neoplasms (MPN) are a heterogeneous but related group of clonal stem cell disorders characterised by the proliferation of one or more myeloid lineage in the bone marrow (BM). Megakaryocytes (MK) are the most pathological cells in all MPN entities, characterised by hyperplasia with varying degrees of pleiomorphism and atypia. We aimed to assess the proliferation, signalling and apoptotic defects in MK to better understand the mechanism of megakaryocytic hyperplasia in MPN.

### Methods

Immunocytochemical staining was performed on BM trephines of 51 MPN (with and without *JAK2*<sup>V617F</sup>) and 12 normal controls to evaluate expression of molecules associated with proliferation (Ki67), apoptosis (BCL-XL, BNIP-3) and signalling (pSTAT5, pAkt). Expression was assessed by light microscopy and visualisation of antigen expression in morphologically identified MK.

### Results

The MPN had a 2.4-fold greater number of Ki67-positive MK (26 ± 12%) than controls (11 ± 10%; P = 0.0001). Anti-apoptotic BCL-XL was more frequently expressed in MPN than controls (MPN: 72 ± 17; controls: 40 ± 18, P < 0.0001). There were significantly fewer MK positive for pro-apoptotic BNIP-3 in MPN (35 ± 22) compared with controls (77 ± 11, P < 0.0001). MPN with *JAK2*<sup>V617F</sup> had a significantly higher percentage of pSTAT5-positive MK (mean 78.5%; range 56-100%) than MPN without (mean 55%, range 16-85%; P < 0.0001) and controls (P < 0.001). There were also significantly more pAkt positive MK in *JAK2*<sup>V617F</sup>-positive patients (mean 79.2, range 58–95%, P < 0.0001).

### Conclusions

Megakaryocytic hyperplasia in MPN is a result of both increased proliferation (Ki67 positivity) and reduced apoptosis, as evidenced by increased BCL-XL and reduced BNIP-3 expression. This deregulation of production and destruction may be a result of excess and constitutive activation of cell signalling pathways, such as the JAK-STAT cascade.

## **052. CALR mutation detection in JAK2 (V617F) negative myeloproliferative neoplasms: Validation of a next generation sequencing (NGS) assay for mutation testing by comparison with sanger sequencing**

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### **Aim**

CALR mutations have recently been described in Myeloproliferative Neoplasms (MPN) as mutually exclusive of JAK2 & MPL mutations. Mutation testing has quickly become important for the diagnosis & prognosis of these disorders. The aim of our study was to optimise and validate CALR mutation detection by parallel testing a traditional Sanger Sequencing (SS) approach with a targeted amplicon based NGS assay.

### **Method**

Histologically confirmed JAK2 negative MPN cases were analysed by SS. Subsequent analysis with an in-house designed 27 gene myeloid panel by NGS using a targeted amplicon assay was undertaken. Library preparation was done on Fluidigm® Access Array (Multiplexed PCR library enrichment) & sequencing was performed on Illumina MiSeq®. These two analysis strategies were then compared for concordance, sensitivity and mutation interpretation.

### **Results**

Fifty JAK2 (V617F) negative MPN cases comprising 32 cases of essential thrombocythaemia & 18 cases of primary myelofibrosis were analysed by SS. M: F= 0.72:1, median age= 67 years (38-85yrs). CALR mutations were detected in 60% (30/50) of these cases. 52bp deletion (18/30) & 5bp insertions (6/30) were the most frequent mutations detected, however, other mutations observed were 34bp deletion (2/30), 19bp deletion (2/30), 31bp deletion (1/30) & 1bp (1/30) deletion. To date, 30 CALR positive and 10 CALR wildtype cases have undergone NGS analysis with 100% concordance for mutation detection between NGS & SS. Additional mutational analysis of the full myeloid panel is ongoing.

### **Conclusion**

Our preliminary results demonstrate an excellent correlation between NGS and SS. Detection of large insertions and deletions by NGS can be technically demanding but with thoughtful oligonucleotide design and bioinformatics support, this new technology has been shown to be equivalent to SS. NGS once optimised & validated is quicker, more sensitive, less labour intensive & easy to interpret as compared to traditional SS.

## **053. IKAROS is down-regulated in the Accelerated and Blast Phases of Chronic Myeloid Leukaemia**

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### **Aims**

Accelerated phase chronic myeloid leukaemia (CML-AP) is marked by a progressive leucocytosis and basophilia whilst on maintenance treatment, increasing blast cells and rapid progression to blast phase (CML-BP). It is thought this results from genomic instability of the initial clone. Genetic alterations resulting in loss of IKAROS activity are associated with the development of BCR-ABL1+ myeloid and lymphoid leukemias, and BCR-ABL1-negative myeloproliferative neoplasms. We have compared IKAROS expression in CML-AP/BC with AML.

### **Methods**

In this study we assessed the level of IKAROS protein in primitive cells in normal bone marrow, CML-chronic phase (CP), CML-AP and myeloid BP (total n=40) and BCR-ABL1-negative Acute Myeloid Leukaemia (AML: n=59). Bone marrow trephine sections were stained using an immunoalkaline phosphatase method and IKAROS antibody (clone SP108). Following haematoxylin counterstaining assessment was by light microscopy.

### **Results**

Primitive cells in both normal and CML-CP were universally strongly positive for IKAROS protein. In the primitive cells in CML-AP/BC there was much greater heterogeneity. There were cases with reduced number of positive primitive cells (n=17), weaker expression (n=4) or total absence (n=9) of IKAROS stain. Particularly striking were cases of CML-AP where IKAROS was undetectable in the blast cells in the expanded paratrabeular region, but could be clearly visualised in the maturing residual chronic phase myeloid cells in the intertrabeular regions. The AML cases had uniformly strong IKAROS staining (58/59) (CML-AP/BC versus AML; P=<0.0001).

### **Conclusion**

Loss or reduced expression of IKAROS protein appears to be a common feature of CML-AP/BC and is different from the ubiquitous strong expression in AML. This demonstrates that suppression of IKAROS is a frequent step and potential diagnostic harbinger of progressing myeloid disease in CML. This data supports studies that have shown direct suppression of IKAROS activity in CD34+ cells from CML-CP leads to rapid development of AP.

## **054. Allograft transplantation for chronic myeloid leukaemia (CML) in the era of tyrosine kinase inhibitors (TKIs): an Australasian Bone Marrow Transplant Recipient Registry (ABMTRR) study**

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### **Aim**

Treatment for CML has dramatically changed following the advent of TKIs in the late 1990's. This study analysed adult first allografts for CML performed in Australian and New Zealand transplant centres in 2005-10 (inclusive), and compared them with allografts performed in 2000-04.

### **Method**

Patient and transplant details were retrieved from the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR), and additional data on patient, disease and transplant characteristics were retrieved from a customised spreadsheet questionnaire distributed to participating centres.

### **Results**

In the years 2005-10, 80 allografts were performed, to 60% male and 40% female recipients with a median age at transplant of 40 years. Half (n=40) of the graft donors were siblings, and n=39 of the balance were unrelated volunteers. The majority (n=57, 72%) of recipients were in chronic phase at transplant. Numbers of first allografts for CML declined sharply from a high of 71 in 2000 to 23 in 2004, and stayed at around 10–15 annually during 2005–12. The majority (65%) of allografts in the period 2000-04 were performed while the recipient was in first chronic phase, however this proportion decreased to 38% in 2005-10, with higher proportions of patients transplanted in second or third chronic or accelerated phases in the latter period. The overall 5-year survival probability for the earlier group of patients was 58.1%, similar to that of the later group (60.9%, P=0.9).

### **Conclusions**

Following the advent of TKIs as standard treatment for patients with CML, a reduced number of allografts are being performed, with a lower proportion of patients in first chronic phase and a higher proportion in more advanced disease states. Despite this the overall post transplant survival has not changed significantly, indicating that a large proportion of patients who fail or do not receive TKIs can be successfully treated with transplant.

## **055. Fertility outcomes in pre-menopausal women following BEAM conditioning and autologous stem cell transplantation**

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### **Aim**

There is minimal data on fertility outcomes in young women undergoing autologous stem cell transplant (ASCT) with carmustine, etoposide, cytarabine and melphalan (BEAM) conditioning. We performed a retrospective analysis of menstrual recovery and fertility outcomes post BEAM/ASCT.

### **Methods**

We performed a retrospective, multicentre analysis of pre-menopausal females between 18 and 40 years of age undergoing BEAM/ASCT for lymphoma at 4 transplant centres between 1995 and 2011. To be eligible, patients had to not have received sterilising chemotherapy prior to conditioning, be in remission at 12 months and have adequate documentation of fertility preservation methods, recovery of menses, conception and pregnancy outcomes.

### **Results**

Out of 41 pre-menopausal women who underwent BEAM transplant, 25 met the inclusion criteria. Eighteen had HL and 7 had NHL. Median number of chemotherapy regimens pre-transplant was 2 (1-3). The median age at transplant was 28 years (range 17-40). Eighteen women (72%) with a median age at transplant of 26 years (range 17-33) recovered their menses. Fourteen patients underwent pre-transplant fertility preservation with GnRH-analogue, ovarian tissue or embryo cryopreservation. Ten patients (40%) had 11 naturally conceived pregnancies resulting in healthy babies in ten and miscarriage in one. Median age at transplant was 24 years (range 17-30). Additionally, one patient had a successful pregnancy after embryo implantation. The time between transplant and first conception ranged between 1 and 13 years. Chemotherapy regimens, prior fertility preservation and lymphoma type did not obviously influence the incidence of menses recovery or conception.

### **Conclusion**

The incidence of recovery of menses and potential fertility in pre-menopausal women undergoing BEAM/ASCT is substantial. Younger age at transplant correlates with superior fertility outcomes. These observations are useful for counselling women in this context. Strategies such as GnRH analogues and assessment of ovarian reserve using anti-Mullerian hormone levels may also impact on fertility outcomes in this patient group.

## **056. A survey of the current use of infection prophylaxis post autologous stem cell transplant (ASCT)**

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### **Aim**

There is currently inadequate evidence supporting the necessity or recommended duration of *Pneumocystis jiroveci* (PJP) prophylaxis post ASCT. Raser et al. (ASH 2013 #3372) reported only 5 PJP cases (all on concomitant steroids) in 1191 patients post ASCT in the absence of prophylaxis. There are also limited data supporting antiviral prophylaxis and re-vaccination post ASCT. In this context, we conducted a survey overview of local infection prophylaxis practice post ASCT.

### **Methods**

Thirty-four surveys were sent electronically to ASCT centres in Australasia

### **Results**

Twenty-six centres responded. A median of 30 ASCT (range 15-90) are performed per centre annually. PJP prophylaxis is routinely used in 20 centres (77%) using sulfamethoxazole/trimethoprim 800/160mg (bd twice weekly in 50%[n=10], daily thrice weekly in 32%[n=7]). Prophylaxis is commenced from time of engraftment in 16 centres (80%). Duration of prophylaxis varied from <3 months (n=3;15%), 3 months (n=8;40%), 3-6 months (n=2;10%) and 6 months (n=7;35%). CD4+ count only influenced the duration in 3 centres. Only 9 centres (47%) continued prophylaxis during maintenance for thalidomine/prednisolone-based protocols. With the limitations of retrospective memory, responders could recall only 6 cases of PJP infections – 5 within 6 months of ASCT, including 2 cases while on prophylaxis. Twenty-one centres (81%) indicated willingness to be involved in a prospective prophylaxis study.

Twenty-two centres used antiviral prophylaxis with most (n=14) using valaciclovir 500mg daily. The majority commenced prophylaxis around time of admission (n=17) and continued for 1 month (n=8), 3 months (n=7), 6 months (n=2) and 12 months (n=5). Despite published European and local guidelines, only 16 centres (62%) implemented routine revaccination policy post ASCT.

### **Conclusion**

There is substantial variation in infection prophylaxis and revaccination policy post ASCT. The apparent low incidence of PJP in the absence of prophylaxis, suggests that routine prophylaxis, which is not without side effects including myelosuppression, may not be warranted except perhaps in the context of concomitant immunosuppression. A prospective study investigating the use of no routine PJP prophylaxis post ASCT is being designed. Similarly, the necessity of routine revaccination post ASCT is worth of further study.

## **057. Comparison of biosimilar filgrastim Nivestim™ with filgrastim Neupogen® for peripheral blood stem cell mobilisation and engraftment in patients with multiple myeloma undergoing autologous stem cell transplantation**

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### **Aim**

Nivestim™ is approved for the same indications as Neupogen® including the mobilisation of autologous peripheral blood stem cells (PBSC). The clinical efficacy and safety of Nivestim™ for this use however have not been formally assessed in clinical trials. Our single-centre study aimed to compare the efficacy and safety of Nivestim™ to Neupogen® for PBSC mobilisation and engraftment of patients with multiple myeloma undergoing autologous stem cell transplantation (ASCT).

### **Method**

Retrospective data was collected from 60 consecutive patients mobilised with Nivestim™ from 2011 to 2013 and 39 patients mobilised between 2010 and 2011 with Neupogen®. Parameters of PBSC mobilisation (peripheral blood CD34+ cells/ $\mu$ L, successful or failed collections, yield of CD34+ cells/kg) and engraftment (neutrophils and platelets) were analysed. Results were considered statistically significant if p values were <0.05.

### **Result**

We found no statistically significant difference between Nivestim™ and Neupogen® in peripheral blood CD34+ on day 5 at first leukapheresis (47 vs. 59 cells/ $\mu$ L,  $p=0.65$ ), successful collections  $>2.0 \times 10^6$ /kg CD34+ cells (51 vs. 32,  $p=0.78$ ), failed collections  $<2.0 \times 10^6$ /kg CD34+ cells (4 vs. 2,  $p=1$ ) and the CD34+ yield (5.37 vs. 4.66  $\times 10^6$ /kg,  $p=0.23$ ). 82 patients (51 Nivestim™ and 31 Neupogen® mobilised) went on to transplant. Median time to both neutrophil engraftment (15 vs. 13,  $p=0.09$ ) and platelet engraftment (20 vs. 18 days,  $p=0.009$ ) was 2 days longer in the Nivestim™ mobilised group. After controlling for induction therapy (thalidomide, bortezomib, lenalidomide and/or chemotherapy) and melphalan dose, the association of Nivestim™ with delayed time to platelet engraftment remained statistically significant ( $p=0.005$ ). Nivestim™ was well tolerated with no unexpected safety concerns related to its use.

### **Conclusion**

Nivestim™ is as effective as Neupogen® for PBSC mobilisation, however, its use was associated with a delay in both neutrophil and platelet recovery. Further prospective randomised controlled studies are required to confirm these findings.

## 058. A study of immune reconstitution following autologous stem cell transplantation for multiple sclerosis

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### Background

Autologous stem cell transplantation (ASCT) has shown promise as a treatment strategy for multiple sclerosis (MS), possibly by eradicating self reactive immune cells and resetting of the immune repertoire upon post-transplant reconstitution. However, the precise mechanism is unclear and we undertook a detailed analysis of lymphocyte subsets following ASCT for MS.

### Method

Between 2010 and 2012, 11 patients with progressive MS unresponsive to other therapies underwent ASCT. Stem cell mobilisation was with cyclophosphamide 2g/m<sup>2</sup> and G-CSF 5ug/kg bd. Conditioning chemotherapy was with cyclophosphamide 50mg/kg and rabbit ATG 1mg/kg days -5 to -2. Lymphocyte subsets including CD4, CD8, naïve T-cells, recent thymic emigrants, T-regulatory cells, TH1, TH2 and TH17 cells were assessed by flow cytometry for 2 years following ASCT. Eleven healthy volunteers were used as controls.

### Results

Outlined below is the mean absolute number (X10<sup>6</sup>/L) of lymphocytes, along with subsets expressed as percentage of CD4 lymphocytes, up to 24months post ASCT. In the short term there is a clear shift in favour of CD8 cells and Th1 cells. Naïve T-cells and RTEs fall initially but re-establish by 12 months. TH17 cells appear to emerge with time. Statistical analysis is pending.

	Control	Pre-aHSCT	1m	3m	6m	9m	12m	18m	24m
CD4	905	479	257	220	246	349	400	486	402
CD8	456	425	1051	758	775	990	780	818	429
T-Naive%	46	28	2	6	14	25	35	30	33
T-RTE%	29	19	1	3	11	22	30	26	27
T-Reg%	4	7	8	7	6	5	5	5	3
Th1%	15	26	48	56	46	38	25	24	21
Th2%	2	7	11	8	7	5	4	4	4
Th17%	1	2	2	2	3	2	3	5	6

### Conclusion

ASCT for MS results in major changes in lymphocyte subsets upon immune reconstitution. These changes may alter autoreactive immunological memory and confer clinical benefit.

## 059. Consolidation and maintenance therapy in Myeloma

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Initial treatment of MM is based on the induction therapy followed by single ASCT in transplant eligible patients, with a median PFS approximately of 36 months. Several attempts to improve the PFS with 4 drugs-based regimens have failed; and improvements in the conditioning regimen, currently high dose melphalan, have yet to demonstrate any impact on PFS. Recent progresses were observed with post transplant continuous therapeutic approaches, either of consolidation or maintenance types. These progresses are in line with recent understanding of the biology of Myeloma that showed that further debulking post ASCT, for the patients with sensitive clones, increases the chance to reach the goal of CR and possibly MRD negative status of the disease. For patients with selected clones of potential adverse prognostic due to the selective pressure of the therapeutics, and other causes yet to be demonstrated, the control (ideally further destruction) of these clones would be ideal and might be obtained applying a continuous therapeutic approach as well.

The terms consolidation and maintenance therapy are often used synonymously in the discussion about treatment options post ASCT; however, they reflect different treatment phases and very likely different objectives in terms of control of the biology of Myeloma cells. On a simplistic understanding, one would consider that Consolidation is short term therapy and enhances the rate and depth of previously obtained response, while the goal of Maintenance therapy is the extension of response duration, and ultimately of PFS and survival. It is possible that the 2 concepts may overlap in their objectives at least for some of the patients.

Several studies were reported in the literature considering the role of lenalidomide and bortezomib in the consolidation treatment after single or tandem ASCT. It seems that consolidation might be better based on a triplet-based regimen, possibly bortezomib backbone-based, with the goal to increase the CR rate further, with limited alteration of the safety profile. Therefore the length of the consolidation varied from 2 cycles to 1 year approximately depending on the regimen used. In some studies consolidation seemed more beneficial to patients not achieving at least VGPR particularly when using tandem ASCT upfront, other have showed that bortezomib-based consolidation was more beneficial to patients sensitive to bortezomib-based induction regimen. A phase 3 randomized multicenter open label study from the BMT group named CTN 0702 might help sort this out.

Maintenance therapy with the objective to cure (operational cure) some patients with very sensitive myeloma disease, or in general, to prevent of tumor progression and thus to prolong PFS and possibly OS, was studied for years. The first trials have evaluated years ago MP as well as corticosteroids and interferon maintenance therapy then thalidomide, but it was often associated with significant side effects and modest activity. Lenalidomide seems to be a promising drug for maintenance therapy because of its stronger antimyeloma effect, its immunomodulatory mechanism of action on the long run, and more importantly its favorable toxicity profile when compared to thalidomide. Bortezomib IV was also studied and appeared very active particularly in some subgroups with adverse profiles, although quite neurotoxic. It would be interesting to study the balance efficacy/safety using Bortezomib sub cutaneous way. Most studies have to date revealed increased progression free survival after consolidation and maintenance therapy, but few demonstrated prolonged overall survival.

To date, although continuous therapeutic appears to be the new concept to prolong further PFS, and possibly OS in many NDMM, it has yet to be approved in many countries and have yet to be considered as wide accepted standards; consolidation and maintenance therapy after ASCT remain controversial indeed. However, more and more studies will certainly demonstrate that this approach is

one of the cornerstones to improve survival of NDMM in the couple of years to come, and will help in the treatment decision to be based on the individual patient estimated benefits and risks.

## **060. Relapsed/refractory myeloma**

**Lonial S**

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Despite improvements in depth of response and duration of first remission (PFS) following induction therapy for myeloma, most patients will eventually relapse and require some form of salvage therapy. Choice of therapy at this time remains somewhat arbitrary, though there may be patient or disease based factors that can help to guide decisions such as side effects during prior therapy, duration of therapeutic benefit, and in some instances, the use of genetics and FISH testing may help to provide insight into choices of therapy for relapsed myeloma. Correct identification of relapse vs refractory vs primary refractory can also be useful when considering whether the use of single agents or combinations are best suited for the management of this disease.

## **061. Final analysis of a phase II study of inpatient dose-escalation of eltrombopag in patients receiving azacitidine for myelodysplasia/AML.**

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### **Background**

Pre-existing thrombocytopenia in MDS/AML is frequently worsened during the initial cycles of azacitidine (AZA), increasing probability of bleeding and platelet transfusion. Eltrombopag (EPG) is an oral TPO-receptor agonist. In vitro, it has anti-proliferative effects on AML blasts.

### **Aim**

To assess the safety of escalating doses of EPG in patients receiving AZA for MDS/AML

### **Method**

An investigator-initiated phase-II, single arm, study of EPG with AZA. Inclusion: relapsed or de-novo MDS/CMML/AML (blasts 5-30%); or symptomatic cytopenia; or blasts 31-50% if  $\geq 65$  years or previously-treated disease; and platelets  $\leq 150 \times 10^9/L$ . Primary endpoint: rate of treatment-related grade III/IV non-haematological events. Secondary endpoints: AE rates, overall response, and survival. EPG was administered at doses of 50-300mg/day, AZA 75mg/m<sup>2</sup> d1-5,8-9 (q28d) Patients with baseline PLT $<100$  received EPG monotherapy for 2w prior to AZA. Eltrombopag was ceased after 6Mo.

### **Result**

Of 25 patients, 10 had prior therapy (7 chemo); 15 had blasts  $\geq 10\%$ . Median entry platelet count was  $38 \times 10^9/L$  (range 8-127). A median 11(2-24) cycles AZA and 6 cycles of EPG were delivered. One patient developed GII EPG-related LFT abnormalities (resolved). Grade 3 fatigue was attributed to the combination in one patient. Uncomplicated thrombocytosis ( $>450 \times 10^9/L$ ) leading to EPG cessation occurred in 6 patients (at 50, 50, 150 and three at 200mg). 10 patients experienced reversible skin yellowing. Response/Improvement was seen in 18 (72%): 7CR, 3CRm, 5HI-P, 1 HI-N, 2 with  $>50\%$  blast reduction from  $>20\%$  (8%). 5 patients had disease progression at first assessment. Platelet improvement was seen in 54% (13/24) of patients with baseline platelets  $<100$  at median (range) at 46d (7-107) following commencement of AZA. Only one patient (4%) had an improvement in platelets following the monotherapy phase.

### **Conclusion**

Eltrombopag could be safely delivered at these doses. 12 month survival outcomes will be presented. Based on this promising efficacy, a phase III international study has commenced to better define the role of this combination as supportive care for patients undergoing treatment with azacitidine.

## 062. Developing CD300f antibodies as novel AML therapeutics

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### Aim

There is a major need for new effective treatments in Acute Myeloid Leukaemia (AML). The CD300 molecules are a family of cell surface glycoproteins, which modulate diverse cellular processes via their triggering and inhibitory functions. CD300f is expressed only on myeloid leukocytes and thus presents as a potential novel target for a therapeutic anti-AML antibody. We aimed to characterise the expression of CD300f on a panel of AML samples and develop novel therapeutic antibodies to this target.

### Method

We have generated novel monoclonal antibodies (mAbs) that bind human CD300f and used these, along with a commercially available mAb, to phenotype CD300f expression in AML blood and bone marrow samples. We have used phage display to express one CD300f mAb as a single chain variable fragment (scFv).

### Result

Of 35 samples tested to date using multi-parameter flow cytometry, CD300f was found to be upregulated on CD45<sup>+</sup> blasts in 27/35 (77%) of cases. Importantly, CD300f was also found on CD34<sup>+</sup>/CD38<sup>-</sup> population in the same proportion of cases, 17/22 (77%). This cell fraction is generally enriched with leukemic stem cells (LSC), a population of chemo-resistant cells thought to be responsible for relapse in AML. Interestingly the proportion of CD300f positive AML samples varied depending on the antibody used, suggesting the presence of a particular "leukaemic epitope" which is most suitable for targeting.

### Conclusion

The characteristics of CD300f as a cell surface molecule, expressed on myeloid leukocytes and primary AML samples, supports its further investigation as a novel target for an AML therapeutic. We now plan engineer the scFv to make humanized reformatted antibody fragments with the functional ability to target and destroy leukemic cells. These fully human anti CD300f mAbs will be tested in xenogeneic models of AML to establish their *in vivo* ability to treat leukaemia.

## **063. CDK9 inhibition with Dinaciclib potently suppresses Mcl-1 transcription to induce curative apoptotic responses in aggressive Myc-driven lymphoma**

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### **Aim**

MYC dysregulation conveys poor prognosis in DLBCL, and effective therapeutic strategies are lacking in relapsed/refractory DLBCL and Burkitt lymphoma. Critical MYC gene targets (e.g. anti-apoptotic Mcl-1) are transcribed by RNA Pol-II, which requires activation by CDK9. We therefore hypothesized that CDK9 inhibition by Dinaciclib would have efficacy in aggressive, MYC-driven disease.

### **Method**

Molecular determinants of sensitivity to Dinaciclib-induced apoptosis were interrogated using an extended panel of independently derived and genetically modified murine E $\mu$ -Myc lymphomas and human Burkitt cell lines. In vivo efficacy was assessed by treating tumour-bearing mice transplanted with the same lymphomas.

### **Result**

Dinaciclib induced p53 independent growth arrest and apoptosis of murine and human Burkitt lymphomas at on target, single digit nanomolar concentrations corresponding to CDK9 inhibition (as demonstrated by dephosphorylation of RNAPII). Dinaciclib rapidly and selectively suppressed Mcl-1 (but not Bcl-2) transcription and protein levels in an apoptotic response that could be rescued by forced retroviral Mcl-1 over-expression. In vivo treatment of C57Bl/6 mice bearing E $\mu$ -Myc lymphoma resulted in brisk apoptotic responses associated with markedly increased overall survival including cures achieved in 20% of mice. This survival advantage maintained in the absence of functional p53 but markedly attenuated in isogenic Mcl-1 overexpressing lymphoma.

### **Conclusion**

CDK9 inhibition by Dinaciclib is highly effective in 'poor-risk' p53 null, aggressive MYC-driven B-cell lymphoma via selective inhibition of critical MYC-targets including Mcl-1 (which is 'undruggable' with existing BH3 mimetics). Rapid clinical translation of CDK9 inhibitors to MYC-dysregulated lymphoid malignancy should now be pursued.

## 064. Introducing Dlk1, a new player in stem cell biology and leukaemia

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### Aim

DLK1 (delta-like homologue 1) is a notch-like cell surface protein that is overexpressed in many acute leukaemias (>50% of primary acute leukaemias in one study). Whether it is important in the development of leukaemia or simply a marker of transformation is unknown. Similarly the role of Dlk1 in adult haematopoietic stem cell (HSC) biology remains to be defined.

### Method

Human acute leukaemia cell lines examined by FACS to separate DLK1 positive from DLK1 negative cells, and their biological properties were examined. A Dlk1 knockout mouse was created, and its adult haematopoietic stem cells and progenitors were studied. The effect of Dlk1 overexpression by fetal liver retroviral infection and haemopoietic reconstitution following transplantation was also undertaken. Student's *t*-test was used for statistical analysis.

### Result

DLK1 positive leukaemic cells among K562 (human erythroleukaemia cell line) showed increased ability to form colonies when cultured ( $p < 0.05$ ). Dlk1 knockout mice showed normal full blood counts with a trend toward higher platelets ( $p = 0.06$ ). Haematopoietic stem cells and progenitors from knockout and wild type mice were studied using several methods including semi-solid agar culture colony assays. Bone marrow cells from young Dlk1 knockout mice yielded higher numbers of haematopoietic colonies overall as well as megakaryocytic colonies ( $p < 0.05$ ). Notably, constitutive overexpression of Dlk1 in mice to date has not resulted in development of leukaemia within 6 months of transplantation, nor significant abnormalities in peripheral blood counts. Further follow up of transplanted mice is ongoing.

### Conclusion

In a leukaemic cell line, DLK1 expression is associated with enhanced clonogenic potential. However, its precise role in normal haemopoiesis and leukaemogenesis remains unclear and is the subject of on-going investigation.

## **065. Prophylactic infusion of multi-virus specific T cells for management of viral reactivation and infection in patients post Allogeneic Hematopoietic Stem Cell Transplantation (HSCT): A clinical update**

**Ma C**<sup>1,2</sup>, Blyth E<sup>1,2,3</sup>, Clancy L<sup>1</sup>, Simms R<sup>1</sup>, Burgess J<sup>1</sup>, Micklethwaite K<sup>1,2,3</sup>, Gottlieb D<sup>1,2,3</sup>

<sup>1</sup> Westmead Millennium Institute, <sup>2</sup> University of Sydney, <sup>3</sup> Haematology Department, Westmead Hospital

### **Aim**

We present an update on a phase I/II clinical trial administering multi-virus specific T cells prophylactically to patients who underwent HSCT.

### **Methods**

Donor derived CMV, EBV, adenoviral and VZV specific T cells were generated according to standard operating procedures. HSCT recipients received  $2 \times 10^7/m^2$  virus specific T cells at or after day 35 post transplant and were monitored for viral reactivation and graft versus host disease (GVHD). Outcome measures were compared with a contemporaneous cohort using landmark analysis.

### **Results**

10 patients were infused with virus specific T cells and followed up for median of 12 months. No infusion related adverse events were noted. 8 patients had CMV reactivation post-transplant: 2 patients reactivated prior to T cell infusion only, 4 patients reactivated CMV before and after T cell infusion and 2 patients reactivated CMV only after T cell infusion in the context of acute GVHD. Median peak CMV DNA titre was 600 copies/ml after T cell infusion. 2 patients required ganciclovir prior to T cell infusion. Only 1 of 6 patients (16%) reactivating CMV after infusion received treatment. No patient developed CMV disease. No clinical EBV, adenoviral and VZV reactivation or disease was seen. 3 patients developed grade II-IV acute GVHD, and 4 patients developed extensive chronic GVHD. In contrast, 9 patients in the control cohort reactivated CMV with a median peak CMV titre of 2840copies/ml and 6 (67%) required antiviral therapy. 1 patient developed CMV colitis. Adenovirus infection, EBV reactivation and a VZV positive vesicular rash developed in 1, 1 and 1 patient respectively in the control group but not in any of the T cell treated patients.

### **Conclusion**

Multi-virus specific T cell therapy appears safe in the setting of HSCT. Following T cell therapy there was a reduction in the need for antiviral treatment in patients who reactivated CMV.

## **066. A novel chemoresistance function for the lipid phosphatase INPP4B in acute myeloid leukaemia**

**Rijal S**<sup>1</sup>, Cummings N<sup>1</sup>, Fleming S<sup>3</sup>, K Rynkiewicz N<sup>2</sup>, Ooms L<sup>2</sup>, Nugyen N<sup>1</sup>, Teh T<sup>1</sup>, Avery S<sup>3</sup>, McManus J<sup>3</sup>, Mclean C<sup>4</sup>, Papenfuss T<sup>5</sup>, Guthridge M<sup>1</sup>, Mitchell\* C<sup>2</sup>, Wei\* A<sup>1,3</sup>, \*Co-corresponding Authors

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### **Aim and background**

Acute myeloid leukemia (AML) is an aggressive blood cancer that may be fatal if disease is not responsive to intensive chemotherapy. Activation of the phosphoinositide 3-kinase (PI3-K)/AKT pathway is prevalent in AML and regulated by a triad of lipid phosphatases, known functionally as inositol polyphosphatase (INPP) enzymes, such as PTEN (3-phosphatase), SHIP (5-phosphatase) and INPP4B (4-phosphatase). The aim of this work was to examine the biological significance of lipid phosphatases in AML, which is currently unknown.

### **Methods and Results**

Sequenom MassArray® quantitative gene expression profiling of 12 lipid phosphatases conducted using mRNA from 25 patient AML bone marrow (BM) and 6 normal BM identified significantly increased expression of the 4-phosphatase INPP4B in AML ( $p=0.02$ ). Immunohistochemistry of an expanded panel of 204 AML cases at diagnosis confirmed overexpression of INPP4B protein ( $\geq 50\%$  blasts positive) in 11% of the cases. INPP4B overexpression was associated with poor response to intensive chemotherapy leading to significantly shorter leukaemia-free survival ( $p=0.02$ ) and overall survival ( $p<0.001$ ). Ectopic overexpression of INPP4B in MV4;11 and HEL AML cells conferred resistance to standard cytotoxic drugs used to treat AML, including ara-C, daunorubicin and etoposide, using in vitro cell death and colony assays. INPP4B overexpression also led to impaired clearance of bone marrow blasts by ara-C in vivo, as well as significantly reduced overall survival in human xenograft models of AML. Although INPP4B phosphatase function was proven to be catalytically active in primary AML, expression of a phosphatase inactive mutant (INPP4B C842A) did not abrogate chemoresistance in vitro or in vivo. In contrast, siRNA-mediated knockdown of endogenously overexpressed INPP4B in KG1 and OCI-AML3 AML sensitized leukemic cells to ara-C.

### **Conclusion**

These findings infer 1) the presence of a novel phosphatase-independent function for INPP4B and 2) a previously unsuspected role for INPP4B overexpression in mediating chemoresistance and poor clinical outcome in a subset of AML patients.

## **067. AML MRD by Flow Cytometry (FCM) : A melody or harmony?**

**Westerman, D**

*Peter MacCallum Cancer Centre, Melbourne, VIC*

Myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) are the predominant myeloid neoplasms where FCM has both a diagnostic and prognostic role in addition to minimal residual disease (MRD) testing. Utilising our understanding and delineation of normal antigen maturation and expression patterns of myelomonocytic precursors enables the detection of aberrancy in immature and mature populations. This forms the basis of reporting AML MRD FCM.

There are numerous publications demonstrating prognostic significance of MRD at both post induction and post consolidation time points in adults and paediatric AML. Most of these are single institution studies, highlighting the sophistication required by laboratories. Although FCM is a standard method used in the diagnosis of AML, MRD testing is far from routine in Australia. It requires a significant laboratory commitment, technical expertise on multiparameter cytometers and thorough cellular interrogation and analysis. These prerequisites and challenges have thus far deterred laboratories from implementing AML MRD analysis. In recognition of these challenges, harmonisation and standardisation efforts are underway in Australia and Europe.

## **068. Diagnostic difficulties in Myelodysplastic**

**Juneja, S**

*Royal Melbourne Hospital, Melbourne, VIC*

The diagnosis of MDS is straightforward in majority of cases. However, in a small but significant minority of cases, their diagnosis, distinction from other myeloid neoplasms & dysplasia due to non-clonal causes can be challenging. In this presentation categories of MDS which often pose diagnostic difficulties like hypoplastic MDS, myelodysplastic syndromes with marrow fibrosis, overlap between myeloproliferative & MDS syndromes, childhood MDS, heavy metal poisonings & metal deficiencies, cytokine therapy & iatrogenic causes will be presented. The role of immunohistochemistry, flow cytometry, cytogenetics & molecular studies in facilitating the diagnosis particularly in diagnostically challenging cases will be also discussed.

## **069. Overview of the pathology of myeloproliferative neoplasms**

**Erber, W**

*The University of Western Australia*

Myeloproliferative neoplasms (MPN) are clonal haematopoietic stem cell disorders characterised by proliferation of one or more of the myeloid lineages. Despite advances in our understanding of the genetic basis of MPN (e.g. JAK2, CALR, MPL mutations), examination of the bone marrow remains a crucial component in diagnosis and classification. Histological features include marrow cellularity, megakaryocytic morphology and topography and stromal changes. These will be discussed in light of the clinical investigations.

## **070. Myelofibrosis: diagnosis, prognosis and management**

**Reiter A**

*University Medical Centre Mannheim of the Ruprecht-Karls University Heidelberg, Germany*

Our understanding of the pathobiology and optimized management of patients with myelofibrosis (MF) continues to evolve. Guidelines have been established for diagnosis and prognostication based on the WHO classification and the recommendations of international working groups and cooperations. Dysregulated activation of the JAK/STAT signalling pathway is a hallmark of MF and related myeloproliferative neoplasms (MPN). In the majority of patients, the constitutive activation of JAK/STAT signalling is caused by a single point mutation (JAK2 V617F). However, functionally similar molecular aberrations may be present in JAK2 V617F negative patients acting in the JAK-STAT signalling pathway downstream of EpoR or Tpo/MPL signalling and making JAK2 an appealing target for therapy. Ruxolitinib, a potent and selective JAK1 /JAK2 inhibitor, has obtained approval from regulatory agencies for the treatment of intermediate and high-risk MF (US), and for the treatment of disease-related splenomegaly and symptoms (EU), based on the results of two phase-III trials: COMFORT-I (ruxolitinib vs. placebo) and COMFORT-II (ruxolitinib vs. best available therapy-BAT). In both trials, ruxolitinib was significantly more effective in reducing splenomegaly and constitutional symptoms, improving the patients' quality of life and potentially also overall survival. Ruxolitinib treatment was well tolerated, with anaemia and thrombocytopenia as the most common on-target side effects. Of note, both, JAK2 V617F mutated and JAK2 wild-type patients responded equally well to treatment. Although the exact mechanisms underlying the clinical efficacy of ruxolitinib and other JAK1/JAK2 inhibitors, e.g. momelotinib, are not yet completely understood, the approval of the first targeted therapy for MF has provided a new and promising treatment option for many MF patients with critical unmet medical needs. In addition to targeting the JAK-STAT pathway, there is growing interest in other targeted agents that could be used alone or in combination with JAK1/JAK2 inhibitors. However, the therapeutic approach to MF is becoming more challenging than anticipated due to the complexity of molecular defects that affect a multitude of genes involved in intracellular signalling, epigenetic regulation and spliceosome at the least. Thus moving away from single drug to combination therapies may be the way forward in the treatment of MF and related MPNs.

**071. Predicting and managing TKI failure**

**Apperley J**

*Hammersmith Hospital, London, UK*

**ABSTRACT NOT SUBMITTED**

## **072. RCPA guidelines for standardised reporting of haemoglobin studies**

**Maxwell, E**

*Melbourne Pathology, Melbourne, VIC*

The Royal College of Pathologists of Australasia (RCPA) remains active in developing position statements and reporting protocols as both an educational tool to assist pathologists and to support clinical management and treatment. Structured reports also facilitate disease notification and registration as well as enabling aggregated data analysis. In 2008 funding was received to initiate structured cancer reporting, the Pathology Units and Terminology Standardisation (PUTS) project commenced in 2011 and the first edition of the Iron Studies Standardised Reporting Protocol was released in May 2013.

Inadequate report interpretation, as highlighted in case studies presented at a Transfusion Outcomes Research Collaborative seminar in 2013, prompted a recommendation from the fellowship that haemoglobinopathy studies be targeted for guideline development. This presentation will report on the progress of the national committee convened in March 2014. Efforts have focused on inclusion of content and interpretation sufficient to inform correct management by a general practice referrer but not limiting detail required by the tertiary referral laboratory, in order to streamline subsequent investigation and/or counselling of the patient, partners or relatives.

## **073. Diagnostic challenges in haemoglobin disorders**

**Finlayson, J**

*PathWest Laboratory medicine, Nedlands, WA*

The haemoglobinopathies comprise a heterogeneous group of disorders, broadly categorised into two main groups – the thalassaemias and the haemoglobin variants. Within these groups, syndromes of major clinical significance include Haemoglobin Barts hydrops fetalis, beta thalassaemia major and sickle cell disease.

Laboratory testing is important in identifying carriers who may be at risk for these severe syndromes in their offspring.

Most cases of uncomplicated haemoglobinopathies can be diagnosed using standard methodologies, however resolution of complex cases requires access to molecular testing and a variety of methodologies. This presentation will focus on the use of molecular testing in the investigation of such cases.

**074. Late side effects of Hodgkin therapy**

**Engert A**

*Uniklinik Köln, Köln, Germany*

**ABSTRACT NOT SUBMITTED**

## **075. Options for preserving fertility during therapy**

**Hart, R**

*The University of Western Australia, Perth, WA*

The talk will cover the options available to men, women and children after a diagnosis of a haematological malignancy. For men the option of sperm freezing is relatively straightforward, however often the quality of the sperm is poor or even absent. The options available to women are more challenging; consisting of ovarian suppression with GnRH analogues, ovarian tissue cryopreservation or if time allows a rapid cycle of IVF to freeze eggs or embryos. However the options available to children are very limited and should only be offered in the setting of a research protocol.

**076. Managing leukaemia in pregnancy**

**Apperley J**

*Hammersmith Hospital, London, UK*

**ABSTRACT NOT SUBMITTED**

## **077. QAP Morphology Session**

**Crichton G<sup>1</sup>**, Juneja S<sup>2</sup>, Marsden K<sup>3</sup>

*<sup>1</sup>Royal Children's Hospital, Melbourne, VIC, <sup>2</sup>Royal Melbourne Hospital, Melbourne, VIC, <sup>3</sup>Royal Hobart Hospital, Hobart, TAS*

This session will follow the standard format of the previous 17 years. There will be presentation and discussion of 3 RCPA Haematology Quality Assurance Program (RCPAQAP)-Morphology cases, followed by review of 6 interesting morphology cases.

In the QAP section, the cases from the last RCPAQAP Morphology Program sent out prior to the HAA meeting will be discussed. This component of the session focuses on the review of submissions from all QAP participants and discussion around those cases.

The cases in the non-QAP morphology presentation will be available for review in a specially allocated room from the day before the session. Participants are encouraged to review these cases themselves.

The non-QAP cases will include both paediatric and adult cases in one or more of the following categories:

rare cases that are presented to a large number of attendees who may not see any or many of such cases

rare, but clinically important morphological diagnoses

an update or new developments on common diagnoses

new or recently described entities

The presentation and discussion during this session aims to be interactive and active audience participation is strongly encouraged.

## **078. New biomarkers in Hodgkin Lymphoma**

**Gandhi, M**

*Princess Alexandra Hospital, Brisbane, QLD*

In this session, Professor Gandhi will review the current status of circulating and tissue based prognosticators and disease response biomarkers in Hodgkin Lymphoma. Emphasis will be on novel biomarkers, and future avenues of research.

## **079. Response adapted therapy in Hodgkin Lymphoma: The role of PET**

**Hutchings, M**

*Copenhagen University Hospital, Copenhagen, Denmark*

PET/CT has emerged as the most accurate tool for staging, treatment monitoring and response evaluation in Hodgkin lymphoma (HL). Accurate staging and restaging are very important for the optimal management of HL, but we are only beginning to understand how to use PET/CT to improve our patients' treatment outcomes. More precise determination of disease extent may result in more precise pre-treatment risk stratification, and is also essential to the minimal and highly individualized radiotherapy volumes of the present era. A number of recently completed and currently running trials investigate the use of PET/CT for early response-adapted therapy, with therapeutic stratification based on interim PET/CT results. Post-treatment PET/CT is a cornerstone of the revised response criteria and enables selection of advanced stage patients without the need for consolidation radiotherapy. Once remission is achieved after first line therapy, PET/CT seems to have little or no role in the routine surveillance of HL patients. PET/CT looks promising for guidance of therapy in relapsed and refractory disease, where a good metabolic response to induction chemotherapy seems to be the most important determinant of outcome after high-dose chemotherapy with autologous stem cell support.

**080. Treatment of advanced stage disease**

**Engert A**

*Uniklinik Köln, Köln, Germany*

**ABSTRACT NOT SUBMITTED**

## **081. Allogeneic stem cell transplant in chronic myelomonocytic leukemia - Multicentre Australian experience**

**Bajel A**<sup>1</sup>, Curley C<sup>2</sup>, Lim A<sup>1</sup>, Handunnetti S<sup>3</sup>, Getta B<sup>4</sup>, Thompson P<sup>1</sup>, Wright M<sup>5</sup>, Greenwood M<sup>6</sup>, Hertzberg M<sup>4</sup>, Curtis D<sup>3</sup>, Szer J<sup>1</sup>, Kennedy G<sup>2</sup>, Ritchie D<sup>1</sup>

<sup>1</sup> Royal Melbourne Hospital, <sup>2</sup> Royal Brisbane Hospital, <sup>3</sup> The Alfred, <sup>4</sup> Westmead Hospital, <sup>5</sup> Royal Perth Hospital, <sup>6</sup> Royal North Shore Hospital

### **Aim**

Chronic myelomonocytic leukemia (CMML) is an uncommon and heterogeneous disease with a varying clinical course. Treatment strategies for CMML are expanding however allogeneic stem cell transplant (allo SCT) remains the only curative therapy. Transplant outcome data for CMML are limited and often presented combined with other myelodysplasia subgroups. We evaluated our outcomes for CMML patients who received alloSCT.

### **Methods**

A retrospective review of CMML patients transplanted in Australia was undertaken. Data analysed included recipient demographics, donor/graft characteristics, conditioning, graft versus host disease (GVHD), overall survival (OS), progression free survival (PFS), non-relapse mortality (NRM) and relapse/progression.

### **Results**

57 patients with CMML underwent 60 alloSCT between Jan 2000 and Dec 2013. Median age of the recipients was 53 years (y) (range 15-68 years). 29(50%) of the patients had transformation to acute leukemia prior to transplant. 33(55%) were matched related donor transplants. The graft source used was peripheral blood stem cells in 54(90%) transplants. Myeloablative conditioning was used in 28 (47%) transplants. Median follow up of the survivors was 40 months (11-155 months). OS and PFS at 6 years was 39% (95% CI 27- 58 %) and 26 % (95% CI 15-46 %) respectively. NRM and incidence of relapse at 6 years were 39% (95% CI 23-54%) and 36% (95% CI 21-51%) respectively. Cumulative incidence of aGVHD grade 3-4 at day 100 was 25% (95% CI 13-36%) and of extensive cGVHD at 1 year, 25% (95% CI 13-37%). In multivariate analysis, age < 50y was an adverse risk factor. There was no impact of transplant year or prior leukemic progression on PFS.

### **Conclusion**

Allogeneic SCT is a curative treatment for CMML but the high NRM and relapse rates continue to remain significant issues. The current transplant outcomes are similar to those previously published and are superior to outcomes with hypomethylating agents.

## **082. Outcomes of allogeneic haematopoietic progenitor cell transplantation in relapsed and/or refractory chronic lymphocytic leukaemia (CLL).**

Yue M, Morris K, Collins J, Curley C, Kennedy G

*Royal Brisbane and Women's Hospital*

### **Aim**

To assess the outcomes of allogeneic haematopoietic progenitor cell transplantation (HPCT) for CLL at our institution.

### **Method**

All HPCT performed at our institution between 2000 and 2013 for CLL were identified retrospectively from an institutional database and assessed for outcomes including relapse free survival (RFS), overall survival (OS) and non-relapse mortality (NRM). Selection for HPCT during this time period was based on EBMT criteria. Conditioning regimen was based on individual physician discretion; all were T-cell replete.

### **Results**

In total, 37 patients underwent allogeneic HPCT for CLL in the study period. Median age was 56yrs (range: 42-67yrs), with 70% male. Overall, 43% patients had chemosensitive disease (4 in CR, 12 in PR) at the time of transplant. Peripheral blood stem cells (PBSC) were used as stem cell source in 36 cases (97%); 17 patients received grafts from matched sibling donors (46%) and 20 patients from unrelated donors (URD); 16 matched, 4 mismatched. Conditioning was with predominantly non-ablative regimens, including fludarabine/cyclophosphamide in 19 cases (51%), fludarabine/TBI in 9 (24%), fludarabine/melphalan in 6 (16%) and cyclophosphamide/TBI in 3 (8%). At a median follow up post-HPCT of 12 months (range 1-162months), median RFS is not reached and median OS is 24 months. Overall NRM was 49%, with a majority of NRM deaths (56%) occurring beyond 6 months secondary to chronic GVHD or infections. Although no clinical or transplant parameter was associated with improved OS, including conditioning intensity, donor type (sibling vs URD) and era of HPCT (pre Vs post 2010); a strong trend towards improved OS was seen in patients with chemosensitive disease at HPCT ( $p=0.07$ ).

### **Conclusion**

Outcomes of allogeneic HPCT in patients with relapsed/refractory CLL remain suboptimal. There is ongoing risk of late NRM due to chronic GVHD or infection. Patients with chemosensitive disease at HPCT may be associated with improved survival.

## **083. Graft transit time has no effect on outcome in unrelated donor haematopoietic cell transplants (HCT) performed in Australia and New Zealand: An Australasian Bone Marrow Transplant Recipient Registry (ABMTRR) study**

Nivison-Smith I<sup>1</sup>, Bardy P<sup>2</sup>, Dodds A<sup>3</sup>, Ma D<sup>3</sup>, Szer J<sup>4</sup>, Blacklock H<sup>5</sup>, Carter J<sup>6</sup>, Butler A<sup>7</sup>, Watson H<sup>8</sup>, Cannell P<sup>9</sup>, Antonenas V<sup>10</sup>, Patton N<sup>8</sup>, Spencer A, Lewis I, Mechinaud F, O'Brien T

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### **Aim**

This study examined whether outcomes of unrelated donor allografts performed in Australia and New Zealand were measurably affected by the length of time that the donated graft spent in transit.

### **Method**

Patient and transplant details were retrieved from the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR), and additional data were retrieved from a customised spreadsheet questionnaire distributed to participating centres. The effect of independent variables, including transit time, on transplant outcomes were measured using cumulative incidence, Kaplan-Meier survival curves and multivariate Cox regression.

### **Results**

A total of 234 patients who underwent first myeloablative allograft for good risk ALL, AML, CML or MDS between 2001 and 2009 in Australia and New Zealand with an unrelated donor and a bone marrow or peripheral blood graft were included in this study. The median transit time (from end of collection to receipt at transplant centre) of grafts travelling from overseas to Australia or New Zealand centres was 32 hours (range 5 – 111). Median neutrophil engraftment time was 20 days for bone marrow (BM) HCT and 16 days for peripheral blood (PB) HCT. Median platelet engraftment was 26 days for bone marrow HCT and 20 days for peripheral blood HCT. Overall survival was 62% at 1 year and 46% at 5 years for BM HCT, and 67% at 1 year and 59% at 5 years for PB HCT. Total elapsed time from end of collection to receipt at transplant centre had no significant effect on engraftment, acute graft-versus-host disease, early (100 day) death or overall survival. The same held true for total elapsed time from receipt at transplant centre to infusion in patient and total elapsed time from collection to infusion.

### **Conclusions**

Graft transit time had no measurable effect on transplant outcome in this group of unrelated donor HCT recipients.

## **084. Causes and effects of methotrexate dose alterations in allogeneic haematopoietic cell transplantation**

**Ramanan R**, Lim A, Mason K, Szer J, Ritchie D

*Department of Clinical Haematology and Bone Marrow Transplant Service, The Royal Melbourne Hospital, Parkville, Victoria, Australia*

### **Aim**

To identify the causes and consequences of omission and/or reduction of methotrexate (MTX) doses in graft-versus-host disease (GVHD) prophylaxis used during allogeneic haematopoietic cell transplantation (alloHCT).

### **Method**

We conducted a retrospective medical record review of 125 alloHCTs (2011-2013) at our hospital where MTX (15,10,10,10 mg/m<sup>2</sup> on day [D] +1, D+3, D+6, D+11 respectively) is used with cyclosporin as GVHD prophylaxis. The association of MTX dose omission with overall survival (OS), non-relapse mortality (NRM) and GVHD, was evaluated with univariate and multivariate analysis.

### **Result**

Of the 116 eligible patients, 85 (73%) received all four full doses of MTX. 22 had a dose omission at D+11, and 2 at both D+6 and D+11. 43 were given folinic acid rescue (FAR). Reasons for MTX alteration were mucositis (22), fluid overload (10), liver impairment (9), renal impairment (8) and sepsis (1). MTX omission was associated with poorer 12 month OS (48% vs 90%, P<0.001) and higher NRM (39% vs 5%, P<0.001). A pattern of ongoing NRM was observed even beyond 3 months. Strikingly, those patients who received all four full doses of MTX had NRM of 0% at 100 days and <5% at 12 months. There was no difference in rates of grade II-IV (24% vs 22%) or III-IV (9% vs 11%) acute GVHD, or relapse (20% vs 17%), at 3 months.

### **Conclusion**

MTX dose reduction has no significant impact on GVHD development, suggesting that MTX omissions or other adjustments of GVHD prophylaxis did not lead to enhanced T cell activation. However, it seems that the need to reduce MTX indicates an increased risk of NRM, likely reflecting ongoing organ dysfunction. Older patients or those with pre-transplant co-morbidities may be better served by strategies that lower the likelihood of organ toxicity, including reduced intensity conditioning and lower initial doses of MTX.

## **085. Multi-pathogen cytotoxic T-lymphocytes to enhance immunity post-allogeneic stem cell transplantation (HSCT)**

**Ma CK** <sup>1,2</sup>, Micklethwaite K <sup>1,2,3</sup>, Deo S<sup>2</sup>, Clancy L <sup>1</sup>, Simms R <sup>1</sup>, Burgess J <sup>1</sup>, Blyth E <sup>1,2,3</sup>, Gottlieb D <sup>1,2,3</sup>

<sup>1</sup> Westmead Millennium Institute, <sup>2</sup> University of Sydney, <sup>3</sup> Haematology Department, Westmead Hospital

### **Aim**

We present preliminary data from a phase I/II clinical trial administering T cells specific for seven common opportunistic pathogens to HSCT patients.

### **Methods**

HSCT recipients were given  $2 \times 10^7/m^2$  donor derived T cells specific for CMV, EBV, adenovirus, BK, VZV, influenza and *Aspergillus fumigatus*  $\geq 28$  post transplant and will be monitored for 12 months for infections and graft versus host disease (GVHD).

### **Results**

To date, 3 patients received multi-pathogen specific T cells with follow up of 4, 3 and 1 month respectively. For patient 1, CMV and EBV DNA were present at low copy number in blood at infusion. She also had BK viraemia with titre of  $1.5 \times 10^9$  copies/ml. After infusion, CMV and EBV DNA became undetectable and BK viraemia reduced to  $3.5 \times 10^3$  copies/ml. No *Aspergillus* infection was detected. Patient 2 (donor CMV seronegative and thus did not receive CMV specific T cells) had EBV reactivation post infusion which resolved spontaneously without therapy. *Aspergillus* DNA was detected before infusion but became negative after infusion. He developed CMV colitis 12 days post infusion which failed to respond to ganciclovir and was rescued with third party CMV specific T cell infusion. Patient 3 had no viral reactivation or *Aspergillus* infection before or after infusion. 1 patient had grade 1 skin acute GVHD which resolved prior to infusion and did not recur. 1 patient developed grade II acute GVHD involving skin and gut 19 days after infusion. Patient 1 died 82 days post infusion from progressive NK leukaemia. The other 2 patients remain alive at 127 and 29 days post infusion.

### **Conclusion**

Multi-pathogen specific T cell therapy has been given following HSCT with no safety signals to date. Treatment of more patients and analysis of infection patterns and immune reconstitution will continue as more patients are recruited to the trial.

## **086. Outcomes of haploidentical and cord blood transplants: A single site experience**

Perram J, Tran S, Milliken S, Dodds A, Ma D, Fay K, Bilmon I, Kwan J, Moore J

*St Vincent's Hospital*

### **Aim**

To report on outcomes of haploidentical and umbilical cord blood transplants performed at a single site from 2003-2013.

### **Method**

All patients who underwent haploidentical (n=9) or umbilical cord blood (n=23) transplants at the centre gave consent for research participation. Single (n=5) and double cord (n=18) transplant as well as haploidentical myeloablative (n=3) and reduced intensity chemotherapy (n=6) groups were assessed with Kaplan Meier survival curves for overall and disease free survival.

### **Result**

Patients who received haploidentical transplants had slightly higher rates of overall survival at 1 year (41%) compared to recipients of umbilical cord blood transplants (35%). Haploidentical transplant recipients had a 1-year disease free survival rate of 41%, compared to 30% for umbilical cord blood transplant recipients. Comparing single and double cord blood transplants, overall survival at 7 years is comparable between double transplant recipients (22%) and single transplant recipients (20%). Of haploidentical transplant recipients, 1-year survival rates were higher amongst recipients of reduced intensity chemotherapy (53%) than those who underwent myeloablative conditioning (33%).

### **Conclusion**

Haploidentical and cord blood transplants were well tolerated presenting an alternative treatment option in high risk haematological malignancy. Preliminary results in haploidentical transplant recipients suggest improved outcomes in the reduced intensity chemotherapy group. Similar survival rates were seen in single and double cord transplant recipients over 7 years follow up.

## **087. Generation of Herpes simplex virus specific T cells from healthy donors using HLA-A1 and A2 specific epitopes**

**Ma C**<sup>1,2</sup>, Clancy L<sup>1</sup>, Kim M<sup>1,2</sup>, Micklethwaite K<sup>1,2,3</sup>, Gottlieb D<sup>1,2,3</sup>

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### **Aim**

We present data on the generation of HSV specific T cells from healthy donors using HLA-A1 and A2 epitopes from tegument proteins VP11 and VP16, viral capsid, glycoprotein B, and ribonucleotide reductase enzyme.

### **Methods**

Mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation of peripheral blood from healthy HSV seropositive donors. HSV specific T cells were generated by stimulating PBMCs with antigen pulsed monocyte derived dendritic cells and cultured for 21 days with IL2 for the last 14 days. The quality and specificity of cultured HSV specific T cells were tested using immunophenotyping, intracellular cytokine release (CFC), CFSE attenuation using flow cytometry and a Calcein AM cytotoxicity assay.

### **Results**

Cultures from 10 healthy donors were performed, with a mean cell fold expansion of 9.1 (range 1.4-19.95). A mean of 98% (range 82.4-99.2%) were T cells. Within the expanded products, 29% (range 1.83-79.2) were CD8+. The majority of cells had an antigen experienced phenotype (mean 44% and 25% effector and central memory T cells respectively, n=6). Specificity testing using CFC showed a mean of 32% (range 8-73%) of CD8+ T cells secreted IFN- $\gamma$  upon stimulation with HLA specific peptides. Overall there was a median total expansion of 7848 fold (range 98 to 609331 fold) of cytokine producing cells. Cytokine profiling showed a mean of 97%, 53% and 15% of antigen specific T cells secreted IFN- $\gamma$ , TNF- $\alpha$  and IL-2 respectively (n=7). After stimulation with HSV peptides, 31% of cells underwent division, compared to 7.6% in control. HSV specific T cells demonstrated cytotoxicity against autologous peptide-loaded PHA-blasts and HSV infected DCs (mean specific lysis 64% and 18% at E:T ratio of 10:1 respectively).

### **Conclusion**

Generation of HSV specific T cells using HLA-restricted peptides is feasible and expanded T cells demonstrate specificity, proliferation and cytotoxicity when stimulated by HSV specific antigens.

## **088. Use of a three-antigen combination to generate T cell products with HLA-DR dependent activity against pathogenic filamentous fungi and yeasts**

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### **Aim**

Aspergillus, fusarium, zygomycetes, scedosporium and candida are the most important causes of invasive fungal diseases (IFD) in hematology patients. We investigated the potential to generate a T cell product with activity against these fungi for adoptive immunotherapy.

### **Method**

We made lysates from fungal pathogens (*A. flavus*, *A. terreus*, *C. albicans*, *C. krusei*, *F. oxysporum*, *F. solani*, *R. oryzae* and *S. prolificans*) and used these to pulse monocyte derived dendritic cells (MoDC) from PBMC or G-CSF mobilized peripheral blood stem cells (PBSC) from normal donors. MoDCs were then used to stimulate autologous cells on Days 0 and 7. Cultures were expanded using IL-2, IL-7 and IL-15 from Days 7 to 21.

### **Results**

In four cultures from PBMC raised specific to each fungus, expansion occurred with all lysates (range 2.3-109.6 fold) generating 85-97% T cells of which >80% were CD4+ cells. The percentages of fungus-specific (TNF $\alpha$ +) CD4+ cells were *A. flavus* 6.8 $\pm$ 5.5%, *A. terreus* 13.2 $\pm$ 12.8%, *C. albicans* 10.5 $\pm$ 8.5%, *C. krusei* 6.4 $\pm$ 6.8%, *F. oxysporum* 6.4 $\pm$ 3.8%, *F. solani* 5.2 $\pm$ 7.8%, *R. oryzae* 7.6 $\pm$ 6.4 and *S. prolificans* 4.4 $\pm$ 3.8% (n=4). A three-antigen combination was selected based on cross-reactivity analyses to generate multifungus T cell products from PBMC (n=8) and PBSC (n=3). There were 7.0 (0.7-12.7) and 12.1 (8.7-15.0) fold-increase in cell numbers in cultures from PBMC and PBSC respectively. The percentage of total CD4+ cells were 61.2 $\pm$ 26.2% and 81.2 $\pm$ 3.5%, and of fungus-specific CD4+ cells were 14.9 $\pm$ 13.9% and 17.0 $\pm$ 8.1% in cultures from PBMC and PBSC respectively. Antifungal activity was mediated through HLA-DR alleles and was maintained when MoDC from partially HLA-DRB1-matched allogeneic donors were used to present fungal antigens to cultured T cells.

### **Conclusion**

We demonstrate a method for manufacturing a T cell product with activity against multiple clinically relevant fungi from blood and stem cells of healthy donors. Adoptive transfer of fungus-specific T cells may be used to prevent and treat IFD in immunocompromised hematology patients.

## 089. The Australian cohort of the multinational registry of patients with atypical haemolytic-uraemic syndrome (aHUS)

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### Aim

Atypical haemolytic-uraemic syndrome (aHUS) is a rare, life-threatening disease caused by chronic complement activation characterised by thrombotic microangiopathy leading to renal and other end-organ damage. There is little information on incidence, treatment and course of patients with aHUS. Since April 2012 the multinational aHUS Registry has been prospectively collecting information on aHUS patients. Herein we report on the Australian aHUS patient cohort enrolled in the global aHUS Registry.

### Methods

Patients with clinical diagnosis of aHUS, with or without an identified complement regulatory factor genetic abnormality or anti-complement factor antibody and ADAMTS13 > 5% are eligible for enrolment in the Registry. The Registry collects data on demographic, disease history, laboratory measures, treatments and outcomes at baseline and every 6 months thereafter.

### Results

Within 2 years, 28 patients from 5 Australian centres were enrolled in the Registry. Twenty-five (89%) patients were ≥18 years and 20 (71%) patients were female. Six patients (21%) had familial aHUS and 5 (16%) had a complement factor gene mutation or auto-antibody identified. At entry in the Registry, 86% of patients had received plasma exchange or infusion, 71% had renal impairment, 68% required dialysis and 36% had received a kidney transplant. Central nervous system (12%) and gastrointestinal (12%) manifestations were the most common extrarenal sites of aHUS involvement. Ten patients (36%) received eculizumab therapy with a median duration of treatment of 1.4 years (0.3–3.6). Four patients (14%) died during the observation period, 1 (10%) in the eculizumab-treated cohort and 3 (17%) in the eculizumab-naïve cohort (chi-square=0.59).

### Conclusions

This analysis demonstrates that in the Australian aHUS cohort the incidence of end-stage kidney disease and annual mortality rates are very high. The Registry is providing a better understanding of aHUS and its progression, and may help optimize management of patients with this life-threatening disease.

## **090. Autologous stem cell transplantation (ASCT) in multiple sclerosis: Results from a single centre**

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### **Aim**

To review the outcome of ASCT for multiple sclerosis at Sir Charles Gairdner Hospital, Western Australia.

### **Methods**

Eligibility criteria for ASCT were (1) progression of sustained disability with EDSS increase of more than 1/10 over a 12 month period, (2) advanced MS with threatened loss of ambulation and (3) rapidly progressive disease not adequately assessed by EDSS. The primary aim was to slow or halt neurological deterioration. Patients were identified by one of two neurologists and then presented to a haematologist. Stem cell mobilization was with cyclophosphamide 2g/m<sup>2</sup> and G-CSF 5ug/kg bd. Conditioning chemotherapy was with cyclophosphamide 50mg/kg and rabbit ATG 1mg/kg days -5 to -2. Patients were assessed at 3, 6, 12 and 24 months post-transplant.

### **Results**

Fourteen patients underwent ASCT. Median age was 47 (22-60). Median time from diagnosis to transplant was 12 years (3-30). Diagnosis at transplant was secondary progressive MS (12), primary progressive MS (1) and neuromyelitis optica (1). Median CD34+ cells re-infused was 6 x10<sup>6</sup>/kg. Median time to neutrophils >1.0 was 10 days and unsupported platelets > 20 was 9 days. Common peri-transplant complications included fluid retention, asymptomatic haematuria and febrile neutropenia. Two patients had worsening of neurological function during chemotherapy which resolved spontaneously. Unexpected complications include one episode each of JK virus cystitis, CMV reactivation and late ITP. The EDSS at 24 months was unchanged in 13 patients and had deteriorated 1.0 in 1 patient. More detailed assessment revealed about half the cohort were neurologically stable at 24 months while the remainder had some neurological deterioration. Two patients had meaningful improvement in bladder function. Follow-up MRI showed no Gd-enhancing lesions, but two patients developed new cerebral lesions on T2 weighted imaging.

### **Conclusions**

In this group of patients with advanced MS, neurological function 24 months post-ASCT was essentially stable in half the cohort while the remainder experienced clinical progression. It is not possible to conclude whether ASCT altered the natural history of the disease.

## **091. Baseline characteristics and outcomes following eculizumab treatment in Australian patients enrolled in the International Paroxysmal Nocturnal Haemoglobinuria (PNH) Registry**

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### **Aim**

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare haemopoietic stem-cell disorder characterised by chronic, complement-mediated haemolysis, life-threatening thrombosis and is associated with 35% mortality at 5 years following diagnosis. Eculizumab, a complement C5 inhibitor, has been demonstrated to reduce both intravascular haemolysis and thrombosis, and to improve survival in PNH patients. This analysis describes the baseline characteristics and clinical outcomes of Australian patients enrolled in the International PNH Registry.

### **Method**

A descriptive analysis of important laboratory and clinical measures at baseline and following eculizumab commencement was performed for Australian patients enrolled in the PNH Registry.

### **Results**

As of February 2014, 82 Australian patients were enrolled in the PNH Registry. 26 patients were eculizumab-naïve (EN) and 56 patients were treated with eculizumab (ET). Median age of all patients was 39 years and sex was evenly distributed. At baseline, 9% of EN patients had a PNH granulocyte clone size >50% compared to 83% of ET patients, while median lactate dehydrogenase (LDH) ratio was 1.1 x upper limit of normal (ULN) in EN patients and 5.2 x ULN in ET patients. 69% of the EN patients had a concomitant bone marrow disorder and none had a prior history of a thromboembolic event (TE) whereas in the ET patients 31% had a concomitant bone marrow disorder and 41% had a history of a TE. Following commencement of eculizumab, there was an improvement in the median LDH ratio to 1.1 x ULN by 6 months which was maintained at 12 and 24 months. 3 TEs and 2 meningococcal infections occurred corresponding to 1.7 and 1.1 events/100 patient-years respectively, while no patient deaths occurred.

### **Conclusion**

Data from the Australian cohort of patients participating in the PNH Registry confirm that ET patients tended to have more significant PNH than EN patients, and responded positively to eculizumab treatment.

## **092. Proplatelet formation capacity of megakaryocytes is impaired by antiplatelet antibodies in immune thrombocytopenia**

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### **Aim**

Primary ITP is caused by production of antiplatelet antibodies that result in platelet destruction and suboptimal platelet production. According to in vitro studies, the later can be related to suppression of megakaryocyte (MK) production and/ or maturation. However, the crucial MK function proplatelet formation and the accompanied platelet release have not been investigated. Therefore, the main aim of this study is to determine the effect of ITP antibodies on MK proplatelet formation and platelet release, with relation to MK production and late differentiation.

### **Methods**

19 ITP patients and 9 healthy controls were included. Cord blood hematopoietic stem cells were isolated and cultured with 50ng/mL human TPO. At day 8 or 9 of culture, 10% ITP or normal serum/ IgG were added. At different times later, the followings were evaluated: the number of proplatelet-bearing MK (inverted microscopy), the number of platelets and MKs total number, ploidy and maturation (all by flow cytometry). For statistical analysis unpaired t-test was used.

### **Results**

Based on their effect on MK, ITP patients were divided into 2 groups: group A autoantibodies significantly suppressed the number of proplatelet forming MK (n = 13, P < 0.0001) while group B autoantibodies didn't induce significant changes (n = 6, P = 0.0599). Other megakaryocytic features including total numbers and maturation were not significantly affected. Interestingly, addition of TPO receptor agonist "romiplostim" had countered the effect of ITP antibodies on proplatelet formation (0.01 < p < 0.05).

ITP antibodies restrain the proplatelet formation capacity of MKs and hence the platelets formation without affecting MKs viability or maturation. Romiplostim has the power to restore the proplatelet formation ability of MKs in the presence of ITP antibodies, without altering the total cell mass. These findings contribute for better understanding of the pathophysiologic mechanisms in ITP and for improving treatment options.

## 093. Next generation sequencing of suspected myelodysplastic syndromes

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### Background and Aims

The myelodysplastic syndromes (MDS) are a diverse group of clonal myeloid disorders. Application of next generation sequencing (NGS) techniques has added to our understanding of their pathogenesis by identifying mutations in genes involved in many parts of DNA regulation. We hypothesise that mutational 'profiling' will be a useful adjunct in the diagnosis of MDS, particularly where bone marrow (BM) findings are insufficient to reach a diagnosis.

### Method

Patients undergoing bone marrow examination for investigation of unexplained cytopenias were recruited at Sir Charles Gairdner Hospital. Somatic DNA was extracted from EDTA-anticoagulated BM and constitutional DNA from buccal swabs. A custom AmpliSeq Primer Panel (Ion AmpliSeq Designer, Life Technologies), comprised of approximately 1500 primer pairs, was designed to assess 127 genes known to be associated with MDS and other haematological malignancies. Genomic DNA was fragmented before target gene sequences were amplified in a massively multiplex PCR reaction using this primer pool. The amplified PCR products were sequenced on an Ion Personal Genome machine (PGM) semiconductor sequencer (Ion Torrent, Life Technologies).

### Result

We report sequencing results from 21 of 30 patients recruited patients for whom we had evaluable matched buccal and marrow specimens (4 normal, 3 morphologically inconclusive, 12 MDS, 2 AML). A total of 28 somatic variants were identified in post-sequencing analysis. 23/28 (82%) were identified in genes known to be associated with MDS, 6 (26%) of which had previously been reported. The majority of variants (19/28, 68%) were demonstrated in patients with confirmed MDS (n= 15) or AML (n=4). Two variants were identified at high allele frequency in patients with inconclusive marrow morphology.

### Conclusion

NGS can identify somatic mutations in patients with cytopenias secondary to BM dysfunction (57% of cases). This suggests an analytical NGS 'pipeline' is technically feasible and capable of demonstrating variants in BM. Further evaluation of peripheral blood and its concordance with BM will be performed given the eventual goal of performing sequencing studies on peripheral blood alone.

## 094. Modelling resistance to epigenetic therapies

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The BET inhibitors are first-in-class, epigenetic targeted therapies that deliver a new therapeutic paradigm by directly targeting protein-protein interactions at chromatin. Early clinical trials have shown significant promise, especially in AML, suggesting that these compounds are likely to form an important component of future anti-cancer regimes. Therapeutic resistance is an inevitable consequence of most cancer therapies, therefore the evaluation of resistance mechanisms is of utmost importance in order to optimise the clinical utility of this novel class of drugs.

Using primary murine stem and progenitor cells immortalised with MLL-AF9, we have developed a novel approach to generate several clones stably resistant to the prototypical BET inhibitor, IBET151. In parallel, we have maintained matched vehicle treated clones and the parental cell line. Resistant cells maintain their clonogenic capacity in IBET and are also impervious to IBET induced cell-cycle arrest and apoptosis. Importantly, resistance to IBET confers cross-resistance to other chemically distinct BET inhibitors such as JQ1 and resistance to genetic knockdown of BET proteins. Moreover, resistance appears to be stably maintained across subsequent cell generations in the absence of ongoing selective pressure. Using a range of sophisticated in vitro and in vivo studies we conclusively demonstrate that resistance emerges from leukaemia stem cells. We will present the molecular mechanisms of resistance, including upregulation of compensatory pathways, molecular events at chromatin and the results of a high-throughput / high-content compound library screen designed to identify best-in-class targeted therapeutics currently in clinical use that overcome resistance to BET inhibitors.

Acquired resistance to cancer therapeutics is widely assumed to arise from cancer stem cells but conclusive scientific evidence for this is scant outside the setting of resistance to TKIs in CML. Our data provides novel insights into the biology of AML, molecular mechanisms of resistance to emerging epigenetic therapies and provides an unprecedented opportunity to study leukaemia stem cells and develop therapeutic strategies to eradicate them.

## **095. Development of a high throughput immunoFISH imaging flow cytometry protocol for the analysis of haematological malignancies**

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### **Aim**

Fluorescence in situ hybridisation (FISH) is a microscopy technique which uses fluorescent probes to detect DNA sequences (e.g. 14q32/IGH in B lymphoid malignancies). FISH is clinically relevant for the diagnosis of a number of diseases however interpretation becomes difficult where the number of genetically abnormal cells is low since morphology cannot be readily visualised. Integration of immunophenotyping and interphase FISH can be applied to air-dried cellular samples or tissue biopsies. Imaging flow cytometry has the potential for immunoFISH to be performed on whole intact cells in suspension and for chromosomal abnormalities to be quantified in cells identified by their morphology and phenotype. We have optimised an imaging flow cytometry protocol for FISH analysis in combination with immunophenotyping.

### **Methods**

Blood mononuclear cells from lymphoma and normals were stained with fluorescently conjugated antibodies to cell surface antigens, fixed and permeabilised. DNA was denatured followed by overnight probe hybridisation with a single colour or split signal probe, after which cells were stained with Hoechst 33342 and analysed.

### **Results**

We evaluated 10,000 events per sample of which 85-90% were single cells suitable for probe spot counting. A minimum of 1,000 phenotyped cells were analysed with 70-80% containing the expected 1-2 probe signals. Inclusion of Hoechst nuclear marker allowed proliferating cells to be excluded and increased spot counting accuracy.

### **Conclusion**

This is the first method to combine immunophenotyping with FISH analysis by imaging flow cytometry. It enables the automated FISH analysis of large numbers of cells identified by their cell phenotype, even when they only make up a subset of cells in the sample. ImmunoFISH has potential to be applied to the detection of aneuploidy as well as deletions, translocations or fusions in phenotypically identified cells. This could be used for staging of malignancies and detection of minimal residual disease detection following therapy.

## 096. Utilising DNA from peripheral blood granulocytes for SNP-microarray in Myelodysplastic Syndromes (MDS)

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### Aim

Conventional bone marrow (BM) metaphase cytogenetics (MC) detects chromosomal abnormality in only 40-50% MDS cases. BM MC fails in 20-30% cases either due to inadequate sample or cells fails to divide. Attempts to use peripheral blood (PB)-MNC for MC showed limited utility due to inability of PB cells to divide.

SNP-array improves detection of chromosomal abnormalities from 44% to 74% (Tiu et al *Blood*. 2011;117(17):4552-4560) In this pilot study we compared the use of DNA from PB-granulocytes (PB-Gran), PB-mononuclear cells (PB-MNC) and BM-MNC in cytogenomic array studies in MDS.

### Method

DNA extracted from PB-Gran, PB-MNC and BM-MNC from 17 MDS patients was used on the Affymetrix CytoScanHD array platform. Karyotyping was performed contemporaneously on whole bone marrow.

### Result

Of the 17 patients tested, 4 showed karyotypic aneuploidy, 1 had a reciprocal translocation and 12 showed normal cytogenetics. All of the karyotypic aneuploidies were confirmed by SNP-array. Cytogenomic change was demonstrated in 3 patients with normal karyotype and further cytogenomic change in 2 patients with abnormal karyotype. 9 of the 10 abnormalities detected by SNP-array in the PB-Gran showed equivalent, or higher, levels of abnormality than in the other two sample types (Table1).

### Conclusion

These results demonstrate the potential of PB-Gran SNP-microarray in MDS patients who are too elderly or frail to undergo BM biopsy, in hypoplastic MDS where sample is limited and in patients whose BM cytogenetics fails. The use of PB-Gran DNA may prove useful in assessing clonal evolution over time in a relatively non-invasive manner. Table 1: Comparison of abnormal clone size detected by SNP Array in BM-MNC, PB-MNC and PB-Gran

		Size of abnormal clone (calculated from array data)		
Case	Abnormality detected on SNP	BM-MNC	PB-MNC	PB-Gran
MDS-1	LOH7q	51	49	60
MDS-2	12p-	78	44	67
MDS-3	20q-	25	20	25
MDS-4	dup8q	60	70	0

MDS-5	LOH7q	67	37	67
MDS-6	+8	10	0	36
MDS-7	LOH7q	75	68	92
MDS-8	9q-	26	15	65
MDS-9	22q-	29	17	45
MDS-10	20q-	92	54	96
MDS 11-17	Normal Karyotype	Normal Karyotype	Normal Karyotype	Normal Karyotype

## **097. Microarray analysis of erythroid progenitors in individuals with beta-thalassaemia**

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### **Aims**

$\beta$ -thalassaemia is a disorder of globin gene synthesis resulting in reduced or absent production of the  $\beta$ -globin chain in red blood cells. Traditionally, the pathogenesis of  $\beta$ -thalassaemia has been attributed to ineffective erythropoiesis with intramedullary apoptosis of the erythroid progenitors. Recently, studies in mouse models have challenged this hypothesis with the concept of delayed progenitor maturation sited as a contributing factor to the ineffective erythropoiesis.

This study uses microarray technology to examine the erythroid progenitor mRNA of patients with transfusion dependent  $\beta$ -thalassaemia and compare it to erythroid progenitor mRNA from healthy controls.

### **Methods**

Haematopoietic stem cells were isolated from the peripheral blood of 6 healthy controls and 6 transfusion dependant  $\beta$ -thalassaemia patients. Following 7 and 14 days in culture early- and late-erythroblasts were isolated and purified. After RNA isolation and linear amplification, gene expression analyses were performed using microarray technology. The generated data were analysed by two methods; the brb-ArrayTools platform and with the Bioconductor platform using bead level data.

### **Results**

Morphological difference in maturation was not observed following 7 days in culture, while a pronounced delayed maturation was observed in the patient group after 14 days. For both analyses, following 7 days in culture there was no difference in gene expression between the control and patient groups. After 14 days in culture, 275 and 156 differentially expressed genes were identified by each method including 47 genes identified by both methods. Interrogating these gene lists with gene ontology tools a subset of 86 genes was selected whose results were confirmed by Quantitative-Real-Time-PCR.

### **Conclusion**

The changes in gene activity and development associated with the phenotype of  $\beta$ -thalassaemia occur late in the maturation process of erythroid-lineage cells. We believe that these changes in gene expression are due to delayed erythropoiesis in erythroblasts of  $\beta$ -thalassaemic patients as a result of their reduced  $\beta$ -globin expression.

## **098. Modification of the Vk\*MYC mouse model to target insertional mutagenesis to the B cell compartment generates mature B cell lymphomas but not multiple myeloma**

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Transposon insertional mutagenesis (IM) provides a powerful approach for the identification and validation of cancer driver mutations that compliments human sequencing efforts. The Vk\*MYC mouse model generated highly penetrant plasma cell tumours which accurately recapitulated the clinical features of human multiple myeloma (MM). The Vk\*MYC transgene was designed to be specific for the mature B cell compartment as its activation was thought to be dependent on reversion of a stop codon by somatic hypermutation during germinal centre B cell development.

### **Aims and Methods**

We adapted the Vk\*MYC model to generate two novel lines in which piggyBac (PB) IM is targeted to the mature B cell compartment. In the first, termed Vk\*hPB, the coding exons of MYC were replaced by the PB transposase. In the second (Vk\*MYC-TA-hPB), MYC and the transposase were expressed together from the same cistron using a T2A peptide linker that hydrolyses soon after translation.

### **Results**

IM mice had increased lymphoma associated mortality with median survival of 75.4 weeks in the Vk\*MYC-TA-hPB and 71.9 in the Vk\*hPB mice, compared to 91.1 weeks in the non-mutagenesis controls. In both IM cohorts approximately half the mice had B cell lymphoma at death however plasma cell neoplasms were not a feature. The morphology of the lymphomas was pleomorphic with follicular and diffuse patterns and variation in cell size from small to large cell both between and within tumours. By flow cytometry the vast majority of B cell lymphomas had a mature phenotype and BCR repertoire analysis revealed they were clonally re-arranged and had undergone somatic hypermutation. However, although the transposase was active, we could not find evidence that the stop codon had been reverted, and the Vk\*MYC-TA-hPB tumours were not universally MYC dependent. Common integration site analysis identified recurrent transposon integrations in known and novel lymphoma associated genes.

### **Conclusion**

IM mice had an increased B cell lymphoma associated mortality and several candidate genes of interest were identified.

## **099. Novel therapeutics in myeloma**

**Lonial S**

*UPMC Université, Paris, France*

Proteasome inhibitors and Immunomodulatory agents have dramatically changed the landscape of treatment for myeloma, but biology based preclinical investigations have identified several additional new targets that are currently undergoing clinical testing. Among these, several new classes of agents including histone deacetylase inhibitors (HDAC's), monoclonal antibodies (targeting CD38, SLAMF7, CD138, CD56 and others), kinesin spindle protein inhibitors (KSPi's), cyclin dependent kinase inhibitors, as well as agents targeting bcl-2, and many other new agents are in early clinical development and hold great promise as treatments in the near future. Incorporation of these treatments using biomarkers or genetics based assessments represents the next steps of clinical investigation and will help to maximally control or potentially cure myeloma in a larger fraction of patients.

**100. New drugs in patients with relapsed and refractory Hodgkins Lymphoma**

**Engert A**

*Uniklinik Köln, Köln, Germany*

**ABSTRACT NOT SUBMITTED**

## **101. Novel therapy for blood cancers: The role of NK cell lines**

**Keating, A**

*University of Toronto, Toronto, ON, Canada*

We have been interested in cells of the innate immune system, especially NK cells, for adoptive immunotherapy of blood cancers. Given that primary, and particularly autologous, NK cells have significant practical and scientific limitations, we have focused instead on permanent malignant NK cell lines to treat hematological malignancies. We have shown high levels of cytotoxicity with the lines, NK-92 and KHYG-1, against a variety of cancers. It is noteworthy that cytotoxic activity is retained after irradiation, rendering them incapable of cell division, hence obviating the likelihood of a NK malignancy arising in the recipient. We are completing a phase I trial of NK-92 cells in patients with advanced blood cancers and preliminary evidence suggests that the cells can be safely administered. We have investigated mechanisms of NK cytotoxicity against blood cancers and implicated activating receptors in killing myeloma cells, and for NK-92, have shown that NKp30, DNAM-1 and NKG2D are involved. In addition, we have shown that the lines NK-92 and KHYG-1 exhibit preferential killing of clonogenic malignant cells in multiple myeloma and acute myeloid leukemia. We are currently modifying the NK cells to further enhance their cytotoxicity against blood cancer targets. Our data raise the possibility that NK lines can be used in the setting of minimal residual disease for selected blood cancers to establish or enhance cure rates.

## **102. Blood on the battlefield**

**Branley J**

*University of Sydney, Sydney, NSW*

The Australian Defence Force (ADF) is required to provide military and humanitarian support both within Australia and in designated locations abroad. These operations, by their nature, carry a significant risk of trauma to military personnel and civilians. The provision of blood products to support operations provides challenges for both the logistic supply chain and the laboratory systems required to ensure safe delivery of resuscitative capacity.

The challenge to supply multiple component blood products to distant and remote locations within small windows of time is difficult. In addition the need for intermittent large volumes of blood combined with periods of minimal need results in a large amount of product wastage.

Deep freezing significantly extend the lifespan of blood products. Deep frozen RBCs last up to 10 years and frozen platelets may last more than 2 years. The experience of working with international forces, and in particular the Dutch military who use such products has informed and expanded the thinking about blood product provision in remote locations.

The combined ADF and ARCBS project looking for frozen blood solutions to these problems will be discussed with some clinical examples. The lessons learnt in the military context may be important for future civilian use of these products.

**103. Advances in blood delivery**

**Irving D**

*Australian Red Cross Blood Service, Alexandria, NSW*

**ABSTRACT NOT SUBMITTED**

**104. Rural and remote trauma**

**Speaker TBA**

**ABSTRACT NOT SUBMITTED**

## **106. Transfusion: What is the hold-up?**

**Bolton-Maggs, P**

*Manchester Blood Centre, Manchester, UK*

Haemovigilance schemes focus on adverse reactions and events in donors and recipients following transfusion of blood and its components. However, patients may also suffer adverse consequences if transfusion does not take place in a timely manner or is inadequate. Between 2005 and 2010 the National Reporting and Learning Scheme in England received reports of 11 deaths and 83 incidents in which patients were harmed as a result of delayed provision of blood in an emergency. A 'Rapid Response Report' followed in October 2010 with immediate action by hospitals to be completed by April 2011, including review of their local practices for requesting and obtaining blood in an emergency.

SHOT has collected reports of delays or inadequate transfusion since 2010 and accepts any report where the clinician noted 'delay', for example delay resulting from reluctance to transfuse overnight despite clear clinical indications. The number of such reports has increased each year. These are seriously ill patients with a high mortality (21/69, 30.4%) and in some cases (10/69, 14.5%), this was related to the delayed transfusion. The majority of these events were emergencies.

Key features identified from these reports included lack of knowledge about major haemorrhage protocols (MHP), and also serious errors: short cuts in procedures resulting in failure to correctly identify patients, poor sample labelling so that repeat samples were required, selection of wrong blood components and transfusion without the final bedside checks. Poor handover contributed to delay. There were unexpected gaps in training and lessons from fire alarms.

It is clear that further education and training is needed, particularly to ensure junior medical staff can recognise haemodynamic compromise and are facilitated to escalate to senior colleagues. The initiation and operation of MHPs will improve patient care because a structured approach can reduce panic and errors from cutting corners. However, to be effective appropriate training and drills are required in all areas of medical and surgical practice. Transfusion laboratories must receive clear notification of urgent requests in order to prioritise their work and make available appropriate components, and also be informed when the MHP is stood down.

## **107. Establishing electronic patient sample safety in Emergency Departments**

**Bayley K**

*PathWest, Nedlands, WA*

Emergency Departments are very urgent and demanding environments which make it very susceptible to simple errors that can impact on patient safety. The most common pathology related errors made in the Emergency Departments are patient identification errors, including the critical error of 'Wrong Blood in Tube' which can lead to possible blood transfusion errors.

PathWest Laboratory Medicine WA has received commonwealth funding to establish a bedside Identification, Labelling and Pathology ordering tool in Metropolitan Emergency Departments. This system is called eOrder and uses:

Barcode technology to ensure Positive Patient Identification

Electronic pathology ordering

Collection type guidance

Print sample labels at the bedside

This overall system will reduce patient identification errors as well as other pathology related errors, while streamlining workflows in the Emergency Departments and in the laboratory.

The project has spent significant time and effort consulting with the Emergency Departments and mapping workflow to ensure the introduced process will fit or is adaptable to the general Emergency Department workflow and will not inadvertently introduce new errors or risks into the system. Due to the eOrder system relying on the patient's identification band barcode to confirm positive patient identification, the project also influenced how and when ID bands are issued to patients, resulting in a safer and quicker process.

## **108. How less is so much more: The Pittsburgh experience of PBM**

**Yazer, M**

*University of Pittsburgh, Pittsburgh, PA, USA*

Patient blood management (PBM) is without question the hottest thing in modern transfusion medicine. While its instantiation can occur in many ways at different facilities, there are several fundamental elements. These include adopting evidence based transfusion thresholds and enforcing them, auditing transfusion practices to identify areas for improvement or to reduce wastage, educating everyone involved in the transfusion process about their role in providing a safe transfusion, and various methods of reducing blood loss during surgery and optimizing the patient before an invasive procedure to avoid a transfusion. Some advocates of PBM say that the best transfusion is the one that is not administered; while transfusions certainly have some risks to the recipients, this sentiment suggests that transfusions never have any benefit to recipients. Perhaps a more rational approach to PBM is that patients should be optimized before they get to the point where they need a transfusion, and if one becomes necessary then it should be administered according to the best evidence and at the lowest possible dose. With this philosophy in mind, reductions in transfusion are inevitable with the potential for better patient outcomes.

For PBM, information is key. Information takes many forms – reading and correctly interpreting the literature, disseminating evidence based transfusion practices amongst the myriad specialties that use blood products, and information technology itself. Understanding where the blood is being used in the hospital, what indications are prompting its use, where and why wastage is occurring, and measuring and following up on patient laboratory parameters are all essential pieces of information in developing and implementing a PBM strategy. To this end, close collaboration with the hospital system's information technology department is just as important as collaborations with hematologists, surgeons and internists. Information systems can be designed to help in PBM, but they alone are probably not sufficient to curtail non-evidence based or wasteful practices.

## 109. Sorting out iron metabolism disorders and iron overload: Pitfalls and perils of Ferritin

Olynyk J

*The University of Western Australia, Perth, WA*

Serum ferritin levels are commonly used for assessment of iron stores but are influenced by other factors including obesity and chronic disease. Published reference ranges have remained unchanged for decades and upper limits are progressively being exceeded by greater numbers of individuals, prompting evaluation for potential iron overload. Population studies involving the Busselton and Raine study cohorts together with cohort studies of subjects with elevated iron parameters in the community have shown that between 1995 and 2005, age-adjusted ferritin levels have risen by 21% in males and 10% in females, paralleling increases in adiposity. Body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup> was associated with higher ferritin levels amongst males  $\geq 35$  years and postmenopausal females ( $P \leq 0.002$ ). Serum  $\gamma$ -glutamyltransferase (GGT) was the principal biomarker correlating with serum ferritin ( $P < 0.0001$ ). The estimated 95th centile for males varied from 353 to 495  $\mu\text{g/L}$  ( $< 35$  years), 350 to 511  $\mu\text{g/L}$  ( $\geq 35$  years, BMI  $< 25$  kg/m<sup>2</sup>), and 413 to 696  $\mu\text{g/L}$  ( $\geq 35$  years, BMI  $\geq 25$  kg/m<sup>2</sup>) over GGT of 10-75 IU/L; for females, this centile varied from 106 to 235  $\mu\text{g/L}$  (premenopausal), 222 to 323  $\mu\text{g/L}$  (postmenopausal, BMI  $< 25$  kg/m<sup>2</sup>) and 249 to 422  $\mu\text{g/L}$  (postmenopausal, BMI  $\geq 25$  kg/m<sup>2</sup>) over GGT of 8-45 IU/L. In the absence of causative HFE gene mutations or a history of exposure to iron or blood products, almost all subjects with ferritin elevated above the upper end of the reference range do not have clinically significant iron overload.

**Conclusion:** Serum ferritin levels have significantly increased in recent years. Reference ranges which accommodate demographic and biomedical variation will assist clinicians in the correct identification of individuals requiring further evaluation for iron overload.

## **110. Iron in ICU**

**Litton, E**

*The University of Western Australia, Perth, WA*

Nearly 20% of all allogenic red blood cell (RBC) units dispensed in Australia are provided to critically ill patients in the Intensive Care Unit (ICU). The majority of these units are transfused for anaemia despite high concordance with restrictive transfusion threshold guidelines. Novel interventions are therefore required to reduce anaemia and RBC transfusion, both associated with increased morbidity and mortality in ICU.

Intravenous (IV) iron has been shown to increase haemoglobin (HB) and reduce RBC transfusion requirement in a variety of clinical settings. Iron-restricted erythropoiesis is common in the ICU due to the high prevalence of absolute iron deficiency in the population as well as inflammation-mediated iron sequestration. Determining the population, timing, type and dose of IV iron likely to be effective in the critically ill, as well as examining safety, in particular infection and oxidative stress, are key research priorities in order to improve patient-centered outcomes in ICU blood management.

**111. Can you use observational data to assess impact of Transfusion  
Methodology: Naturally, of course, your first choice**

**van de Watering, L**

*Sanquin - Leiden University Medical Centre, Leiden, The Netherlands*

Like with all types of research, also with assessing the impact of transfusion methodology you should use observational data as your first choice. As the data is more readily available than with new prospective intervention studies, the answers will also be more readily available.

Currently, every topic seems to need an RCT or even a meta-analysis of RCT's to finally come to a conclusion. RCT's are by many considered the mandatory pinnacle in generating evidence. However, RCT's are very time-consuming, very expensive, supply limited answers, are often not needed and are sometimes even unethical or impossible to perform. They are definitely not a condition-sine-quo-non for making decisions.

**112. Can you use observational data to assess impact of Transfusion Methodology- DEBATE**  
**No way, of course not, are you kidding?**

**Carson, J**

*Rutgers Robert Wood Johnson Medical School*

There is little debate that clinical trials are the best research methodology to assess the effect of most therapies. However clinical trials are difficult to perform and are very expensive to carry out. Therefore, investigators often perform observational studies since these studies are much easier to perform and often less expensive. It is not surprising that 100's of observational studies have been performed evaluating the impact of transfusion on clinical outcomes. The question we will debate today is whether these studies provide reliable evidence to base clinical decisions on. My answer is NO.

There are many reasons why observational studies are not reliable when evaluating the effect of transfusion. Some of these include 1) confounding by indication where the sickest patients are transfused and less ill patients are not transfused. 2) inability to measure some of the key confounders in most data that are used in observational studies, 3) inability of observational studies to detect small to moderate effects in an unbiased way, 4) most studies have not examined impact transfusion at different hemoglobin levels, and 5) empiric evidence that observational studies have overestimated the risk of transfusion.

Only clinical trials can provide unbiased reliable evidence to guide transfusion decisions

**113. Your say: to the floor!**

**Speaker TBA**

**ABSTRACT NOT SUBMITTED**

## 114. Supply planning and challenges

**Chesneau S**

*Australian Red Cross Blood Service, Melbourne, VIC*

The Australian Red Cross Blood Service (the Blood Service) issued 703,374 red cells to Health Providers in 2013/14, which was a -7.9% reduction on the prior financial year. This decrease was not matched in other product lines, in fact demand for certain products increased over the same period. As many of the blood products supplied by the Blood Service are derived from the same source collections, these contrasting trends in usage are creating a degree of pressure that is difficult to manage. This is why it is imperative to produce accurate demand forecasts by product and to have clear production plans and mitigating strategies.

The Blood Service operates a policy whereby a whole blood (WB) donation is only ever taken with the intent of producing a usable red cell. The plasma and buffy coat that can also be sourced from that donation are considered secondary but they are vital in the production of clinical plasma and pooled platelets. Cryoprecipitate is a product that is heavily reliant on WB donations - 93% of all Australian Cryoprecipitate came from WB in 13/14 - and it is possible to manufacture 28 units for every 100 WB donations. Using this ratio, it would have been possible to manufacture 219,335 WB-derived cryoprecipitate units (all ABO) across the whole of the last financial year, which exceeds the number of units that were requested by customers. However, cryoprecipitate demand grew in 13/14 (+5.3%) and is forecast to continue to grow in 14/15 and beyond, not least through the broadening rollout of the ROTEM platform. The balance of supply and demand is always most keenly felt with group AB, due to its universality, but the larger blood groups could also come under pressure. One scenario represents a continuing modest decline in red cell demand and a growth of 3% for cryoprecipitate. It suggests that the demand for group A WB-derived cryoprecipitate will exceed the Blood Service's manufacturing capacity by 2017. This raises a number of questions to consider in relation to forecast accuracy, Health provider reliance on WB derived product, blood sector costs, risk mitigation strategies, collection efficiency and product development.

## **115. Strategic planning for blood transfusion in Australia**

**McJames, L**

*National Blood Authority, Canberra, ACT*

Governments are committed to promoting safe, high quality management and use of blood products, blood related products and blood related services in Australia. In support of this objective they have approved a wide ranging agenda to support improvements in the sector, encompassing research and development, haemovigilance, development and implementation of patient blood management at a hospital level, education and training and collection of data. This presentation outlines the strategic planning agenda to date and into the future.

## **116. Obstetric haemorrhage, the not so small transfusions**

**Leung, Y**

*The University of Western Australia, Perth, WA*

Obstetric haemorrhage may be anticipated, as in cases of abnormal placentation, or unexpected. The underlying causes may include acute coagulopathies, obstetric or surgical trauma, uterine atony or multifactorial. Regardless of the cause, the quantity of blood loss is often significant enough to initiate the Massive Transfusion Protocol. The management of obstetric haemorrhage is best undertaken by a multidisciplinary team.

This presentation will review the role of various members of the multidisciplinary team with the support of contemporary data.

## **117. Transfusion for children: What do we need to know?**

**Bolton-Maggs P**

*Manchester Blood Centre, Manchester, UK*

Transfusion in children is less common than in adults where there is a clear increase with age, most components being transfused to people over 60 years of age. The groups of children most likely to require transfusion are premature neonates, children with haemoglobinopathies, and children of any age with haematological malignancies. Neonates have specific requirements for their blood products and in some circumstances may have been transfused in utero, when irradiated concentrated red cells are required. Children with thalassaemia or sickle cell disease should be phenotyped prior to the first transfusion, and then receive units matched for Rh and Kell. Cardiac surgery is increasingly common in neonates and often requires cardiac bypass with transfusion.

The specific needs for children resulted in national paediatric guidelines published in 2004, currently being updated. SHOT has 17 years' data and has identified some increased risks in paediatric practice. There is an increased risk of adverse events due to error, usually failure to request or supply appropriate components. The UK national clinical audit programme has also demonstrated areas of poor practice in red cell transfusion, and inappropriate use of fresh frozen plasma for apparent coagulation disturbances without bleeding where clinicians may have used inappropriate reference ranges to make their transfusion decisions. Problems are also identified with transfusion volumes and techniques. In 2013 69% paediatric reports to SHOT were error-related.

Paediatric data are analysed separately each year offering the opportunity for specific lessons to be learned and shared with paediatricians (annual reports can be viewed at [www.shotuk.org](http://www.shotuk.org)). In 2012 a case of graft versus host disease was identified, the first for more than a decade of leucodepletion which carries a high level (but not complete) of protection against this fatal complication. A child received an intrauterine transfusion from the mother (therefore non-irradiated, not leucodepleted) and subsequently died 3 months after birth. Review of this case led to changes in national practice with education for fetomaternal units and improved communication between clinicians and the blood services.

Despite the great success with prevention of Rh haemolytic disease of the fetus and newborn (HDFN) errors in interpretation of laboratory results and in administration are common. Failure to recognise D-sensitisation in pregnancy has resulted in suboptimal management. Nine of 19 cases resulted in HDFN, one neonatal death and 3 requiring transfusion.

Audit and haemovigilance provide useful information contributing to guideline production and improved clinical practice.

**118. NOACS and bleeding**

**Young L**

*Auckland City Hospital, Auckland, New Zealand*

**ABSTRACT NOT SUBMITTED**

## 119. Snake venom procoagulants: Cerebral hemorrhage, AAFP fuel and weird factor studies

Isbister G

*The University of Newcastle, Callaghan, NSW*

Snake venoms containing a range of procoagulant toxins, including prothrombin activators (Australasian elapids, carpet and saw-scaled vipers), factor X and V activators (Russell's viper) and thrombin-like enzymes (pit vipers). These toxins cause clot formation *in vitro*, but *in vivo* they cause venom induced consumption coagulopathy (VICC), which manifests as a deficiency of one or more clotting factors depending on the toxin. Patients envenomed by these snakes will develop VICC and are therefore at risk of bleeding. Fortunately the proportion of patients who develop major haemorrhage is low and less than 5% in Australian elapids. Intracerebral haemorrhage is the most serious and almost universally fatal complication of VICC. Over the last decade a number of intracranial haemorrhages from snake envenoming have been reported to the Australian Snakebite Project (ASP). These have better defined the time course and potential mechanism of intracranial bleeding in VICC. A number of recent cases suggest that patients may not develop intracranial haemorrhage immediately after the development of coagulopathy. Fresh frozen plasma remains a controversial treatment for VICC with concerns that the administration of exogenous clotting factors will just act to fuel the consumption process. A recent randomised controlled trial of fresh frozen plasma (FFP) in VICC found that administration of FFP within 4 hours of antivenom resulted in more patients having an INR < 2 6hr post-antivenom. However, FFP given with 6 hours of the bite (but post-antivenom) appeared to worsen the coagulopathy. Large observational studies of factor levels in patients with VICC support that multiple factor deficiencies occur with Australian elapids and with Russell's viper envenoming. A novel finding in VICC due to Russell's viper bites was very high factor VII levels on admission before antivenom. Further analysis demonstrated that factor VII levels appears to mirror the venom concentrations measured using venom specific enzyme immunoassay and that factor VII level were a surrogate measure of venom activity. Further study of the pathophysiology of procoagulant venoms in humans will improve potential interventions for VICC.

## 120. What's the problem?

van de Watering L

*Sanquin - Leiden University Medical Centre, Leiden, The Netherlands*

It has already been known for ages that to-be-transfused RBC deteriorate during storage. That's why there always were maximum storage times. In-vitro test have shown, among others, changes in the concentrations of ATP, and 2,3-DPG and in the distribution of  $K^+$ , but also morphologic changes to the cells and changes in the composition and quality of the cell membrane. Some of these effects may be caused by "natural aging", but most are likely the result of the non-physiological storage condition, *A red cell has not evolved to be stored in a plastic bag in a refrigerator*. A lot of research has been performed to register and minimize these changes using different storage solutions and conditions. What changes are the most important to monitor is still unknown and may differ per patient. The covert large variety in RBC production protocols doesn't help to answer the question whether the maximum storage times currently used may result in RBC transfusions doing more harm than good.

## 122. Developing transfusion guidelines

**Carson J**

*Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, US*

High quality guidelines have the potential to improve the quality of care patients receive and therefore are being developed widely in all fields of medicine. The numbers of published guidelines are growing rapidly and clinicians are challenged with knowing which ones are performed using high quality methods. The US Institute of Medicine has proposed standards to produce high quality guidelines. These principles were used to develop AABB guidelines that were recently published. The AABB guideline committee had representatives from the transfusion community, surgery, hematology, anesthesia, cardiology, and others. The guidelines were based on a systematic review of the literature, which focused on clinical trials. Observational studies were not used. The quality of literature was evaluated using the GRADE methodology.

Four questions were posed. 1) In hospitalized, hemodynamically stable patients, at what hemoglobin concentration should a decision to transfuse RBCs be considered? 2) In hospitalized, hemodynamically stable patients with preexisting cardiovascular disease, at what hemoglobin concentration should a decision to transfuse RBCs be considered? 3) In hospitalized, hemodynamically stable patients with the acute coronary syndrome, at what hemoglobin concentration should an RBC transfusion be considered? 4) In hospitalized, hemodynamically stable patients, should transfusion be guided by symptoms rather than hemoglobin concentration?

A restrictive transfusion strategy was recommended in most patients except those with acute coronary syndrome where adequate is not available. The guidelines also emphasized the other limitations of our knowledge especially in high-risk patients, which have not been evaluated in clinical. It is important that guidelines identify gaps in knowledge and not extend recommendations when the optimal treatment is unknown. Guidelines have the potential to improve patient care but only if they are carefully developed based on high quality evidence and applied to the appropriate patients.

## **123. Development and implementation of the national guidelines in Cambodia**

**Saxon B**

*Australian Red Cross Blood Service, Adelaide, SA*

Transfusion practice in the Kingdom of Cambodia is highly variable based on training and background of health practitioners, availability of blood products, access to health facilities and health providers, and financial constraints. Within this context the Australian Red Cross Blood Service has been working with the Cambodian governments, health institutions, Cambodian National Blood Transfusion Centre and other NGOs on a PEPFAR sponsored vein to vein blood program. One aspect was the writing, publishing and implementation of national transfusion guidelines. I shall discuss development of these guidelines and potential lessons for similar work in other developing countries.

## **124. Coagulopathy in major trauma patients is an independent predictor of mortality**

Hall D, P'Ng S, Rao S, Burrell M, Craven M

*Royal Perth Hospital, Perth, WA*

### **Aim**

Royal Perth Hospital (RPH) is the State Level 1 Trauma Service receiving patients evacuated from an area covering over 2 million square kilometres. These trauma patients typically present with combinations of injuries often associated with significant blood loss and a unique trauma coagulopathy. The aim of this study was to assess the impact of coagulopathy on patient outcomes amongst major trauma patients, in particular focusing on mortality, blood product usage and hospital length of stay.

### **Method**

A retrospective audit was performed on trauma patients evacuated to RPH between 2008 and 2012. Major trauma was defined by an Injury Severity Score (ISS) of >15. Coagulopathy was defined as an INR >1.5 and/or Fibrinogen <1g/L, massive transfusion as >10units of packed red blood cells within 24hours. Statistical analysis was performed utilising multivariable logistic regressions.

### **Results**

285 major trauma patients were identified, 22% of whom were coagulopathic. Coagulopathic patients had a mortality rate of 33%, or 5.6 times higher odds of death than non coagulopathic patients after adjusting for age, ISS and head injury ( $P<0.001$ , 95% CI 2.6-12.5) and were 7.8 times more likely to receive a massive transfusion ( $P<0.001$ , 95% CI 2.9-20.8). ISS and age were also independently associated with mortality, and ISS was independently associated with massive transfusion. Coagulopathy was not associated with hospital length of stay. Transit time was not included in a full multivariable analysis due to missing data (25%), however amongst patients with a known transit time, no association with mortality, massive transfusion nor length of stay was found.

### **Conclusion**

Amongst major trauma patients evacuated to Royal Perth Hospital, coagulopathy is an independent predictor of mortality and massive transfusion requirement after adjusting for ISS, head injury, age and transit time.

## 125. Intravenous iron use during pregnancy: Comparison of ferric carboxymaltose and iron sucrose

Kidson-Gerber G<sup>1,2,3</sup>, Boughton S<sup>2</sup>, Amanda H<sup>2,3</sup>

<sup>1</sup> SEALS, Prince of Wales Hospital, Randwick, NSW, <sup>2</sup> Royal Hospital for Women, Randwick, NSW, <sup>3</sup> University of New South Wales, Sydney, NSW

### Aim

To compare the effectiveness and tolerability of the contemporary formulations iron sucrose (FeS) and ferric carboxymaltose (FCM) in pregnant patients with iron deficiency anaemia (IDA).

### Method

Retrospective audit of all pregnant women receiving intravenous FeS or FCM at an Australian tertiary maternity hospital January 2007-July 2013. Data collected were demographics, maternal history, haematological details, infusion details including adverse events, and pregnancy outcome.

### Results

A total of 94 infusions (38 FeS infused over 4 hours in Acute Care, 56 FCM infused over less than 2 hours in Day Stay) were administered. There were no significant demographic differences between groups with average maternal age 30.4 vs. 31.6 years, 37% vs 44% nulliparous and average gestation at first infusion 32.0 vs. 33.2 weeks for FCM and FeS respectively.

Pre-infusion Hb and ferritin were 98.2g/L  $\pm$  12.0g/L and 7.2 $\mu$ g/L  $\pm$  2.5 $\mu$ g/L (FeS) vs. 95.7g/L  $\pm$  17.9g/L and 10 $\mu$ g/L  $\pm$  6.8 $\mu$ g/L (FCM). For all first infusions, FCM had a lower risk of minor adverse events (16% vs. 34%,  $p = 0.049$ ). No unplanned admission secondary to adverse events occurred in either group. Post infusion, both groups had significantly greater haemoglobin (14.6g/L improvement FCM and 8.9g/L FeS,  $p < 0.001$ ), MCV (2.32fL improvement FCM vs 1.18fL FeS,  $p < 0.001$ ) and ferritin (295.8 $\mu$ g/L improvement FCM vs 104.8 $\mu$ g/L FeS,  $p < 0.001$ ) with ferritin levels showing greater improvement in FCM ( $p = 0.002$ ). Maternal outcomes were similar regarding postnatal length of stay, mode of birth, and rate of post-partum haemorrhage.

### Conclusion

Ferric carboxymaltose administered in pregnancy had a lower risk of infusion-related adverse events and superior haematological efficacy compared to iron sucrose. Pregnancy outcomes were similar. Given the favourable safety and haematological profile, ease of administration and decreased monitoring/infusion time of ferric carboxymaltose, compared to iron sucrose, it is likely to be the preferred IV formulation for IDA treatment in pregnancy.

## **126. Impact of fibrinogen (cryoprecipitate or fibrinogen concentrate) transfusion on postoperative thromboembolism or infection in patients undergoing thoracic aortic surgery**

**Maeda T**<sup>1</sup>, Miyata S<sup>1</sup>, Okita Y<sup>2</sup>, Usui A<sup>3</sup>, Shimizu H<sup>4</sup>, Sasaki H<sup>1</sup>, Kimitoshi N<sup>3</sup>, Katori N<sup>4</sup>, Ohnishi Y<sup>1</sup>, Matsushita T<sup>3</sup>, Kano H<sup>2</sup>, Takahashi K<sup>1</sup>, Ueda Y<sup>5</sup>

<sup>1</sup> National Cerebral and Cardiovascular Center, <sup>2</sup> Kobe University, <sup>3</sup> Nagoya University School of Medicine, <sup>4</sup> Keio University, <sup>5</sup> Nara Prefecture General Medical Center

### **Aim**

There is increasing evidence that acute hypofibrinogenemia plays a crucial role in massive bleeding. The use of fibrinogen products (cryoprecipitate or fibrinogen concentrate) is increasing, since recent publications have suggested the efficacy of a higher dose of transfused fibrinogen with a higher plasma fibrinogen concentration threshold (up to 2g/L) for transfusion. However, there is concern that transfusion of fibrinogen products may worsen outcomes, possibly by inducing thromboembolic and infectious events.

### **Aim and Methods**

To investigate whether transfusion of fibrinogen products could be a risk factor for postoperative thromboembolism (stroke/TIA, limb ischemia, myocardial infarction, pulmonary embolism) or infection (sepsis, surgical site or urinary-tract infection, pneumonia), we performed a multicenter retrospective cohort study of patients undergoing thoracic aortic surgery. Clinical information was obtained from the medical records and the Japan Adult Cardiovascular Surgery Database.

### **Result**

A total of 1062 patients were enrolled at 4 institutions, of which 283 (26.8%) were treated with fibrinogen concentrate or cryoprecipitate. During the postoperative period, 59 and 120 patients suffered from thromboembolism and infection, respectively. The transfusion of fibrinogen products was identified as a risk factor for thromboembolism ( $p=0.012$ ) or infection ( $p=0.0006$ ) by univariate logistic regression. Step-wise logistic regression identified history of cerebrovascular disease and use of intra-aortic balloon pump as independent risk factors for thromboembolism, and identified age, perfusion time, and history of chronic obstructive pulmonary disease for infection. However, transfusion of fibrinogen products was not an independent risk factor for thromboembolism (odds ratio (OR): 1.5, 95% confidence interval (CI): 0.9–2.4;  $p=0.09$ ) or for infection (OR: 1.6, 95% CI: 0.94–2.6;  $p=0.08$ ). No cases of hepatitis B, C, and HIV transmission were reported.

### **Conclusion**

Although fibrinogen products tend to be transfused in more complex surgical cases, transfusion of fibrinogen products seems not to be an independent risk factor for thromboembolism or infection in patients undergoing thoracic aortic surgery.

## **127. Maternal anti-platelet $\beta$ 3 integrin antibodies impair angiogenesis and cause intracranial hemorrhage in fetal and neonatal alloimmune thrombocytopenia**

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Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a life-threatening disease in which intracranial hemorrhage (ICH) is the major risk. Although thrombocytopenia caused by maternal antibodies against  $\beta$ 3 integrin and occasionally against other platelet antigens (e.g. GPIIb/IIIa) has long been assumed to be the cause of bleeding, the mechanism of ICH has never been adequately explored. Utilizing murine models of FNAIT and a high frequency ultrasound imaging system, we found that ICH only occurred in fetuses and neonates with anti- $\beta$ 3 integrin- but not anti-GPIIb/IIIa-mediated FNAIT, despite similar thrombocytopenia in both groups. Only anti- $\beta$ 3 integrin-mediated FNAIT reduced brain and retina vessel density, impaired angiogenic signalling, and increased endothelial cell apoptosis; which were abrogated by maternal administration of intravenous immunoglobulin (IVIG). ICH and impairment of retinal angiogenesis was further reproduced in neonates by injection of anti- $\beta$ 3 integrin- but not anti-GPIIb/IIIa-antisera. Utilizing cultured human endothelial cells, we found that cell proliferation, network formation, and Akt phosphorylation were inhibited only by murine anti- $\beta$ 3 integrin-antisera and human anti-HPA-1a IgG purified from mothers with FNAIT children. Our data suggest fetal hemostasis is unique in that impairment of angiogenesis rather than thrombocytopenia is likely the cause of ICH; importantly maternal IVIG therapy can effectively prevent this devastating disorder.

## **128. Assessment of transfusion triggers based on thromboelastometry and standard coagulation tests for patients undergoing orthotopic liver transplantation**

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### **Aim**

The use of thromboelastometry (TEM) and associated transfusion algorithms has been reported to contribute to more appropriate use of blood products during orthotopic liver transplantation (OLT) compared to transfusion therapy based on standard coagulation tests (International normalised ratio [INR], partial thromboplastin time [aPTT], fibrinogen, platelet count). The aim of the study was to determine the points at which transfusion would be triggered using TEM, currently a standard practice at Flinders Medical Centre, versus proposed therapy based on standard coagulation tests for patients undergoing OLT.

### **Method**

Standard coagulation and TEM tests were performed at four intraoperative phases of OLT. Transfusion of blood products was guided by an algorithm based on TEM results. Various TEM sample points that triggered transfusion were selected for analysis. The equivalent coagulation test sample points based on platelet count, INR and fibrinogen were analysed to predict the need for transfusion if only standard coagulation tests had been available to guide therapy.

### **Result**

A total of 162 sample points for TEM were included in the study. EXTEM Maximum Clot Firmness (MCF) had a moderate correlation with platelet count ( $\rho=0.53$ ) and FIBTEM MCF had a good correlation with fibrinogen level ( $\rho=0.68$ ). 73/162 TEM sample points triggered transfusion of fresh frozen plasma (FFP) or platelets or cryoprecipitate or all three. 56 of the 73 sample points had equivalent coagulation tests available for analysis. Transfusion rates and predicted transfusion rates based on TEM and standard coagulation test triggers (platelet count  $<50 \times 10^9/L$ , fibrinogen  $<1.3g/L$ , INR  $\geq 1.5$ ) was 42.9% vs. 78.8% ( $p < 0.001$ ) for FFP and 48.2% vs. 37.5% ( $p = 0.25$ ) for platelets and cryoprecipitate.

### **Conclusion**

Using thromboelastometry in comparison to standard coagulation tests to guide or product use, the indication for FFP was lower, however for platelets and cryoprecipitate was higher which is reflective of the functional assessment of platelets and fibrinogen by thromboelastometry.

## 129. ABO isohaemagglutinin titrations of PAS platelets

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### Aim

To determine the titres of IgG and IgM anti-A and -B in supernatant from platelets suspended in PAS (PAS platelets) to assess the potential for acute haemolysis with minor ABO-mismatched PAS platelets.

### Method

Anti-A and -B titrations were performed on native plasma, apheresis plasma, and PAS platelets from each donor. IgG and IgM titres  $\geq 128$  or 64 respectively are considered critical. A  $\geq 2$  tube difference was considered to be significant. IgG and IgM levels were measured by standard nephelometric methods.

### Results

Mean titres and IgG / IgM levels in the 3 types of specimen.

	Anti-A titre		Anti-B titre		IgG (g/L)	IgM (g/L) <sup>#</sup>
	IgG	IgM	IgG	IgM		
<b>Native plasma</b>	329	30	219	23	11*	1.44 <sup>#</sup>
<b>Apheresis plasma</b>	331*	28*	256*	19*	8*	1.1 <sup>#</sup>
<b>PAS platelets</b>	138	11	89	7	3*	0.41 <sup>#</sup>

\*n = 20; #n = 2; rest, n = 58

Comparisons of IgG and IgM anti-A and -B titres of PAS platelets and native plasma.

Anti-A				Anti-B			
IgG		IgM		IgG		IgM	
Less	Equivalent	Less	Equivalent	Less	Equivalent	Less	Equivalent
25	33	29	29	29	29	36	22

In native plasma, IgG anti-A and -B titres were  $\geq 128$  in 47 (81%) and 41 (71%) donors respectively. Corresponding figures for PAS platelets were 26 (45%) and 16 (27%) donors respectively. In native plasma, IgM anti-A and -B titres were  $\geq 64$  in 9 (16%) and 7 (12%) donors respectively. Corresponding figures for PAS platelets were 1 (1.7%) and 0 (0%) donors respectively.

### Conclusions

Though IgG and IgM levels reduced as expected in PAS platelets and apheresis plasma taking in to account dilution with anticoagulant +/- PAS, reduction in titres was variable. In approx. 50% (except with IgM anti-B), these were similar in the PAS platelets and the corresponding native plasma. In many PAS platelets, titres remained above what are considered critical values. Unlike with standard platelets, clinical haemolysis has not been reported with ABO-mismatched PAS platelets but its safety from this perspective is uncertain. The reason for the less than expected reduction in titres in some donors is unclear. Better predictors of acute haemolysis in this setting – antibody avidity, complement activation and recipient variables – are needed.

## 130. CMV in Australian blood donors

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Cytomegalovirus (CMV) represents a significant cause of morbidity and mortality amongst neonates and immunosuppressed adults. As a blood-borne virus, transfusion-transmitted CMV (TT-CMV) is well-recognised. To establish component inventories with reduced infection risk, the Australian Red Cross Blood Service (Blood Service) currently utilizes both universal leukodepletion and donor seroscreening.

This study aimed to evaluate CMV seroepidemiology in Australian blood donors, including demographic influences and temporal trends. It also aimed to model trends in the acquisition and demand for CMV negative blood components, in order to estimate the ability to meet future demand for CMV negative components.

Existing data was extracted from Blood Service databases regarding donor demographics, component donations, CMV serology results and component issues for the 5 financial years from 2008/09 to 2012/13, inclusive. Population estimates were also extracted from the Australian Bureau of Statistics for the calculation of age-weighted seroprevalence estimates. Linear regression was used to model trends in component acquisition and demand, and statistical analysis was performed using ANOVA and Student's t test.

This study estimates the age-weighted CMV seroprevalence in 20-69 y/o to be  $76.12 \pm 0.13\%$ , with higher rates in females and older donors. Donor seroprevalence was found to decrease over the study period. CMV negative component demand increased throughout the study period, and without actions to influence the current trends, it is predicted that supply may be insufficient by FY 2017/18.

This study represents a comprehensive evaluation of CMV seroepidemiology in Australia, and forms a basis to predict the future status of CMV negative component inventories. These results will serve to guide Blood Service operations, inform current debate regarding optimal strategies for the provision of CMV-safe blood components, and potentially guide the development of a future CMV immunisation program.

## 131. A pair-wise comparison of lyophilised and fresh frozen plasma plasma following reconstitution and refrigerated storage

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### Background and aim

Two major advantages of lyophilised plasma (LyP) over fresh frozen plasma (FFP) are that LyP can be stored at room-temperature, and reconstitution of LyP is significantly faster than thawing FFP. However the lyophilisation process is time consuming and may result in product loss. The aim of this study was to evaluate the stability of coagulation factors in reconstituted and stored LyP.

### Method

Apheresis plasma donations were split and frozen to give equivalent pairs of FFP (n=13 pairs). One of each pair was lyophilised by HemCon (USA). Coagulation factors (STA Compact), complement C5a and C3a (cytometric bead array), and immunoglobulin concentration (biochemistry analyser) of LyP were measured immediately after reconstitution, and after 1 and 5 days storage at 2-6 °C. Results were compared to thawed FFP (control) using a two-way repeated measures ANOVA with post-hoc tests.

### Result

Reconstitution of LyP ( $2.9 \pm 0.9$  minutes) was 33-fold faster than the FFP thawing time ( $36.6 \pm 5.8$  minutes). The activity of factors V ( $p=0.0005$ ) and VIII ( $p=0.0412$ ), but not factor VII were significantly reduced in reconstituted LyP over storage compared to FFP. The prothrombin time ( $p=0.0280$ ), activated partial thromboplastin time ( $p=0.0032$ ), procoagulant phospholipid clotting time ( $p<0.0001$ ), and activated complement C5a ( $p=0.0200$ ) and C3a ( $p<0.0001$ ) concentration were significantly higher in reconstituted LyP over storage. However, there were no significant differences in all tested variables immediately after reconstitution or thawing, except the procoagulant time (LyP,  $25 \pm 3$ ; FFP,  $23 \pm 2$  seconds) and C5a concentration (LyP,  $9.2 \pm 2.3$ ; FFP,  $6.8 \pm 2.3$  ng/mL).

### Conclusion

Our data suggest that coagulation factors in freshly reconstituted LyP were comparable to freshly thawed FFP; however they were reduced during storage. Given that LyP can be reconstituted rapidly, the need to reconstitute LyP in advance and to use it after storage can be negated.

## 132. Unraveling a 22-year-old “cold” case: the MNS hybrid glycophorin GP.Kip

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### Background

Genetic exchanges between Glycophorin A (GP.A) and Glycophorin B (GP.B) produce (A-B-A) and (B-A-B), hybrid glycoporphins, which display distinct profiles of neoantigens. A serological profile MUT, Mur, Hil and “Kip”/an Anek-like antigen for a GP(B-A-B) hybrid was reported for an Australian donor at the ASBT (now ANZSBT) meeting in 1992. An example of similar serological profile was also reported in 2012 for a proposed BAB-hybrid, “GP.Yak”.

### Aim

To genotype an Australian example reported as “Kip positive”, to define, at a molecular level, the genetic exchange that defines this hybrid GP.

### Methods

DNA from donor CH was sequenced, targeting the regions in which breakpoints for hybrid GPs have been reported and novel features identified by comparison with other hybrid glycoporphins.

### Results

Sequencing confirmed a BAB crossover with a 35 nucleotide *GYP*A insertion from nucleotide c.220 to IVS3+25 (GenBank KF501485) now *GYP*\*506. In the sequence contributed by *GYP*B codon TCC (c.208-210) Ser70 is predicted to be the defining feature of the Kip antigen, now recognised by the ISBT as MNS48 (predicted sequence <sup>63</sup>EISVTTVSPP<sup>72</sup>).

### Conclusion

These data demonstrated that GP.Kip (and the superseded Gp.Yak) are the same, GPB(1-26)-GPΨB(27-54)-GPA(55-57)-GPB(58-103), with three GP.A residues, 55-57, the shortest BAB insert reported. The ISBT has assigned allele *GYP*\*506 to GP.Kip now the internationally accepted terminology for this hybrid. For this family their blood group is now named and a 22 year-old “cold case” resolved. The ISBT assignment of Kip as antigen MNS48 highlights the extreme diversity in this system with, on average, one antigen per four amino acids. This hybrid has been reported in several populations (Japan, Australia and Europe) suggesting a world-wide distribution. Antibodies to antigens found in hybrid glycoporphins have been implicated in transfusion reactions and HDFN and accurate typing techniques, possibly only available via genotyping, are a key requirement for future transfusion practice.

### **133. Genetic classification of donors with the RHD alleles associated with weak D: evidence base for confidence in managing donors with reduced D antigen expression**

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#### **Background**

Donors with weak RhD antigen serology agglutination scores (less than 2+ on 0-4+ scale) are classified by applying a published genetic classification system which numbers *RHD* gene variants from weak D type 1 to 76. The distribution of weak D types varies across ethnic groups. The aim of this study is to classify donors reported as weak D and to define the distribution in our current donor demographic.

#### **Methods**

Donor blood samples (n=32) reported as weak D following automated and manual serology testing were forwarded to Progenika Inc for testing on a SNP platform (BLOODCHIP Reference). Diagast monoclonal reagents were used for D-epitope mapping.

#### **Results**

Genotyping showed 28/32 samples were hemizygous for the *RHD* gene with a single nucleotide variant (SNV) and classified as Weak D: type-1 (16/32, 50.0%), type-2 (9/32, 28.1%), type-3 (3/32, 9.4%). Two samples (2/32, 6.3%) were hemizygous for an *RHD* gene with two SNVs classified as Weak D type-1.1. One sample (1/32, 3.1%) was a compound heterozygote comprising Weak D type-1 and Partial-DVII and one (1/32, 3.1%) showed no variant by SNP profiling. All monoclonal anti-D reacted with the compound heterozygote and a Weak D type-1, except for one monoclonal, designated HM10, which showed no detectable agglutination with a Weak D type-1 sample.

#### **Discussion**

The distribution of Weak D associated alleles reflects reports for Caucasian donor populations in Europe. The basis for one Weak D antigen remains unresolved. This data extends an earlier Australian study using PCR based methods (*Vox Sanguinis*, Vol 79. P 251-252, 2000). The detection of Weak D type-1.1 and Partial-DVII/Weak D type-1 demonstrates the expanded capability of current molecular genotyping platforms. This ongoing genetic study provides an evidence base for the management of Weak D donors.

## **134. Clinical benefits of genotyping**

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Sickle cell disease (SCD) patients pose special challenges to the blood bank as collectively they tend to be a highly alloimmunized population. Although there currently is no standard of care for providing RBCs to SCD patients either when they are experiencing a crisis or for a prophylactic transfusion, many centers provide all SCD patients with Rh and K matched RBCs; if an antibody is produced then more complete antigen matching is offered when possible. This strategy is a good compromise between being able to provide RBCs quickly when they are needed and the risk of alloimmunization. However, in order for this strategy to be successful, a few parameters need to be satisfied: The recipient's RBC phenotype must be known, as must that of the donor. As SCD patients can be highly transfused and often seek care at different hospitals, having their phenotype readily available is not always possible. Plus, the serological phenotyping reagents are becoming increasingly more expensive and hard to find.

Enter RBC genotyping. While it will not solve the problem of having the recipient's RBC information available everywhere they might be transfused, nor the need for increased minority donors, it can be performed on recently transfused recipients and can provide far more information than is routinely available with serological techniques. Variable throughput genotyping machines are now commercially available and the American regulatory approval process is underway. Several recent studies have demonstrated that a genotyped inventory is manageable and that impressive RBC order fill fractions can be achieved – even in difficult to match recipients. In fact, any patient who has produced, or is at risk of producing, an antibody will benefit from the blood bank's ability to rapidly locate antigen negative units. This presentation will discuss some strategies to prevent alloimmunization in highly transfused recipients and will explore some of the future directions of genotyping.

## **135. Our changing population and what this means for "rare" phenotypes in our patient and donors**

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*Australian Red Cross Blood Service, QLD*

The cultural and ethnic diversity of the Australian population has been reshaped over many years by migration with over one quarter of our resident population in 2013 being born overseas. Historically, the strongest influence has been from the United Kingdom, but, although still strong, emigration from this region is in decline. The highest rate of increase in annual growth between 2003 and 2013 is people born in Nepal, followed by India, Pakistan, Bangladesh and Sudan. This change in demographics for our population presents some challenges for the Blood Service as we work towards a blood donor population that reflects more closely the diversity of our population. In the past few years we have seen an increase in patients requiring transfusion support for phenotypes that are considered very uncommon in our predominantly Caucasian population.

To support these requests the Australian Red Cross Blood Service has a small but very helpful rare donor panel and maintains an inventory of frozen rare red cell donations. On occasion to assist in supporting these requests we look to recruit family members and members of the community with similar ethnic backgrounds as blood donors. This increases the chances of finding a donor with the same phenotype as the patient and will also assist in increasing the diversity within our blood donor population. Where we are unable to support these patients with donations from Australian Blood Donors we are able to access donors listed on the International Rare Donor Register to import suitable blood products via international blood services.

This changing population has also presented challenges in our antenatal patients with an increasing number of women with antibodies not previously seen in our population. The clinical significance of these antibodies varies depending on the specificity and the Blood Service Team works closely with the clinician to support the management of these pregnancies.

Whilst as a scientist, this changing population and the new antibodies and blood groups it brings with it are exciting scientifically it remains a constant challenge to ensure we are able to provide the necessary blood components for transfusion when required. We must continue to actively recruit donors from the minor ethnic groups to meet such future demands.

**136. PBM in cardiothoracic surgery**

**Pavey W**

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**ABSTRACT NOT SUBMITTED**

## **137. Transfusion Triggers: More or less blood**

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Millions of units are transfused worldwide yet the indications for transfusion have only recently been explored in depth. There are over 18 clinical trials that have randomly allocated patients to either liberal transfusion threshold (more blood) versus restrictive transfusion threshold (less blood).

In over 7000 patients who have enrolled in these trials most demonstrate no advantage of liberal transfusion. These trials do show that about 40% less blood is used with restrictive transfusion. Some evidence points to higher risk of bacterial infection and borderline increase risk of death at 30 days. In one trial in gastrointestinal bleeding, mortality was statistically higher in the liberal transfusion group (9 g/dL) compared to the restrictive transfusion group (7 g/dL).

However, there remains substantial uncertainty regarding in several subgroups of patients including those with acute coronary syndrome and haematological malignancies.

Most transfusion guidelines recommend 7-8 g/dL threshold in most patients although some suggest even lower thresholds. Evidence that transfusion should be guided by symptoms is sparse but reasonable.

## **138. Near miss transfusion errors: What do they teach us?**

**Bolton-Maggs P**

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A near miss incident in relation to transfusion is defined as any error which, if undetected, could result in the determination of a wrong blood group or transfusion of an incorrect component, but was recognised before the transfusion took place. The UK national haemovigilance scheme, Serious Hazards of Transfusion (SHOT) fully analysed these for the last 4 years. Near miss reports make up about a third of the total every year. Review of these common events gives insight into the root causes and informs transfusion practice. Errors, or human factors, are universal; it is not possible to eliminate all errors but rather to focus on means of neutralising them.

Transfusion of an incorrect blood component is the most dangerous transfusion error, particularly ABO-incompatible red cell transfusion which may cause death. Over a 4-year period 3919 near miss events were recorded. A large number of all possible wrong component transfusions were prevented (2532/2873, 88.13%) by detection before transfusion. Blood sampling errors make up about half all the near miss events. More than 90% of these are as a result of 'wrong blood in tube' (WBIT) incidents, detected by the vigilance of staff, particularly in transfusion laboratories when the blood group gives a result discrepant with a historical group. About 100 near miss WBITs are detected for every one that results in a wrong transfusion (the tip of the iceberg). Common causes are failure to identify the patient and failure to label the sample at the bedside. Doctors are responsible for taking these samples in 45%, the largest single staff group. It is better to focus on changing this behaviour as a result of the warning given from these many near miss events than to only react when there is a serious event: ABO-incompatible transfusions are fortunately uncommon, 9 in 2013 (one death and 3 cases of major morbidity), but review of near miss events showed that at least 125 could have resulted in ABO-incompatible transfusion if not detected. This demonstrates that the problem is indeed larger than the actual events demonstrate.

The quality management systems within hospitals, particularly those in the transfusion laboratories, are detecting many errors and preventing unsafe transfusions. However, there are parts of the transfusion process where quality systems might be improved to detect more errors before they lead to patient harm. Near misses are "free lessons" that cause no danger to patients, so increased reporting of these may highlight where quality improvements could be made.

## **139. Blood transfusion and HLA antibodies: Mechanisms of sensitization and implications for prevention**

**D'Orsogna L**

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Both leucocytes and red blood cells carry a significant HLA antigen load. While HLA sensitization following pregnancy or transplantation is considered unavoidable, it is transfusions given to these patients that most often leads to broad HLA sensitization and potentially graft failure following allogeneic HSCT. Residual leucocytes and/or red cell HLA expression may explain why leukocyte-reduced units are unable to fully prevent HLA sensitization, and therefore prevention of HLA sensitization requires active management by the haematology physician. In this lecture the mechanisms of HLA sensitization following blood transfusion will be reviewed, with a focus on strategies that can be used to prevent HLA antibody formation. In particular, methods to avoid transfusions, HLA matched blood transfusions, immunosuppression and the HLAMatchmaker computer algorithm as tools to prevent sensitization will be discussed. While the potential clinical benefits are large these additional measures are not logistically straightforward or devoid of risks. However remaining as passive observers while patients become highly sensitized to HLA antigens should not be considered acceptable.

## **140. Third party blood transfusion before and after renal transplantation: A powerful predictor of rejection and transplant outcome**

**Fidler S**

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Third party blood transfusion prior to transplant is immunomodulatory and associated with a lower risk of rejection in historic series but its contemporary significance and the effect of post-transplant transfusion is rarely studied. The aim of this study was to evaluate the relationship between blood transfusion and transplant outcome in renal transplant recipients (RTR).

We determined the transfusion history of 256 RTR and examined its association with relevant clinical and demographic factors, and patient and graft outcomes. Transfusion after transplant was defined as within the first 30 post-operative days.

105 RTR (41%) never received a transfusion, 50 (19%) received a transfusion pre-transplant only, 44 (17%) received a transfusion post-transplant only and 57 (22%) received both pre and post-transplant transfusions. Factors associated with transfusion included recipient gender (female), increasing donor and recipient age, re-transplant, delayed graft function (DGF), CMV disease and cadaveric donation. Compared with those never transfused, the univariate HR for rejection was 0.74 (pre), 1.2 (Post) and 2.0 (both)  $P=0.012$ , and Graft loss 0.64 (pre), 1.5 (post) and 5.1 both ( $P=0.026$ ). After adjusting for age, gender, donor type, DGF, DR match, CNI use, and re-transplant the HRs for rejection were 0.95 (pre) 1.6 (post) and 2.2 (both) and graft loss 0.77 (pre) 2 (post) and 5.2 (both). eGFR at last follow up was 49 (never), 55 (pre), 49 (post) and 43 ml/min (both)  $P=0.03$ .

Transfusion pre- and post-transplant are clinically determined and associated with recipient gender, donor and recipient age and donor type. Compared with those never transfused or those transfused pre- or post-transplant only, previously transfused RTR receiving a transfusion within the first 30 days of surgery have significantly increased risk of rejection, graft loss and reduced long term eGFR.

## 141. New issues in antiphospholipid syndrome

### Ward C

*Northern Blood Research Centre, NSW*

Antiphospholipid antibodies (APL) are heterogeneous and often transient, recognizing proteins associating with negatively-charged phospholipid, including beta<sub>2</sub>-glycoprotein I (GPI). They occur in asymptomatic individuals, and in diverse groups of patients, with autoimmune diseases, unprovoked venous and arterial thrombosis and adverse pregnancy outcomes. The mechanism(s) of APL-induced thrombosis remain poorly defined. APL alone are insufficient to trigger vascular events and an inflammatory "second hit" may do this by increasing beta<sub>2</sub>-GPI levels in the vasculature. Innate immunity (via Toll-like receptor 4 as an endothelial beta<sub>2</sub>-GPI receptor) and complement activation both promote vascular events in experimental models of APS. These findings raise the possibility of new therapeutic targets, including complement inhibition in APS refractory to standard anticoagulation.

Incidental findings of APL is not an indication for thromboprophylaxis. Thrombosis rates are close to population risk in asymptomatic "carriers" of a single APL, and only mildly increased (1.3%/year) in double or triple positive cases. Diagnostic criteria for APL have recently been updated and should be followed in all laboratories. Proposals to improve prognostic significance include screening for antibodies against domain 1 of beta<sub>2</sub>-GPI, and phosphatidylserine-prothrombin complex. Combining laboratory and clinical features in a formal APS risk score shows promise as a prognostic tool.

Clinical APL syndromes remain poorly defined and studied, particularly in pregnancy. Non-ischaemic complications can occur, in multiple organ systems. Most treatment recommendations are based on opinion only, although there is limited evidence supporting heparin in recurrent pregnancy loss. Anticoagulation is the mainstay of therapy, but it is unclear if new oral anticoagulants are as effective as warfarin in APL. Additional agents such as hydroxychloroquine and statins may suppress platelet and endothelial activation. Prospective trials are clearly needed in APL, applying strict diagnostic and clinical criteria.

## 142. Understanding the TFPI / protein S pathway

Hackeng T

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Tissue factor pathway inhibitor is an essential inhibitor of onset of coagulation through the tissue factor or extrinsic pathway (TF/FVIIa). TFPI appears as two isoforms due to alternative splicing: TFPI $\alpha$  and TFPI $\beta$ . In addition, TFPI $\alpha$  is subject to proteolysis in plasma leading to additional truncated variants. All these isoforms have different distribution with TFPI $\alpha$  present in platelets, plasma and in association with the vascular endothelium, and truncated TFPI $\alpha$  coupled to lipoproteins through crossed disulfides. TFPI $\beta$  is attached to the vascular wall through direct GPI anchorage. All forms inhibit TF/FVIIa and FXa to a different extent, whereas it was recently shown that TFPI $\alpha$  has inhibitory properties towards FXa in prothrombinase as well. As TF is believed to be the primary initiator of coagulation in vivo, TFPI might well be an important determinant in bleeding disorders and in pathologic thrombus formation. More recently it was discovered that protein S act as a cofactor for TFPI in the inhibition of FXa and TF/FVIIa. Protein S stimulates TFPI approximately 10-fold, not only in binding and inhibition of FXa, but also in the direct inhibition of TF/FVIIa by TFPI. TFPI is a tight binding, but rather slow inhibitor that is easily overruled by rapid FXa and thrombin generation. In these events the well known APC/protein S anticoagulant system works in conjunction with TFPI/protein S by attenuating FXa and thrombin generation through inactivation of FVa and FVIIIa. Through the resulting dampening of the procoagulant response, APC/protein S enables TFPI/protein S to become active again resulting in an extra down regulation of (unwanted) procoagulant events.

## 143. Fibrinolysis: beyond clot removal

**Medcalf R**

*Monash University, Melbourne, VIC*

Since its inception, the fibrinolytic system has been intimately associated with the removal of fibrin. Indeed, tissue-type plasminogen activator (t-PA), the most renowned plasminogen activating enzyme in the blood, was harnessed for therapeutic development over 30 years ago, initially for use in patients with myocardial infarction and more recently in patients with ischaemic stroke. t-PA went into clinical development ahead of other plasminogen activators (i.e. urokinase) due to the fact that t-PA, and not urokinase, was fibrin-selective, meaning that fibrin bound plasminogen was more efficiently activated by t-PA than free circulating plasminogen. This selectivity allowed t-PA to be targeted to clots to activate plasminogen. Even today, t-PA is still the only approved thrombolytic agent for ischaemic stroke. What has now come to light is that t-PA is maybe fibrin-selective when compared with fibrinogen, but it is not fibrin-specific as many proteins, mostly denatured or misfolded, act in essentially the same manner as fibrin to promote plasminogen activation by t-PA. This is relevant as the “fibrinolytic system” can be harnessed to remove dead cells and tissue being driven by the array of misfolded proteins formed as a consequence of cell death and injury.

In addition to the broadening role of t-PA mediated plasmin formation, the fibrinolytic system has also proven to be particularly important in the extravascular space and in contexts that do not all necessarily involve plasmin formation or perhaps not even proteolysis. One key area is in the brain where t-PA has been linked with memory formation and anxiety, the processing of neurotrophic factors, and in the response to drugs of addiction. While these are physiological responses, t-PA can also potentiate many neurotoxic paradigms, while in models of ischaemic stroke and traumatic brain injury, the absence of t-PA results in smaller infarct volumes and improved recovery times, respectively. t-PA has also been shown to increase permeability of the blood brain barrier (BBB) in humans and in mouse models and this may underlie the ability of t-PA to promote haemorrhage in patients with ischaemic stroke. This presentation will highlight some of these unexpected roles of t-PA and how the fibrinolytic system is no longer fibrin-centric.

## **144. Clinical evaluation of the platelet response: combining new tools with old to drive clinical decision making in the 21st century**

**Brass L**

*Perelman School of Medicine, Pennsylvania, PA, USA*

More than a century after platelets were first described, clinical hematologists are asked to evaluate human platelet function with a tool set that has benefitted only indirectly from the array of tools available in research laboratories. Where research questions have focused on the molecular basis of platelet activation and the use of mouse models, clinical hematologists faced with bleeding and clotting patients are more commonly asked about the separation of intrinsic platelet defects from those caused by von Willebrand's disease or clotting factor deficiencies; the diagnosis and management of platelet-associated bleeding defects that are not due to one of a relative handful of well-characterized, but rare monogenic disorders; and the diagnosis and management of platelet-related prothrombotic phenotypes in cardiovascular and myeloproliferative disorders. This session will focus on the tools that are available in the clinical setting now and those that may be coming on line in the future.

## 145. Mechanisms controlling thrombus growth and stability

Gardiner E

Monash University, Melbourne, VIC

The platelet collagen receptor, glycoprotein (GP)VI of the immunoglobulin (Ig) superfamily, plays a key role in initiating platelet adhesion, activation and thrombus formation following vascular injury. GPVI forms a functional complex with the Immunoreceptor Tyrosine-based Activation Motif (ITAM)-bearing Fc receptor  $\alpha$ -chain (FcR $\alpha$ ), required for surface expression of GPVI/FcR $\alpha$ . Surface expression of GPVI is also regulated by irreversible ectodomain shedding, mediated by the sheddase ADAM10 (of the  $\alpha$  disintegrin and metalloproteinase family). This is based on studies showing ADAM10 (but not ADAM17) cleaves synthetic peptides based on the cleavage site in human GPVI, and the ADAM10-selective hydroxamate inhibitor GI254023 preferentially blocks shedding of GPVI. Western blot analysis with a rabbit polyclonal antibody against the cytoplasmic domain of GPVI (that detects both intact and proteolysed forms of GPVI) reveals that essentially all of the GPVI on healthy circulating platelets is intact, unless shedding is induced by exposure to elevated shear stress *in vitro*, GPVI ligands, platelet activation, coagulation, cholesterol depletion with methyl- $\beta$ -cyclodextrin (M $\beta$ CD), or antiplatelet antibodies acting via the low-affinity IgG receptor, Fc $\gamma$ R11a. Soluble GPVI (sGPVI) is also elevated in plasma from patients with atherothrombotic disease or coagulopathy, immune or non-immune thrombocytopenia, and other diseases. While GPVI shedding is rapidly induced (within seconds to minutes) when human platelets are treated with triggers of shedding, how ADAM10 activity and GPVI expression is regulated during thrombus formation is unknown. To address this question, a fluorescence resonance energy transfer (FRET) substrate (GPVI-Cy3) has been generated, involving a fluorophore and quencher linked by a short peptide with sequence corresponding to the ADAM10 cleavage site in GPVI. GPVI-Cy3 is cleaved by recombinant ADAM10 (rADAM10) but not rADAM17, and by washed human platelets either treated with agents known to upregulate ADAM activity on other cells, or briefly exposed to pathological shear rates using a cone-plate viscometer. Using ADAM10 substrate, fluorescently-labelled antibodies against GPVI (DyLight-1G5 Fab fragment), and confocal imaging of thrombi formed on a collagen surface under flow (input wall shear, 1800 s<sup>-1</sup>), current studies are attempting to unravel mechanisms affecting thrombus growth and stability. Understanding of ADAM10 activity towards GPVI and other substrates of this ubiquitous enzyme may be relevant to bleeding or thrombotic propensity as well as vascular pathology beyond platelets and thrombosis.

## **146. New ITP treatments**

**Bird R**

*Princess Alexandra Hospital, Woolloongabba, QLD*

The management of chronic ITP has taken a huge step forward with the development of thrombopoietin receptor agonists (TRAs). There is still room for improvement in managing newly diagnosed and persistent ITP, which has not progressed substantially since steroids were first used. An intervention to change the natural history of ITP, resulting in less patients progressing to the chronic phase, is the focus of much recent clinical research. Do we need novel therapies, or will manipulating the schedule for currently available agents provide the key?

A review of therapies in development, current and recent trials, and an update on safety data for TRAs will be presented.

## 147. Thrombocytopenia in pregnancy

McLintock C

*Auckland City Hospital, Auckland, New Zealand*

Thrombocytopenia during pregnancy is diagnosed when the platelet count is  $<150 \times 10^9/L$ . Thrombocytopenia during pregnancy has many potential causes. The commonest causes include gestational thrombocytopenia, preeclamptic disorders of pregnancy and immune thrombocytopenic purpura (ITP). Although gestational thrombocytopenia presents no risk of maternal or fetal hemorrhage, preeclampsia and ITP can expose mother and child to potentially life-threatening complications. As the maternal and fetal/neonatal risks for mother and child vary greatly according to the cause of thrombocytopenia, an accurate antenatal diagnosis of the cause of the thrombocytopenia is essential to ensure optimal therapeutic management. ITP is common in women of childbearing age either developing de-novo during pregnancy or having been diagnosed previously.

The major clinical concerns with ITP in pregnancy relate to the risk of maternal bleeding especially at delivery and the implications of thrombocytopenia if it develops in the infant. For the mother, management decisions relate to: determining a "safe" platelet count during pregnancy to prevent spontaneous bleeding, bleeding during delivery and any invasive procedures; if the diagnosis of ITP should influence the mode of delivery; when can regional anesthesia safely be used; and the most appropriate treatment options if treatment is required. For the infant, questions include: the frequency of thrombocytopenia in infants born to women with ITP; whether it is possible to predict which infants are at risk of developing thrombocytopenia; if antenatal testing for the fetal platelet count is required; the potential for clinically severe bleeding in utero and/or during delivery; whether the risk of major bleeding, especially intracranial hemorrhage is modified by mode of delivery; and what treatment options are available for affected infants.

## **148. New approach in management of heparin-induced thrombocytopenia (HIT)**

**Chong B H**

*St George Hospital, Kogarah, NSW*

Heparin-Induced Thrombocytopenia (HIT) is a limb- and life-threatening complication of heparin therapy that affects 1-5% of patients receiving unfractionated heparin. HIT is mediated by an autoantibody that reacts with Platelet Factor 4/ heparin complex. The immune complex formed, binds to platelet Fc $\gamma$ R11a receptors causing platelet activation and thrombin generation, and initiating and driving thrombosis. Current treatment of HIT consists of (1) withdrawal of heparin and (2) suppression of thrombosis using an alternative anticoagulant (danaparoid, lepirudin or argatroban). Recently other anticoagulants such as fondaparinux and the new oral anticoagulants (e.g. dabigatran) have also been used. Anticoagulant treatment however has not been shown to significantly reduce the limb amputation or mortality rate in HIT although it does decrease the development of new thrombosis. This is probably because anticoagulant therapy alone does not suppress or extinguish the HIT antibody-induced platelet activation and thrombin generation which together drive thrombosis in HIT. We believe that is an urgent unmet clinical need for a new approach in the treatment of HIT i.e. one that specifically blocks Fc $\gamma$ R11a receptors and consequently extinguishes HIT antibody-induced platelet activation. We have recently developed a humanized antibody fragment (scFv) (STG3) that strongly inhibits this reaction.

STG3 gene construct was generated by joining the variable heavy chain and light chain domains of a known anti-Fc $\gamma$ R11a receptor antibody with a flexible linker. This construct was then cloned into an expression vector, expressed in E coli, and STG3 protein purified from bacterial lysate. Binding of STG3 scFv to human platelets was detected by confocal microscopy and flow cytometry. It was shown to block strongly platelet aggregation and <sup>14</sup>C-serotonin release induced by the HIT antibodies. In addition, STG showed potent inhibition of platelet thrombus formation in a flow system using a Venaflux device.

In conclusion, these findings suggest that a therapeutic approach that blocks the binding of HIT antibody/antigen complexes to platelet Fc $\gamma$ R11as could potentially improve the treatment outcome of patients with HIT.

## **149. NOACS: Research to practice**

**Lane D**

*University of Birmingham, Birmingham, UK*

In addition to vitamin K antagonists (VKAs) for stroke prevention in atrial fibrillation (AF) patients we now have three (soon to be four) non-VKA oral anticoagulants (NOACs), dabigatran, apixaban, rivaroxaban (and edoxaban), which can be prescribed. This presentation will review the main results from the Phase III trials which compared warfarin to each of these NOACS (RE-LY, ARISTOTLE, ROCKET-AF, and ENGAGE-AF, respectively) and the AVERROES trial, which compared aspirin to apixaban. Many sub-group analyses have also been published and the efficacy and safety of the NOACs in these sub-groups will be summarised. Current clinical guidelines will be discussed and will concentrate on how to operationalize the NOACs in daily practice. The presentation will also focus on the practical management of NOACs in different clinical scenarios, with emphasis on ensuring medication adherence, managing patients with chronic kidney disease and the management of bleeding complications.

## **150. Global haemostatic assay assessment of the direct oral anticoagulants**

**Baker R**

*Western Australian Centre for Thrombosis and Haemostasis, Perth, WA*

The new direct oral anticoagulants (DOAC's) are being progressively prescribed for prevention and treatment of venous and arterial thrombo-embolism. Unlike warfarin, all DOAC's available in Australia (dabigatran, rivaroxaban and apixaban) have a wide therapeutic range and usually do not require routine laboratory monitoring. There are however occasions when laboratory monitoring is desirable such as in situations of haemorrhage, prior to emergency surgery, assessing compliance, when a thrombotic event occurs, with drug overdose or renal impairment. If available, specific drug level can be measured by calibrated plasma using a standardised thrombin time (dabigatran) or anti Xa chromogenic assay (rivaroxaban or apixaban). There is little information as to what the drug level means for haemostasis particularly in a patient with haemorrhage, prior to emergency surgery or when a specific haemostatic agent is given.

Global assays have been developed to assess haemostasis in patients with bleeding or thrombotic diathesis and include those with haemophilia with and without inhibitors, cardiac and liver surgery, arterial disease or those on anticoagulants. The application of thrombin generation and thromboelastography may be useful to assess the DOAC's ability to individually inhibit coagulation activation which could improve the understanding of the relationship between the pharmacokinetic and pharmacodynamic parameters of these new antithrombotics. In addition, when haemorrhage occurs not only is the drug level of the DOAC important for clinical therapeutic decisions, but the pharmacodynamic effect of the anticoagulant may also be relevant. The effect of reversal of the abnormal parameters by haemostatic agents such as Prothrombinex VF, FEIBA or recombinant factor VIIa may be of assistance in guiding the dose and frequency of administration of these agents in clinical situations in patients with active haemorrhage.

Initial data suggest that dabigatran, rivaroxaban and apixaban all have a dose response effect on the calibrated automated thrombogram (CAT) but only dabigatran significantly affects the dynamic thromboelastography parameters with TEG and ROTEM. The pattern of thrombin generation is different between dabigatran and rivaroxaban/apixaban but the rate and extent of thrombin generation can be restored by using Prothrombinex VF, FEIBA or to a lesser degree by recombinant factor VIIa. Global assays are increasingly being evaluated in complex haemostatic scenarios such as in patients with major haemorrhage on DOAC's, but further well designed studies are required to determine whether they will assist in understanding the individual variation in pharmacokinetic and pharmacodynamic of DOAC's and whether they can guide haemostatic decisions in situations of significant haemorrhage or prior to emergency surgery.

## **151. Intracranial haemorrhage, anticoagulation and reversal strategies - what is the evidence?**

**McRae S**

*SA Pathology, Upper Sturt, SA*

Fatal bleeding is the most feared complication of anticoagulation and occurs at an annual frequency of approximately 0.5% of patients receiving warfarin. The location of bleeding is a major determinant of the case fatality rate of major bleeding events and is highest for intracranial bleeding. This talk will discuss the existing evidence regarding the distribution and fatality rate of anticoagulant related major bleeding for the different agents, and the impact that choice of agent is likely to have on the community burden of fatal bleeding events. The impact or otherwise of current reversal strategies on fatal outcome following major bleeding occurring while on anticoagulant therapy will also be examined, and how this may influence choice of anticoagulant agent will be discussed.

## 152. Platelet activation and procoagulant platelet formation appear to be uncoupled

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<sup>1</sup> Adult Cancer Program, Lowy Cancer Research Centre, <sup>2</sup> Prince of Wales Hospital

### Background and Aims

Pathological thrombosis may be related to an excess of procoagulant platelets. Distinct cell death pathways are important in regulating platelet activity, and the procoagulant platelet is proposed to be necrotic. We previously showed that novel platelet necrosis marker, tagged-GSAO, identified necrotic platelets *in-vitro* and *in-vivo*, and may serve as a surrogate marker for procoagulant platelets. This study explores the relationship between platelet activation and necrosis. Understanding this relationship may yield insights into anti-platelet therapy, particularly in circumstances where classical anti-platelet therapy is inadequate for prevention of arterial thrombosis.

### Method and Results

Platelets from healthy volunteers before and after 7-days aspirin therapy, stimulated with dual agonists (thrombin and collagen), and assessed for platelet necrosis, defined by tagged-GSAO/P-selectin positivity by flow cytometry. Rate of onset of necrosis versus mitochondrial depolarisation was compared using time-lapse analysis of platelets after agonist exposure. Necrosis was defined by GSAO-uptake and mitochondrial depolarisation by change in JC-1 fluorescence.

COX-1 inhibition significantly decreased platelet activation as defined by P-selectin exposure ( $p < 0.05$ ,  $n=5$ ), but had no effect on platelet necrosis, indicating that aspirin does not decrease platelet procoagulant formation. Time-lapse studies indicate GSAO labelling occurs within seconds of agonist stimulation. Labelling was maximal within 60s of collagen stimulation and within 12s of dual agonist stimulation. GSAO labelling preceded mitochondrial depolarisation. Correlation of GSAO labelling with mitochondrial depolarisation demonstrated that mitochondrial depolarisation was not confined to GSAO+ve population.

### Conclusion

Our findings suggest a potential uncoupling between platelet activation and procoagulant pathways. The procoagulant necrotic platelet may be a distinct subpopulation generated directly rather than a population that follows the activation pathways that result in  $\alpha$ -granule release and mitochondrial depolarisation. This suggests it may be possible to separately target platelet activation and procoagulant pathways for anti-thrombotic drug development.

## 153. Apolipoprotein A-IV is a novel ligand of platelet beta-3 integrin and an endogenous inhibitor of thrombosis

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Platelet  $\beta 3$  integrins are essential for platelet aggregation and thrombosis. Understanding of  $\beta 3$  integrin-ligand interactions is crucial in elucidating the mechanism of thrombosis. In an effort to isolate new  $\beta 3$  integrin ligands, we identified apolipoprotein A-IV (apoA-IV), a plasma protein that has been reported to inversely correlate with cardiovascular diseases. However, its roles in thrombosis are completely unknown.

Using biomembrane force probe that detects single-molecule interactions, we demonstrated that apoA-IV bound to  $\alpha \text{IIb}\beta 3$  on activated platelets. ApoA-IV also bound to purified activated  $\alpha \text{IIb}\beta 3$  or that expressed on Chinese hamster ovary (CHO) cells. In comparison, apoA-IV did not significantly bind to  $\alpha \text{IIb}\beta 3$  on resting platelets, GPIb-complex on CHO cells,  $\alpha \text{M}\beta 2$  on K562 cells, nor purified  $\alpha 5\beta 1$  and  $\alpha \text{V}\beta 3$  integrins. Importantly, these apoA-IV- $\alpha \text{IIb}\beta 3$  interactions can be completely blocked by an antibody against  $\beta 3$  integrin (M1). These data showed the specificity of apoA-IV for  $\alpha \text{IIb}\beta 3$  integrin. Furthermore, apoA-IV competitively blocked fibrinogen binding to  $\alpha \text{IIb}\beta 3$ , whose 2D affinity for  $\alpha \text{IIb}\beta 3$  is 43% of fibrinogen- $\alpha \text{IIb}\beta 3$ .

Platelet functional studies *in vitro* showed that apoA-IV inhibited both human and mouse platelet aggregation. Consistently, platelet aggregation was enhanced in mice lacking apoA-IV following stimulations. In *ex vivo* perfusion chambers, apoA-IV inhibited human and mouse thrombus growth and dissolved pre-formed thrombi, while absence of apoA-IV enhanced *ex vivo* thrombosis under both low and high shear stresses. Using two *in vivo* intravital microscopy models, and a carotid artery thrombosis model, we demonstrated that apoA-IV significantly inhibited thrombus growth *in vivo*. We found that the two aspartic acid (D) residues (D5 and D13) at apoA-IV N-terminus are required for this inhibition, which are the potential binding sites for  $\alpha \text{IIb}\beta 3$  integrin.

Thus, apoA-IV is identified as a novel endogenous inhibitor of thrombosis and represents a new link between lipoprotein metabolism and platelet function, both of which play critical roles in cardiovascular diseases.

## 154. The *in vitro*, *ex vivo* and *in vivo* effects of tyrosine kinase inhibitors on platelet function

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### Aim

The purpose of this study is to assess the effects of the tyrosine kinase inhibitors (TKIs), imatinib, nilotinib and dasatinib, on platelet function and thrombus formation *in vitro*, *ex vivo* and *in vivo*.

### Methods

*In vitro* platelet aggregation and platelet alpha granule release studies using healthy human platelets were assessed in the presence of increasing concentrations of TKIs by performing light transmission aggregometry and flow cytometry methods, respectively. To further investigate the effect of TKIs in platelet function *ex vivo* and *in vivo*, wild-type mice were orally administered with imatinib (25 mg/kg), nilotinib (25 mg/kg) or dasatinib (5 mg/kg). Thrombi formation was captured in real-time using a Zeiss Axiovert microscope. The significance of these results was determined by statistical tests (e.g. ANOVA, *t*-test) using the PRISM Grafpad software.

### Results

Compared with the untreated control, dasatinib and imatinib but not nilotinib inhibited agonist-induced platelet aggregation. Also, *in vitro* studies showed that dasatinib and imatinib appear to inhibited agonist-mediated alpha granule release while nilotinib significantly potentiated protease activated receptor-mediated alpha granule release. Furthermore, nilotinib but not imatinib or dasatinib potentiated thrombus formation on type I collagen under *in vitro* and *ex vivo* arterial flow conditions. Significantly, wild-type mice treated nilotinib clearly increased thrombus formation and stable thrombi over time compared to the untreated control. In contrast, dasatinib and imatinib exerted inhibitory effects on thrombus growth *in vivo*. Interestingly, the effects of TKIs on thrombus growth *in vivo* 48 hours after oral administration were shown to be reversible.

### Conclusion

The present results show that TKIs behave differently in modulating platelet function and may cause abnormal thrombotic events. The outcomes of this project have provided an insight in into the mechanistic effects of these drugs on platelet function.

## 155. Ventricular assist devices are associated with loss of platelet receptors

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### Aim

Ventricular assist devices (VADs) are associated with bleeding, not fully explained by anticoagulant or anti-platelet use. Acquired von Willebrand syndrome (AVWS) may contribute to bleeding in patients with VADs. We investigated the relationship between AVWS and platelet dysfunction, through the loss of von Willebrand factor (VWF) receptor, GPIb (of the GPIb-IX-V complex) and the major collagen receptor, GPVI. GPIb and GPVI are crucial for platelet function at arterial shear rates.

### Methods

A pilot analysis was performed in 21 VADs patients 0.5-30 months post-implant. Platelet counts, coagulation tests and VWF analyses were performed. Levels of platelet surface and shed receptors were measured by flow cytometry-based assays developed in-house or ELISA. The National Cancer Institute (NCI) bleeding score grouped patients into major (NCI  $\geq 3$ ) or non-major (NCI  $< 3$ ) bleeding.

### Results

We demonstrated loss of high molecular weight VWF multimers in most VADs patients (19 of 21), consistent with AVWS. Platelet receptor shedding was demonstrated by significantly elevated soluble GPVI levels in plasma ( $p=0.025$ ), reduction in surface GPVI levels ( $p=0.0003$ ) and GPIb levels ( $p=0.0008$ ) in VADs patients compared to healthy donors (Table 1). Five patients with VADs (24%) had major bleeding, however, differences in platelet receptor levels were not statistically significant for those who had major bleeds compared with those who did not.

**Table 1. Platelet receptor analyses in VADs patients vs healthy donor**

Test Parameters	Healthy Donors; n=40 Median (range)	VAD; n=21 Median (range)	p value
Surface GPIb	823 (406-1764)	546 (230-1069)	0.0008
Surface CD41a	224 (60-581)	206 (65-482)	NS
Surface GPVI	183 (11-474)	100 (42-292)	0.0003
Soluble GPVI	26 (3.2-50)	30.6 (23.4-66.2)	0.025

### Conclusion

We linked for the first time AVWS and increased platelet receptor shedding in patients with VADs. Loss of platelet surface receptors GPIb or GPVI may negatively regulate platelet adhesion/activation and limit thrombus formation under pathologic shear conditions. Further investigation will elucidate mechanisms of platelet receptor loss.

## 156. Tetraspanin CD151 and ADP purinergic P2Y<sub>12</sub> receptor, participate in a common pathway to regulate thrombus growth and stability

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### Background

CD151 is highly expressed in megakaryocytes and to a lesser extent in platelets (at approximately 3,000 copies/platelet). The ADP purinergic receptor P2Y<sub>12</sub>, which is mainly expressed in platelets, is therapeutically important as a target of several clinically-approved antithrombotic agents.

### Aim

To investigate the functional importance of CD151 and the P2Y<sub>12</sub> receptor in platelet function, using wild-type or CD151-deficient mice treated with either PBS or 50 mg/kg clopidogrel.

### Methods

Platelet granule release was assessed by measuring the release of  $\alpha$  and dense granules. The post-occupancy events of integrin  $\alpha_{IIb}\beta_3$  were determined using clot retraction, platelet aggregation and platelet spreading on fibrinogen. "Inside-out" integrin  $\alpha_{IIb}\beta_3$  signalling was examined using FITC-fibrinogen and JON/A mAb expression. Thrombus formation was assessed using *in vitro* flow shear and induction of *in vivo* vascular injury of mesenteric arterioles by FeCl<sub>3</sub>.

### Results

CD151-deficient mice treated with clopidogrel exhibited further impairment in kinetics of clot retraction, platelet aggregation (at different doses of PAR-4 and collagen), and platelet spreading on fibrinogen compared to solitary CD151 knockout (KO) or P2Y<sub>12</sub> receptor blockade mice. Neither granule secretion ( $\alpha$  or dense) nor "inside-out" integrin  $\alpha_{IIb}\beta_3$  signalling were affected. *Ex vivo* and *in vivo* experiments, however, revealed smaller and less stable thrombi, with increased tendency to embolise in clopidogrel-treated CD151 KO mice than from mice with CD151 KO or P2Y<sub>12</sub> receptor blockade alone.

### Conclusion

These data demonstrated a functional relationship between CD151 and the P2Y<sub>12</sub> receptor in platelets in regulating *in vitro* and *in vivo* thrombus growth and stability.

## 157. PI3KC2 $\alpha$ regulates platelet OCS structure and uncovers a novel link between the platelet internal membrane system, adhesive function, and thrombus stability

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### Aim

Phosphoinositide 3-kinases (PI3Ks) are a family of eight intracellular signalling enzymes important in a range of cells, including platelets. Class I PI3Ks have been well studied and p110 $\beta$  inhibitors are in clinical development as anti-platelet agents. In contrast, little is known about Class II PI3K function. This study aimed to determine the function of Class II PI3Ks in platelets.

### Methods & Results

We detected expression of two of the three Class II PI3Ks – PI3KC2 $\alpha$  and PI3KC2 $\beta$ , but not PI3KC2 $\gamma$  – in human and mouse platelets *via* Western blot. Platelets from PI3KC2 $\beta$ <sup>-/-</sup> mice functioned normally in all assays examined. We generated PI3KC2 $\alpha$ <sup>-/-</sup> mice, which died *in utero* prior to haematopoiesis, preventing analysis of platelet function. To overcome this, we generated a novel RNAi-based *in vivo* mouse model in which inducible expression of a shRNA against PI3KC2 $\alpha$  in adult mice reduced protein expression in platelets to <5% of normal levels. These PI3KC2 $\alpha$ -deficient mice exhibited significantly impaired haemostasis and thrombosis in *in vivo* models. Strikingly, agonist-induced activation and PI3K lipid product accumulation were normal in PI3KC2 $\alpha$ -deficient platelets, but a specific ultrastructural defect was observed in which the open canalicular system (OCS) displayed an altered distribution and a 37% increase in size. Detailed analysis of the adhesive behaviour of PI3KC2 $\alpha$ -deficient platelets revealed that haemodynamic shear stress induced significant changes in the structure of the OCS that was associated with enhanced adhesive function of the major platelet integrin  $\alpha_{IIb}\beta_3$ , and led to accelerated thrombus growth. These dysregulated thrombi were highly unstable, leading to thromboembolism in both *ex vivo* and *in vivo* thrombosis models.

### Conclusion

Our studies have uncovered a role for PI3KC2 $\alpha$  in the haemostatic and thrombotic function of mouse platelets and provide a novel link between the OCS and platelet adhesive function that is important for *in vivo* thrombus stability.

## 158. Control of thrombosis by functional disulphide bonds

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Thrombosis is exquisitely regulated by discrete peptide bond cleavage. Numerous proteins that control platelet adhesion/activation and blood coagulation in injured blood vessels are activated and/or inactivated by proteolysis. There are an increasing number of thrombosis proteins found to be controlled by cleavage of the next most frequent covalent bond linking the polypeptide backbone of proteins – the disulphide bond<sup>1</sup>. Protein disulphide bonds are the links between the sulphur atoms of two cysteine amino acids (the cystine residue) that form as proteins mature in the cell. Blood proteins are rich in disulphide bonds. For instance, the 2,000 or so plasma proteins contain about 10,000 disulphide bonds.

Most of the disulphide bonds, like most of the peptide bonds, perform a structural role. They stabilise the mature protein structure and remain unchanged for the life of the protein. However, some of the disulphide bonds, the allosteric disulphides, control the function of the mature protein in which they reside when they are cleaved. Cleavage of an allosteric disulphide can change ligand binding, substrate hydrolysis, proteolysis, or oligomer formation of a protein. The allosteric disulphide bonds are reduced by the catalytic disulphides of oxidoreductases or by thiol–disulphide exchange. I will highlight an allosteric disulphide that controls ADAMTS13 regulation of von Willebrand factor (VWF) haemostatic activity.

VWF is a multimeric blood protein that tethers platelets to the injured vessel wall during thrombosis. Only the largest multimers are effective at capturing platelets in the shear forces of flowing blood. VWF multimeric size is regulated in the circulation by proteolysis of the A2 domain Tyr1605-Met1606 peptide bond by ADAMTS13. The A2 domain contains an unusual disulphide bond that links adjacent cysteine residues Cys1669 and Cys1670. Mass spectrometry analysis of plasma VWF indicates that the disulphide bond is naturally reduced in about one in two VWF subunits. The reduced VWF is much more efficiently cleaved by ADAMTS13 than the oxidised protein. The disulphide bond has a standard redox potential of -283 mV and Molecular Dynamics simulations revealed that reduction of the disulphide has a pronounced effect on the structure, dynamics and internal stress of the domain. These findings imply that the Cys1669-Cys1670 bond acts as an allosteric switch in VWF by controlling ADAMTS13 regulation of multimer size.

1. Butera D, Cook KM, Chiu J, Wong JW, Hogg PJ. Control of blood proteins by functional disulfide bonds. *Blood*. 2014;123(13):2000-2007.

## 159. Bridging and perioperative anticoagulation made easy?

Curnow J

*Westmead Hospital, Westmead, NSW*

Perioperative anticoagulation requires an individualized approach tailored to patients' circumstances, balancing the Ying and Yang of underlying thrombosis risk, which is transiently increased by anticoagulation cessation and surgery-associated bleeding risk, which is exaggerated by anticoagulation administration. Standardizing procedures ensures advanced planning of all relevant aspects of anticoagulation management. Estimating a patient-specific thrombotic risk will determine the importance of minimizing the period without anticoagulation. This varies according to the indication for anticoagulation; atrial fibrillation, venous thromboembolism or mechanical valve prostheses. Estimating a procedure-specific bleeding risk will determine whether anticoagulation interruption is necessary at all and for what duration. If discontinuation is required, the timing will be determined by the anticoagulant in use. The long half-life of Warfarin necessitates earlier cessation than for the newer direct oral anticoagulants (DOACs), which have much shorter half-lives. Bridging anticoagulation may be indicated pre-operatively, post-operatively or both for warfarinised patients requiring intervals of 5 days off therapy. In patients with high thromboembolic risk, low molecular weight heparin or unfractionated heparin may be used to reduce the interval without anticoagulation but choice of agent, anticoagulant intensity and timing of use must be considered. The predictable offset of action of DOACs makes pre-operative bridging therapy unnecessary. However the DOACs also have a rapid onset of action and since no reversal agents are currently available, there may be circumstances in which an alternative reversible anticoagulant is used for post-operative bridging if bleeding risk is high. Laboratory testing to determine the presence or absence of anticoagulant effect in the perioperative period will also vary according to the agent in use and cut-offs of anticoagulant levels which are safe for urgent surgery are not well-established for DOACs.

## **160. Venous thromboembolism in Northeast Melbourne, Australia: Evaluation of epidemiology, risk factors and treatment strategies in the warfarin era**

Lim H<sup>1</sup>, Chua C<sup>2</sup>, Sleeman M<sup>1</sup>, Tan J<sup>2</sup>, Smith C<sup>1</sup>, Grigg A<sup>1</sup>, Hayes L<sup>2</sup>, Ho P<sup>1,2</sup>

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### **Aim**

Venous thromboembolism (VTE) is a major cause of morbidity and mortality. While most studies have analysed specific aspects of VTE, we aim to provide a holistic evaluation of local VTE management in the warfarin era.

### **Method**

Retrospective evaluation of VTE from July 2011 to December 2012 at Austin and Northern Health, Melbourne including demographics, provoking factors, management, complications and mortality.

### **Result**

1029 episodes were identified including 26 recurrences - 577 (56%) pulmonary embolism (PE), 428 (42%) deep venous thrombosis (DVT). Median age was 63 years with male predominance (52%), including in the DVT subgroup (57% vs 48%, p=0.003) although there was no gender difference for PE. 20% reported prior VTE. Left limb DVT was more common (49% vs 43%, p=0.0008). 247 patients (24.6%) had cancer and were excluded from analysis. In non-cancer patients, 63% had provoked VTE and thrombophilia screen was performed in 41%. The median duration of anticoagulation was 6 and 7 months for DVT and PE respectively. The majority (90%) was on warfarin for long-term anticoagulation. 5% required further interventions – IVC filter (n=28) and thrombolysis (n=15). 38% had end-of-treatment repeat imaging and residual clot was observed in 40%. Clot persistence was associated with increased recurrence risk, with an odds ratio of 2.64 (1.15 – 6.04, p=0.02). 8% had recurrent thrombosis with no difference between provoked versus unprovoked VTE (7.5% vs 9.0%, p=0.45). 5% reported grade III/IV bleeding, independent of duration of anticoagulation. Patients on enoxaparin had higher risk of bleeding (28% vs 10%, p<0.001). The mortality rate in this non-cancer cohort was 11%.

### **Conclusion**

VTE is associated with a significant mortality rate of 11% in non-cancer patients. Risk factors for recurrence identified in this retrospective review include residual clot on repeat imaging. This data will serve as an important baseline for future comparison in the new era of novel oral anticoagulants.

## **161. Interim analysis of the ASTH Anticoagulant Reversal and Events Study (ARES) Collaborative.**

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### **Introduction**

The ARES Collaborative is a large ANZ prospective observational study of consecutive patients who present with haemorrhage, thromboembolism or who need urgent anticoagulant reversal for surgical intervention and who are taking either a direct oral anticoagulants (DOAC- dabigatran, rivaroxaban or apixaban) or warfarin. The clinical context and severity of the event is recorded in addition to haemostatic strategies used for urgent anticoagulant reversal or treatment of thromboembolism.

### **Methods**

Outcome measures from the first 126 patients from the 5 initial centres were analysed and include detailed description of the presenting population demographics and event type, coagulation profile, nature and efficacy assessment of treatment strategies used for haemorrhage, urgent reversal and thromboembolism. A further interim analysis will be presented at the meeting.

### **Results**

DOAC's are increasingly prescribed in ANZ accounting for 25% of ARES cases (Dabigatran 14%, Rivaroxaban 11%). Most patients treated with DOAC's are appropriate (adequate renal function, correct indication) and have similar characteristics (elderly age mean 73.4 years and CHADS score 3) to those enrolled in the pivotal clinical trials. Presenting events include haemorrhage (n=82; 73% major haemorrhage), urgent reversal for surgery (n=28) or thromboembolism (n=12). The gastrointestinal tract was the most prevalent site of haemorrhage and 26% were on concurrent anti-platelet agents. ARES patients who presented with major haemorrhage had an extended length of hospital stay (6.5 days) and high mortality rate (14.6%). Various haemostatic agents were used to improve haemostasis in patients with haemorrhage who took OAC (Prothrombinex-VF, FEIBA, rFVIIa, tranexamic acid) but 40% of DOAC patients did not receive an intervention.

### **Conclusion**

ARES data provide important observational information concerning current anticoagulant practice in patients on DOAC's when compared to those on warfarin. ARES is now recruiting 2000 patients in 20 sites to provide comprehensive data that will improve anticoagulation practice.

## 162. Below knee deep vein thrombosis: A more benign entity or not?

Ho P<sup>1,2</sup>, Lim H<sup>1</sup>, Chua C<sup>2</sup>, Sleeman M<sup>1</sup>, Tan J<sup>2</sup>, Smith C<sup>1</sup>, Grigg A<sup>1</sup>, Hayes L<sup>2</sup>

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### Aim

Below knee deep vein thrombosis (BKDVT) is traditionally associated with less clinical sequelae such as thrombosis recurrence and malignancy, and often treated with shorter duration and lower intensity of anticoagulation. We aim to evaluate the characteristics of BKDVT in our study population.

### Method

Retrospective evaluation of all BKDVT from July 2011 to December 2012 at Austin and Northern Health, Melbourne, including demographics, provoking factors, associations and outcomes.

### Result

Of a total of 1029 venous thromboembolism (VTE) cases, there were 279 (27%) episodes of BKDVT, of which 22% had concurrent pulmonary embolism (PE). Median age was 63 years with male predominance (56% vs 44%,  $p=0.003$ ). Laterality was similar and the majority (96%) was symptomatic. Forty-six patients (16.5%) had active malignancy and they had higher rates of concurrent PE (77% vs 18%,  $p=0.0001$ ). 191 patients had isolated BKDVT without malignancy. Of these, 18% had a prior history of VTE. Three (1.5%) were subsequently diagnosed with cancer, similar prevalence to those with major VTE (1.7%), defined as proximal DVT and/or PE. BKDVT were more likely to be provoked compared to major VTE (72% vs 55%,  $p<0.001$ ). Median duration of anticoagulation was 5.4 months versus 7.0 months for major VTE. Patients with major VTE were more likely to experience grade III/IV bleeding complications (6.3% vs 1.0%,  $p=0.003$ ) despite similar duration of therapy. Recurrence was similar to major VTE (6.8% vs 8.7%,  $p=0.42$ ), with no difference between provoked and unprovoked BKDVT (7.7% vs 9.3%). Mortality rate was 5.5% with no thrombosis-related deaths.

### Conclusion

BKDVT is associated with significant mortality (5.5%) and has comparable rates of recurrence and subsequent cancer detection to major VTE. Given these findings, investigation and treatment of BKDVT should not differ from major VTE. Further studies are required to determine the adequate length of anticoagulation.

## 163. The risk of Venous Thromboembolism (VTE) following elective hip and knee arthroplasty among patients with hereditary bleeding disorders: A retrospective review

Tran H, McCarthy P, Walsh M, Davis A

*The Alfred Hospital*

### Aim

Patients with hereditary bleeding disorders (HBD) are increasingly undergoing lower limb joint arthroplasty for either haemophilic arthropathy or degenerative arthritis as their life expectancy approximates that of the healthy population. These procedures significantly increase the risk of VTE in patients without HBD, but it is controversial whether patients with HBD have similar risks and should receive pharmacological thromboprophylaxis following such procedures, during which their coagulation deficiency is corrected to normal. We aim to evaluate the rate of VTE in patients with HBD undergoing hip and knee arthroplasty at a large haemophilia treatment centre (HTC).

### Method

The medical records of consecutive patients with HBD (without inhibitors) undergoing hip or knee arthroplasty at a single HTC between February 2002 and June 2014 were reviewed. Patient demographics, arthroplasty type, correction of coagulation factor deficiency, rate of VTE (deep vein thrombosis (DVT) and pulmonary embolism (PE)) and bleeding at day 30, and use of thromboprophylaxis were recorded.

### Results

Patients	Number
Male/female	24/0
Age, years (median [range])	55 [30-83]
Haemophilia A	26
Haemophilia B	10
Hip/Knee arthroplasty	Hip: 16 Knee: 20
Factor replacement, modality (infusion/bolus)	Infusion: 6; bolus: 2; infusion/bolus: 26
Duration of treatment, days (median, [range])	14 [8-33]
Measured FVIII or FIX level during replacement therapy, % (NR, 50-150)	29 – 226%
Received pharmacologic thromboprophylaxis (enoxaparin or aspirin)	Enoxaparin 3; aspirin 1
DVT/PE	Nil
Major Bleeds	Haemarthrosis 2 (5.5%); muscle haematoma 1 (2.7%)

### Conclusion

The absence of VTE following lower limb joint arthroplasty in this cohort of corrected factor deficiency HBD patients, mostly without thromboprophylaxis, suggests use of thromboprophylaxis is unnecessary. However, a larger prospective clinical study is necessary to confirm these findings.

## 164. Thrombogenic risk profile among patients undergoing major surgery for cancer: predictor of surgical outcomes

**Burbury K**<sup>1</sup>, Dauer R<sup>2</sup>, Heriot A<sup>1</sup>, Lynch C<sup>1</sup>, Smart P<sup>3</sup>, Wilson S<sup>1</sup>, Westerman D<sup>1</sup>, Collins M<sup>1</sup>, Riedel B<sup>1</sup>

<sup>1</sup> Peter MacCallum Cancer Centre, <sup>2</sup> Alfred Hospital, <sup>3</sup> St Vincents Hospital

Haemostatic and endothelial dysfunction are likely to be important contributors to postoperative outcomes – macro- and microvascular – following major cancer surgery. Routine laboratory tests do not accurately recognise hyper- or hypocoagulable states, nor provide predictive risk stratification. Cellular-based assays, including thromboelastograph (TEG®), may provide a better assessment.

**Aim** Assess TEG® profiles, haemostatic biomarkers and clinical parameters in patients undergoing major cancer surgery, and correlate with clinically significant postoperative events.

**Methods** Prospective correlation of TEG® profiles, haemostatic biomarkers and clinical data in patients undergoing major cancer surgery at PMCC (12-month period), with postoperative outcomes. Biomarkers included: Haemoglobin, WCC, platelet count, APTT, PT, D-dimer, fibrinogen, FVIIIc, vWF-Ag, Fibrin monomers, PF1+2, TAT complex, TEG®. Postoperative complications, included length of stay (hospital and ICU/HDU), wound infection, surgical site bleeding; TE or ischaemic event (up to 6 weeks post-surgery); sepsis; drop Hb>30g/dL; and/or blood products within 72hours and 7days of surgery. Associations of baseline biomarkers with post-operative events were assessed using simple and multiple linear regression and Wilcoxon rank-sum.

**Results** 135 patients were included in the cohort, median age 65years (range 18-91), 64% were lower GI surgery, 62% adenocarcinoma, 68% locally advanced. 30 (22%) had received chemo-and/or radiotherapy within 3 months of surgery. Pre-operative parameters are listed in table 1. More than a third of patients in our cohort demonstrated a procoagulant profile prior to surgery, which was associated with an increased likelihood of postoperative complications. Multiple regression model demonstrated TEG-MA>69 (p=0.0009), platelets>350 (p=0.05) and fibrinogen>4 (p=0.0006) as strong predictors – and the combination profile of fibrinogen and TEG-hypercoagulable profile demonstrated greater association with post-operative complications (60% vs 29%, p=0.0006).

**Discussion** This data provides a potential risk assessment tool to develop algorithms for the identification of at risk patients and application of appropriate peri-operative optimization, including thromboprophylaxis.

**Table 1: Pre-operative biomarkers**

Parameters factors (NR)	Median	Range
Haemoglobin (g/L)	131	69 – 167
White cell count (x10 <sup>9</sup> /L)	6.4	2.8 – 41.0
Platelet count (x10 <sup>9</sup> /L)	264	96 – 992
PT (11.8-14.6sec)	12.7	11.5 – 15.2
APTT (24-34sec)	29	24 – 49
Fibrinogen (2-4g/L)	4.1	1.5 – 9.8
TEG – R (2.0-8.0 min)	5.9	3.3 – 12.5
TEG – K (1.0-3.0 min)	1.8	0.8 – 4.0
TEG – a (55-78 deg)	67.2	42.1 – 78.8
TEG – MA (51-69mm)	66	52.1 – 83.1

TEG-LY30 (0-8%)	0.1	-4.8-16.0
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## **165. Diagnostic challenges in thrombotic microangiopathies (TMAs): Data from a targeted analysis of the Australia / New Zealand Registry**

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### **Introduction**

Advances in understanding TMAs have led to recognition of separate clinical entities including TTP (thrombotic thrombocytopenic purpura) and aHUS (atypical haemolytic uraemic syndrome). A rapid and accurate diagnosis is now critical to enable the use of specific management strategies to improve patient outcomes. Despite the utility of pre-plasma exchange (PE) ADAMTS13 activity <10% in diagnosing TTP, diagnostic challenges remain, such as distinguishing aHUS from secondary TMAs. Potential pitfalls include overlapping clinical symptomology and the lack of timely access to diagnostic tests, such as the identification of pathogenic mutations in complement regulatory proteins particularly for aHUS.

### **Aims**

To collect data from the national TTP registry demonstrating the clinical spectrum of TMAs and to explore clinical and laboratory features useful in discriminating between them.

### **Methods**

Monash University hosts a multicentre registry for TTP and other TMAs. In 2013, senior clinicians undertook validation by case-note review at 13 centres, targeting sites reporting cases with ADAMTS13 activity levels of >10%.

### **Results**

67 registry cases were validated; 17 had TTP, 23 had no ADAMTS13 result (due to failure to obtain before PE, or assessed as not required), and 27 had an ADAMTS13 >10%. From the latter two groups, 11 different diagnoses were made with secondary TMAs outnumbering aHUS, illustrating the marked heterogeneity of cases. There were significant differences at presentation between patients with TTP vs probable aHUS including renal failure (23% vs 94%), neurological symptoms (82% vs 41%), CNS/coronary thrombosis (41% vs 0%), platelet count >30 (0% vs 82%) and response to plasma exchange at 5 days (35% vs 6%).

### **Conclusion**

The TTP Registry data demonstrate significant differences in clinical and laboratory features that may be useful in the development of a diagnostic algorithm to improve early diagnosis of TMAs. However the heterogeneity of these rare disorders continues to present a diagnostic challenge, particularly in discriminating secondary TMAs.

## **166. New ways to think about hemostasis: A systems approach to platelet activation and the effects of antiplatelet agents**

**Brass L**

*Perelman School of Medicine, Pennsylvania, PA, USA*

Vascular injury and vessel wall diseases in the arterial circulation serve as triggers for the accumulation of platelets and fibrin, sometimes with disastrous consequences. Although commonly studied *in vitro* using tools that work best at the level of individual platelets or small groups of platelets, the hemostatic response and pathologic thrombus formation can also be viewed as the products of larger platelet populations which, by coming together, alter their local microenvironment. This population-based perspective has allowed us to use a systems approach to understanding hemostasis and thrombosis, combining experimental, observational and computational methods with a goal of identifying better ways to limit thrombosis without excessively impairing hemostasis. Among the emergent properties that we and others have observed are regional differences in platelet activation and stability. Such differences are associated with variations in platelet packing density and intrathrombus transport rates, which in turn shape agonist concentration gradients and lead to differential activation of the platelet signaling network. Our goal is to understand how these regional differences arise and how they influence thrombus growth and stability.

## **167. Integrin PSI domain has endogenous thiol isomerase function and is a novel anti-thrombotic target**

**Ni H**

*University of Toronto, Toronto, ON, Canada*

Integrins are a large family of adhesion receptors expressed on almost all cells and play key roles in cell-cell and cell-matrix interactions. Integrin conformational changes are required for their activation/function although the underlying mechanisms remain to be further elucidated. The  $\beta$  subunit contains a PSI domain that is highly conserved across integrins and species, though its function is unknown. While the PSI domain contains two CXXC sequences, the active site motif of protein disulfide isomerase (PDI), whether PSI domain has thiol-isomerase activity has not been identified. Using an assay measuring the refolding of reduced, denatured RNase, we found that recombinant murine integrin  $\beta 3$  and human  $\beta 1$  and  $\beta 2$  PSI domains have PDI activity, which can be dose-dependently inhibited by PDI inhibitors, bacitracin and DTNB.

Integrin  $\alpha IIb\beta 3$ , an essential receptor for platelet aggregation and hemostasis/thrombosis, was further studied. We found that mutation of either CXXC motif reduced its PDI-like activity, while removal of both CXXC motifs completely abolished this activity. In a cell-free system (ELISA) bacitracin attenuated fibrinogen and PAC-1 binding to human platelet  $\beta 3$  integrin. We also developed mouse anti-mouse/anti-human  $\beta 3$  PSI domain monoclonal antibodies (mAbs) and found that these mAbs inhibited the PDI-like activity of both the murine recombinant  $\beta 3$  PSI domain and purified human platelet  $\beta 3$  integrin. Furthermore, anti-PSI mAbs inhibited murine and human platelet aggregation *in vitro*, *ex vivo* and thrombus growth *in vivo*, in murine small and large vessel thrombosis models. Treatment with anti-PSI mAbs did not significantly affect tail bleeding times or platelet counts.

Thus, we identified that the PSI domain has thiol-isomerase activity, and  $\beta 3$  integrin PSI domain is a novel target for anti-thrombotic therapies. Since PSI domain is conserved in all integrin  $\beta$  subunits, our discovery may have broad implications for the role of integrins in all cell-cell interactions and many human diseases.

## **168. Fc receptors as targets for novel treatments**

**Hogarth M**

*Burnet Institute, Melbourne, VIC*

The interaction of antibodies and Fc receptors induces powerful effector functions which are essential for normal immunity. In aberrant immunity, immune complexes (complexes of antibody and antigen eg autoantigen or allergen) induce powerful and undesirable destructive responses. On the other hand these same destructive responses can be usefully harnessed by engineered therapeutic monoclonal antibodies (mAb) the eradication of malignancies. Thus the antibody: Fc interaction is being usefully targeted for the treatment of disease. In the treatment of antibody pathologies, strategies to prevent antibody binding to Fc receptors may be a useful therapeutic strategy in, for example lupus or immune thrombocytopaenias. In contrast, better therapeutic mAb may be developed by enhancing FcR binding for more potent Fc receptor-dependant function. Understand FcR antibody interaction underpins these distinct approaches to development of new therapies for different applications. Studies of FcR function in human and nonhuman primates will be discussed in the context the development of small molecule antagonist of Fc receptor function and in the development of engineered therapeutic antibodies. *pmhogarth@burnet.edu.au*

## **169. Peptide and protein based molecular target imaging of cardiovascular disease**

**Hackeng T**

*University Maastricht, Maastricht, The Netherlands*

Molecular imaging of biomarkers of thrombosis offers the possibility for prognosis and prevention as well as image guided therapy of thrombotic disease. Well known pathological events in venous and arterial thrombosis are thrombus formation, plaque macrophage infiltration, and intra plaque angiogenesis. As their respective molecular markers, fibrin, macrophage receptor CCR5, and angiogenic endothelial marker CD13 were chosen as molecular imaging targets. Peptide and protein based molecular imaging agents were designed and synthesized using total chemical synthesis. In comparison with recombinant molecular biology techniques, chemical synthesis of proteins offers clear advantages for the incorporation of non-coded elements, such as unnatural amino acids, and MRI/PET/SPECT or fluorescent labels at single, specific sites. For fibrin detection multimodal  $\alpha_2$ -antiplasmin derived peptides were synthesized that covalently bind to fibrin through FXIIIa cross linking, for CCR5 detection multimodal Rantes variants were synthesized, and for detection of CD13, various cyclic NGR motifs were synthesized as imaging agents. *In vitro* and *in vivo* targeting and imaging of thrombosis and atherosclerosis and angiogenesis in mice show promising results for further development of these imaging agents for prognosis, diagnosis, and treatment of cardiovascular disease.

## **170. New long acting coagulation therapies**

**Tran H**

*The Alfred Hospital, Melbourne, VIC*

Compared with episodic treatment, prophylaxis with factor concentrate to manage patients with severe haemophilia beginning at a young age has been shown to reduce bleeding episodes, less joint damage, and improved quality of life. With a factor VIII half-life of 8-12 hours frequent dosing regime of 3 times weekly or alternate day has been necessary, sometimes compromising compliance in some individuals. Recently, longer-lasting coagulation factor concentrates have been shown to result in lower annualised bleeding rates when dosed prophylactically at once to twice per week. These products have the potential to provide a significant benefit for people with (severe) haemophilia worldwide. This presentation reviews various technologies including glycopegylation, albumin-fusion, Fc fusion, and antibody techniques that results in longer-acting coagulation factors.

## **171. The role of miRNA regulation of coagulation factors**

**Tay J**

*Murdoch University, WA*

microRNAs (miRNAs) are short noncoding RNA species that bind to the 3' untranslated regions (3'UTR) of target genes to downregulate their expression. Accumulating evidence indicate that miRNAs are involved in important physiological pathways, and shown to be deregulated in various diseases. In addition, miRNAs have been demonstrated to be highly stable in circulation and detected in extracellular fractions of blood, including platelets, plasma, microparticles and exosomes, with recent studies showing that specific miRNAs contribute to the regulation of haemostasis. However, the analysis of miRNAs from biofluids remain challenging due to the low amounts of total RNA recovered from serum or plasma samples and the lack of established endogenous housekeeping controls for data normalisation.

High circulating oestrogen levels during pregnancy have been strongly associated with an increased risk of venous thrombosis and acquired Protein S deficiency. We have reported that the miRNA, miR-494 is oestrogen responsive and directly binds within the PROS1-3'UTR to downregulate PROS1 expression in HuH-7 liver cells, indicating a miRNA-mediated mechanism in the development of acquired Protein S deficiency during pregnancy. To further characterise the role of miRNAs in oestrogen-mediated Protein S deficiency, we examined the tissue specificity of oestradiol-mediated miR-494 expression in endothelial, megakaryocyte and trophoblast cell lines, as well as the levels of circulating plasma miR-494 expression pregnant women with high circulating oestrogen levels, using a number of miRNA quantitation strategies (quantitative realtime PCR, digital droplet PCR and nCounter miRNA analysis).

## 172. Platelet receptors in haemostasis... is everything under control?

Andrews R

Monash University, Melbourne, VIC

Platelets' familiar role in haemostasis and thrombosis has continued to expand due to increasing experimental and clinical evidence for a key role for platelets in inflammation, infection and tumour metastasis, and highlighting the crosstalk between these vascular systems. This may, in part, explain why the number of platelets in the normal circulation ( $150\text{--}400 \times 10^9/\text{litre}$ ) derived from megakaryocytes in the bone marrow, appears far greater than required for normal haemostasis. There is a current lack of reliable platelet-based assays that reflect the quality of platelets, that can be used at low platelet count, or in the case of immune or non-immune thrombocytopenia, that can distinguish between decreased platelet production (*eg.* bone marrow defects) or increased platelet clearance (*eg.* autoantibodies). In this regard, platelet count remains the most common clinical measure but does not predict bleeding risk, and does not discriminate production *versus* destruction defects. Analysis of platelet receptor expression and function can potentially provide a new approach for evaluating platelet quality in individuals, and/or for monitoring response to treatment. The platelet-specific adhesion-signalling complex, the ligand-binding glycoprotein (GP)Ib $\alpha$  of the GPIb-IX-V complex, and co-associated GPVI, play a central role in platelet function particularly at elevated shear rates in flowing blood. GPIb $\alpha$  of the leucine-rich repeat (LRR) family, which binds von Willebrand factor (VWF) and GPVI of the immunoglobulin (Ig) superfamily, which binds collagen, initiate platelet adhesion, activation and thrombus formation upon vascular injury, while GPIb $\alpha$  also binds coagulation factors (Factor XII, XI and thrombin), and counter receptors  $\alpha$ M $\beta$ 2 on leukocytes and P-selectin on activated endothelial cells. Metalloproteinase-mediated ectodomain shedding of GPVI (*via* ADAM10) and GPIb $\alpha$  (predominantly *via* ADAM17) regulates expression and ligand-binding function of these key receptors, as well as generating proteolytic fragments as platelet-specific biomarkers. Platelet surface GPVI and shed soluble GPVI (sGPVI) are quantifiable using specific immunoassays. Experimental triggers of shedding, including exposure to elevated shear stress, GPVI ligands, platelet activation, coagulation, antiplatelet antibodies, or apoptosis, are relevant to a range of human diseases, and recent studies show abnormal GPVI expression/shedding in atherothrombosis, immune or non-immune thrombocytopenia, inflammation, myeloproliferative or other disorders. Reference data on plasma sGPVI has been obtained from >500 healthy blood donors. Future analysis of primary platelet receptor expression and shedding in patients with defined clinical outcomes could provide useful information on risk, and help guide use of antiplatelet drugs.

## 173. Adenosine generation and ADP hydrolysis protect from antiphospholipid antibody-induced miscarriages

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### Background

ATP and ADP are extracellular purines that activate inflammation and thrombosis, processes implicated in antiphospholipid antibodies (aPL-ab)-mediated foetal loss. ATP and ADP are hydrolysed by the cell surface enzyme CD39 (NTPDase) to AMP and then to adenosine by the action of another enzyme, CD73. In contrast to the effect of ATP and ADP, adenosine signals to inhibit inflammation and suppress TF expression.

### Aims

To analyse the role of purinergic nucleotides in APS miscarriages.

### Methods

We have established an aPL-ab-induced model of miscarriages by administration of aPL-ab (purified from patients) to pregnant mice.

### Results

We applied this model to mice with modifications of several of the purinergic pathway enzymes:

(A) CD39-Transgenic (CD39-TG on a BALB/c strain) mice with increased hydrolysis of ATP and ADP to AMP and adenosine: demonstrate reduction in aPL-ab-induced miscarriages. Resorption frequency in wild-type (WT-BALB/c) treated with non-immune IgG, 21% ±6 (SEM); WT treated with aPL-ab, 40% ±5; CD39-TG treated with aPL-ab, 14% ± 3; (P = 0.0008, n = 7/group).

(B) CD39-null (CD39<sup>-/-</sup>, on a C57Bl/6 strain, which is more resistant to miscarriages than BALB/c) mice with decreased hydrolysis of ATP and ADP: demonstrate higher frequency of miscarriages. Resorption frequency with aPL-ab: 3%±2 in WT-C57BL/6 and 15%±4 in CD39<sup>-/-</sup>, p=0.036, n=7/group).

(C) CD73<sup>-/-</sup> (C57BL/6 strain) mice cannot hydrolyse AMP further to adenosine: demonstrate higher frequency of miscarriages. Resorption frequency with aPL-ab: 2%±2 in WT-C57BL/6 and 11%±2 in CD73<sup>-/-</sup>, p<0.05, n=7/group).

(D) Adenosine receptor A2AR<sup>-/-</sup> show an increased trend to miscarriages (p=ns as yet).

We demonstrated that TF mRNA expression is more (>2-fold, p<0.05) in cohorts with increased miscarriages. Also, complement activation and TNF expression is reduced in the placentae of CD39-TG mice that have fewer miscarriages as compared with WT.

### Conclusions

Hydrolysis of ATP and ADP and adenosine generation is protective in APS miscarriages by reducing inflammation, TF expression and complement activation.

## 174. High-throughput sequencing for the detection of *ADAMTS13* mutations in patients with congenital thrombotic thrombocytopenic purpura

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### Background

Congenital thrombotic thrombocytopenic purpura (TTP) is caused by loss-of-function mutations in the *ADAMTS13* gene. The diagnosis of congenital TTP is typically confirmed by labour-intensive and time-consuming conventional sequencing of the entire *ADAMTS13* gene.

### Aims

To investigate the feasibility of high-throughput sequencing techniques for the detection of mutations in *ADAMTS13* in patients with congenital TTP. To sequence thrombotic microangiopathy associated genes in patients with congenital TTP in order to investigate the possibility of contribution of other mutations to observed clinical phenotypes.

### Method

Patients with congenital TTP and available DNA samples were identified from the UK TTP registry. All patients had mutations detected previously by conventional sequencing of *ADAMTS13*. DNA samples were sequenced using the Illumina TruSight One Sequencing Panel on an Illumina HiSeq 2500. Only genes related to thrombotic microangiopathy were analysed. Sequencing data was analysed using a validated customised bioinformatic pipeline.

### Results

Samples from 16 patients with congenital TTP and known *ADAMTS13* mutations were tested using the TruSight One assay. 3/16 samples had inadequate read depth for further analysis. For the other 13 samples, the average *ADAMTS13* coverage was 92% (at a minimum of 20x read depth). *ADAMTS13* exons 6, 19, 22 and 23 had consistently suboptimal read depth. All previously identified *ADAMTS13* mutations in the 13 patients were correctly identified using the TruSight One assay and subsequent bioinformatic pipeline. 4/13 samples were found to have an uncommon single nucleotide polymorphism in vWF associated with resistance to cleavage by *ADAMTS13* therefore potentially contributing to clinical phenotype.

### Conclusion

High-throughput sequencing is a useful technique with significant cost and efficiency advantages over conventional sequencing for the detection of *ADAMTS13* mutations in patients with congenital TTP. Moreover, it may provide further insight into the pathogenesis of this condition through the detection of mutations in other thrombotic microangiopathy associated genes.

## 175. miR-494 regulates multiple factors in the Coagulation Pathway

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<sup>1</sup> WACHTH Murdoch University, <sup>2</sup> Royal Perth Hospital

### Aim

Expression of the microRNA, miR-494, was shown to be oestrogen responsive and directly downregulates Protein S (*PROS1*) expression, indicating a role for miR-494 in oestrogen-mediated acquired Protein S deficiency. This study aims to further characterise miR-494 function in coagulation and identify other direct targets of miR-494 that are regulators of the coagulation pathway.

### Method

To identify miR-494 target genes, the 3'UTR sequences of 40 genes involved in the coagulation pathway were analysed using online prediction tools for miRNA binding sites, TargetScan, RegRNA, and miRanda. HuH-7 cells were transiently transfected with miR-494, and the mRNA levels of these 40 coagulation factors were determined by quantitative PCR to identify miR-494 candidate targets. The direct targeting of select coagulation factors by miR-494 was investigated using dual luciferase reporter assays in HuH-7 cells transiently transfected with pMIR-REPORT luciferase vector containing the full length 3'UTR sequence, a *Renilla* luciferase control vector, pRL-SV40, and miR-494 or negative control miRNA precursors.

### Result

Computational analyses of 3'UTR sequences identified miR-494 binding sites in multiple genes such as, Factor 2 (*F2*), Factor 8 (*F8*), fibronectin (*FN1*) and tissue factor pathway inhibitor (*TFPI*). Cotransfection of miR-494 with luciferase reporter vectors inhibited *F2*-3'UTR and *F8*-3'UTR-dependent relative luciferase activity by 26% and 18% respectively, compared to controls in HuH-7 cells. Gene expression analysis of coagulation pathway factors in miR-494-transfected HuH-7 cells showed that mRNA levels of multiple genes were differentially regulated compared to mRNA levels in miR-negative control transfected cells, including genes that did not contain miR-494 binding sites.

### Conclusion

The results show that miR-494 targets multiple anti- and procoagulation factors in addition to *PROS1* to regulate coagulation via direct and indirect mechanisms. These findings also demonstrate the potential effects of oestrogen-induced miR-494 expression during pregnancy on the regulation of important coagulation factors contributing to increased thrombotic risk.

## **176. The 78 kDa glucose-regulated protein (GRP78) binds to thrombomodulin and exhibits novel antithrombotic activities.**

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**Nandurkar H** <sup>1,5</sup>

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Thrombomodulin (TM) is a transmembrane glycoprotein expressed primarily on vascular endothelial cells and exhibits anti-inflammatory and anti-coagulant activities via generation of activated protein C.

### **Aim**

To identify novel proteins that interact with thrombomodulin and regulate haemostasis.

**RESULTS:** We utilized an affinity trap with the N'-terminal lectin-like domain (LLD) of TM as the 'bait' and identified a specific interaction with (GRP78 by proteomic analysis. We generated recombinant GRP78 and demonstrated that LLD and GRP78 can directly interact without the need of other cofactors. GRP78 prolongs TF dependent clotting (prothrombin time-based assay;  $P < 0.0025$ ). GRP78 also inhibits FXa generation (Xa spectrozyme assay) while showing no effect on thrombin time.

We have demonstrated for the first time that recombinant GRP78 (8–10  $\mu\text{g}/\text{mL}$ ) can inhibit platelet aggregation up to 80% (compared to buffer) in response to collagen (1 U/mL), TRAP (1 IM) and ADP (10 IM).

Pre-administration of GRP78 prolonged mouse tail bleeding time (time in min): buffer  $3.1 \pm 0.7$ ; GRP78 1  $\mu\text{g}/\text{g}$   $4.5 \pm 0.9$ ; 2 $\mu\text{g}/\text{g}$   $8.9 \pm 0.8$  and 5 $\mu\text{g}/\text{g}$   $11.2 \pm 1.3$ , respectively,  $P < 0.003$  for buffer v/s 2 $\mu\text{g}/\text{g}$  and 5 $\mu\text{g}/\text{g}$ ,  $n = 6$  each cohort.

GRP78 confers significant protection in a platelet dependent model of acute venous thrombosis, induced by collagen (1.2 $\mu\text{g}/\text{g}$ ) into the jugular vein of anaesthetised mice and monitored for morbidity (percent survival) within 30 min post collagen challenge; buffer 15%; GRP78 4 $\mu\text{g}/\text{g}$  (66%); 8 $\mu\text{g}/\text{g}$  85%,  $P < 0.003$  for buffer v/s GRP78,  $n = 11$  each cohort.

### **Conclusion**

We have identified a novel mechanism wherein GRP78 localizes with thrombomodulin on endothelial surface and regulates haemostasis through its anticoagulant and antiplatelet activities.

## 177. Thrombin generation in the normal population – Impact of age and sex

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<sup>1</sup> Department of Haematology, Northern Health, <sup>2</sup> Austin Pathology, <sup>3</sup> Department of Medical Sciences, RMIT, <sup>4</sup> Florey Institute of Neuroscience and Mental Health

Thrombosis is a major cause of morbidity and mortality in Australia, with age and male sex being major risk factors. Unfortunately, there are no laboratory tests that reflect the true in-vivo coagulation status. Global coagulation assays such as calibrated automated thrombogram (CAT) maybe a better surrogate measure of an individual's cardiovascular risk. However, it is important to understand the impact of these assays in the normal population, particularly the impact of age and sex.

### Methods

Normal controls with no prior history of cardiovascular/thrombotic disease were recruited as part of a biomarkers of thrombosis study at Austin/Northern Health, Melbourne, Victoria. All were evaluated with routine laboratory tests to exclude underlying thrombosis risk factors including full blood examination, thrombophilia screen, von Willebrand studies, fasting lipid profile. All samples were double centrifuged at 2500G and frozen at -80°C within 2 hours of collection. Samples were analysed using the calibrated automated thrombogram using standard 5 pmmol reagent (Stago) .

### Results

32 normal controls (20 females, 12 males) with median age of 45 (range: 24-79) years were recruited. All patients had negative thrombophilia screens without significant cardiovascular risk factors. No controls were on the oral contraceptive pill.

Thrombin generation parameters varied significantly within this controlled population group (table 1) and there was no correlation with age ( $r^2=0.059$ ). There were 2 distinct patterns of thrombin generation curves (Figure 1) – the concave type curve being more common in males. Thrombin generation was higher in females (ETP: 1418 nM, 95% CI: 1294-1542) compared to males (ETP: 1282, 95% CI: 1112-1450). ETP appears to be higher in pre-menopausal women (1524 vs 1410 nM).

### Summary

Thrombin generation varies significantly within the normal population but does not correlate with age. This may reflect the unpredictable thrombotic risk profiles within the normal population. There appears to be two distinct thrombin generation curves, the significance of which is unclear. Females have higher thrombin generation compared to males, which maybe related to underlying hormonal status. Further recruitment and analysis is ongoing.

Figure 1 – Distinct Thrombin Generation Curves

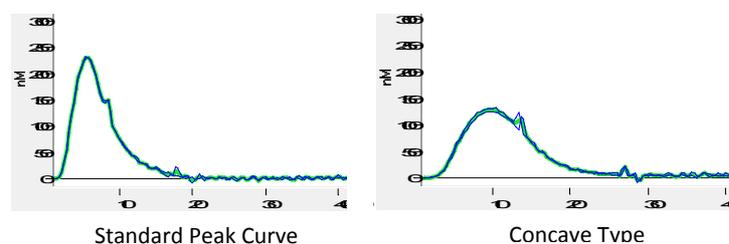


Table 1: Thrombin Generation Parameters (median + range)

	Total (32 controls)	Female controls (20)	Male (12 controls)
Endogenous Thrombin Potential	1330 (944 – 2136)	1373 (1102 – 2136)	1288 (944 - 1543)

<b>(ETP) (nM.min)</b>			
<b>Thrombin Peak (nM)</b>	200 (102 – 357)	227 (102 – 357)	162 (102 - 233)
<b>Velocity Index (nM/min)</b>	50 (16 - 1530)	64 (16 – 153)	34 (21 – 81)
<b>Time to Peak (secs)</b>	7.5 (4.4 – 11.7)	6.9 (4.4 – 11.7)	8.2 (5.9 – 10.1)
<b>Lag Time (secs)</b>	3.3 (2.05 – 6.83)	3.1 (2.05 – 6.8)	3.5 (2.7 – 4.6)

## **178. Correlation of calibrated automated thrombography with lupus anticoagulant in anti-phospholipid patients**

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*National University Hospital, Singapore*

### **Background**

The anti-phospholipid syndrome (APS) is characterized by the occurrence of thrombosis in patients with lupus anticoagulant (LAC). Several mechanisms may explain the thrombogenic effects of LAC. Calibrated automated thrombography (CAT) has been reported for sensitive detection of APS in Caucasian patients with and without anticoagulant treatment. However, the correlation of APS syndrome and thrombin generation times have not been evaluated among Asian population.

### **Aim**

To correlate the presence of LAC with normalised peak time/lag time ratio as measured by CAT among Asian population.

### **Method**

Citrated plasma samples from healthy volunteers (n=20) and APS patients as referred from thrombophilia centers or referred for autoimmune disease testing (n=30) were analyzed for lupus anticoagulant profiles as according to ISTH guidelines. The platelet poor plasma will be stored at -80°C for further analysis using CAT (peak and lag time), added with mixture of phospholipids and tissue factor.

### **Result and Conclusion**

Laboratory measurement and correlation of thrombin generation with LAC provide a potential biomarker for identifying APS patients with thrombotic risk. Further study and long-term follow up are required to determine if the parameter could guide clinical management of patients.

## **179. Medical VTE Prevention: Why? Who? What? How Long?**

### **Gallus A**

*SA Pathology, SA*

Guidelines recommend VTE prophylaxis for 'at risk' medical inpatients because low dose heparins (unfractionated or low molecular weight) reduce the incidence of subclinical DVT (at leg scan or venogram) and symptomatic VTE (including PE, according to most overviews), although not mortality. The price is a small excess of bleeding. This requires risk assessment for thrombosis and bleeding risks, and a heparin if thrombosis risk is high and bleeding risk is not. Support stockings have weak evidence.

The uptake of heparin prophylaxis tends to be less in medical patients than after surgery. Reluctance to apply the guidelines in medical patients probably reflects concerns by critics that (1) thrombosis and bleeding risks in clinical trial populations may not have reflected the average of elderly medical inpatients; (2) they find evidence for clinical benefit from prophylaxis weaker in medical than surgical patients; and (3) harm may exceed benefit. A major conundrum is that thrombosis and bleeding risk assessment models are imprecise.

At least two thirds of VTE are provoked by hospital admission and at least half of these are in medical patients. Clinical value from VTE prophylaxis is more likely than not. If we are not convinced, then we should perform more and better clinical trials of anticoagulants in medical patients – preferably large, simple, focused on risk assessment and symptomatic outcomes, and placebo-controlled.

## **180. Long term anticoagulation: Spoilt by choice - aspirin, warfarin, NOASC or nil?**

**Brighton T**

*SEALS Prince of Wales Hospital, Sydney, NSW*

Recurrent vein thrombosis, either Deep Vein Thrombosis (DVT) or Pulmonary Embolism (PE), accounts for 10-20% of all cases of vein thrombosis. As with first cases of vein thrombosis, recurrent thrombosis is associated with significant morbidity and a defined mortality. Recurrent thrombosis may occur in the context of reversible factors (such as surgery, leg injury, acute illness with immobility, pregnancy and childbirth) and long term anticoagulation is generally not required in such patients. A smaller but significant proportion of patients experience unprovoked thrombosis and are considered to have a "thrombophilia". This "thrombophilia" is not measurable or predicted by laboratory assays. Patients with recurrent unprovoked vein thrombosis generally receive life-long anticoagulation. Guidelines now recommend patients with first unprovoked vein thrombosis also receive long-term anticoagulation unless there is a contraindication to such therapy. Clinical studies of long term anticoagulation demonstrate that while on treatment extended duration anticoagulation is effective, but comes at a significant cost with increased major/fatal bleeding. The inconvenience of long-term warfarin therapy can be overcome with newer oral anticoagulant drugs. In patients unable or unwilling to take long-term anticoagulant therapy low-dose aspirin has shown efficacy in recent studies. This review will examine strategies to select patients for long-term anticoagulation and explore which patients might benefit from warfarin, newer anticoagulant medication, aspirin or just observation.

## **181. Cancer associated venous-thromboembolism in the era of the direct oral anticoagulants**

**Chen V**

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Venous thromboembolism (VTE) remains one of the major causes of morbidity and mortality in patients with cancer. Cancer alone increases the risk of VTE 4.1 fold, chemotherapy increases the HR a further 6.5. Furthermore, the rate of recurrence is 3.2 fold higher for cancer patients and the rates of major bleeding on treatment are increased 2.2 fold. Low molecular weight heparin (LMWH) remains the treatment of choice for initial and long term therapy. Studies comparing LMWH versus vitamin K antagonists (VKA) for secondary prevention of VTE in cancer show consistent evidence for improved efficacy of LMWH. A meta-analysis confirmed a relative risk reduction of 53% for recurrence of VTE with LMWH, while the rates of major and clinically relevant bleeding were no different. The recommended duration of therapy is long term anticoagulation in presence of active cancer. Anticoagulant therapy in cancer considered burdensome, is associated with risk and viewed as having a negative impact on quality of life.

In an era when the options for treatment of non-cancer related VTE are expanding, the limitations in treatment options in cancer are highlighted. In the non-cancer population: three direct FXa inhibitors (rivaroxaban, apixaban and edoxaban) and one direct thrombin inhibitor (dabigatran) have been shown to be non-inferior to VKA for initial treatment and secondary prevention of VTE. Sub-group analysis of cancer patients from phase III trials of rivaroxaban and apixaban indicate that efficacy (rate of recurrence) was equivalent between direct oral inhibitors vs conventional therapy (LMWH followed by VKAs). Major bleeding favoured apixaban therapy. A recent meta-analysis also indicated the direct oral inhibitors were as effective and safe as conventional (VKA based) treatment for prevention of VTE in cancer patients. However, the standard of care in cancer VTE is long term LMWH, not VKA, and there are no randomised trials to date comparing LMWH with direct oral anticoagulants in cancer. Thus while use of the oral agents may appear attractive, data is lacking with regards their role in cancer and ASCO guidelines do not recommend use of these agents in cancer related thrombosis. Clinical trials are still needed to address this question.

## **182. Our Achilles heel: Graft versus host disease**

**Tierney K**

*Stanford School of Medicine, Stanford, CA, USA*

Acute graft versus host disease remains a significant cause of both morbidity and mortality following allogeneic transplantation. This overview will cover the incidence, risk factors, pathophysiology, prognosis, prevention and treatment. An emphasis will be placed on nursing management of the individual with acute graft versus host disease.

## **183. Chronic Graft versus Host Disease: getting to the heart of the matter**

**Panek-Hudson Y**

*Peter MacCallum Cancer Centre, Melbourne, VIC*

Complications associated with chronic graft versus host disease (cGvHD) continue to be the leading cause of significant morbidity and late mortality post allogeneic transplantation. Despite internationally recognized consensus guidelines on the management of cGvHD there remains a dearth of powerful evidence for most available treatment options. Consequently patients endure protracted treatment with cortico-steroids in addition to second and third line immunosuppressive agents resulting in both treatment and disease related morbidity.

Consensually accepted manifestations of cGvHD are graded according to degree of organ involvement and dysfunction. Opportunistic infection is common necessitating a broad approach to prophylaxis and treatment, once again contributing to toxicity.

Utilising case histories and personal accounts this paper will identify and discuss the impact living with cGvHD has on person and family.

## **184. What lies beneath? Caring for people living with Cutaneous Lymphoma**

**Buelens O**

*Peter MacCallum Cancer Centre, Melbourne, VIC*

Cutaneous Lymphoma is a rare condition requiring multidisciplinary collaboration with clinical and pathological correlation. The disease can masquerade as a number of non malignant skin conditions adding to the diagnostic dilemma of Cutaneous Lymphoma. Seventy five percent of all cases of patients with Cutaneous Lymphoma are diagnosed as having CTCL (Cutaneous T Cell Lymphoma) and the remaining have Cutaneous B Cell Lymphoma. Both patient groups experience different symptoms, challenges and treatments related to their dermatological and haematological presentation.

The average number of years taken for a patient with CTCL to be diagnosed is three years. Many patients experience frustration and distress related to the complexity of their disease. As health professionals caring for patients with Cutaneous Lymphoma we are faced with the ongoing challenge of ensuring patients receive consistent, evidence based care in an evolving and dynamic field.

Patients frequently face challenges of living with a chronic skin condition with a haematological overlay. There are a myriad of complexities these patients face including skin pain, pruritus, chronic wounds and infective complications. What lies beneath challenges us as health professionals to look at the whole person taking into account the haematological and dermatological aspects of this patient group. Look at the skin, look at the blood, and look at whole situation including psychological effects. Many patients living with Cutaneous Lymphoma are faced with the challenge of living with a disfiguring disease with significant alteration in their body image.

Symptom management strategies for patients with various types of Skin Lymphoma will be addressed in this presentation. The role of the Nurse Practitioner and the importance of collaborative, multidisciplinary care will also be discussed in this session.

## **185. Setting up a SCIg Program in a Regional Health Service**

Hollis L, Morwood K, Lambooy C

*Sunshine Coast Hospital and Health Service*

### **Aim**

To implement a Subcutaneous (SCIg) Program within the Sunshine Coast Hospital and Health Service (SCHHS).

### **Method**

SCIg (Subcutaneous Immunoglobulin) was approved for use in Australia by the National Blood Authority on March 1st 2013 for patients with primary and secondary immunodeficiency. Administration of immunoglobulin by the subcutaneous route in the home environment is advantageous for both the patient and health service. It reduces patient admission episodes, adverse reactions, produces more stable blood levels and thus decreased infective episodes.

In September 2013 the SCHHS Immunologist, Senior Medical Officer in Haematology and Transfusion CNC approached the Blood Management Committee for approval to submit a Briefing Note to the Executive Leadership Team to implement a SCIg program within our health service.

Consultation with key stakeholders was undertaken in October and November 2013. A cost benefit analysis and a literature review were also undertaken. The Briefing Note was submitted to the Patient Safety and Quality Committee and the Executive Leadership Team in December 2013 and subsequently endorsed.

### **Result**

The Transfusion Clinical Nurse Consultant co-ordinated the implementation of the SCIg program in January 2014. A Training Day was undertaken in January involving 20 key medical, nursing and laboratory staff. Representatives from the product manufacturers also attended.

The first patient commenced SCIg training on the 17th March 2014. The SCHHS currently has recruited 14 adult Immunology and Haematology patients onto the SCIg program using a variety of infusion methods including "push" (7 patients), NIKI Pumps (5 patients) and Springfusers (2 patients). Training sessions range from 1 session to 4 sessions based on patient confidence and competence. A data base has been established to monitor patient outcomes.

### **Conclusion**

The SCHHS was the first health service in QLD to implement a home based SCIg

## **186. Identification of the factors that influence adherence to oral chemotherapy in adults with cancer**

**Jar W**

*Canterbury District Health Board*

### **Aim**

To identify the key factors that influence oral chemotherapy adherence in adults with cancer which will inform clinical practice and future research opportunities

### **Method**

An integrative literature review was undertaken to determine these factors. Using key search terms and limiting the search from January 2002 – May 2012, 244 potential articles were sourced. Once search limitations were applied, a total of 82 articles warranted further scrutiny. After reviewing these articles against eligibility criteria and the Joanna Briggs Institute (JBI) critical appraisal tools, 19 articles progressed through to data analysis.

### **Result**

Within the literature, a total of 103 factors that influence adherence to oral chemotherapy were identified. These factors were then categorised into 14 themes and synthesised further into 3 major findings: patient related factors; treatment related factors and health provider related factors. Key reasons for non-adherence included adverse effects/toxicities, forgetfulness, the need to modify their life style and an inadequate therapeutic relationship with their health care provider.

### **Conclusion**

Nurses have a pivotal role in supporting patients receiving oral chemotherapy. This includes providing education, ensuring timely and accurate communication, and aggressive management of any adverse effects/toxicities and given the life-long commitment of some of treatments, promoting individual self-efficacy.

## **187. The beginnings of a new cancer day hospital in a regional setting**

**Whelan G, Wenta E**

*Icon Cancer Care, Townsville, Queensland, Australia*

### **Background**

Icon Cancer Care is Australia's largest private provider of cancer care, managing more than 75,000 patient visits each year across six day hospitals, with the support of more than 70 doctors. It is well documented that cancer patients living in regional areas often experience poorer outcomes than those in metropolitan areas. Icon Cancer Care Townsville is the first private stand-alone dedicated cancer day-only service offering patients in regional North Queensland the same treatment opportunities as those in metropolitan areas. The new day hospital incorporates an experienced team of haematologists and medical oncologists working alongside pharmacy and pathology services, and specialised oncology nurses.

### **Aim**

To assess the success and learnings related to opening a brand new cancer care service in a regional setting.

### **Method**

Six months after the opening of the day hospital, a staff satisfaction survey was conducted across medical, nursing, pharmacy and administration services. The survey gathered information regarding role transition, job satisfaction, professional support and development, and overall service improvements. The survey was anonymous and voluntary. Data was collected over a two week period.

### **Results**

A total of 31 surveys were distributed. 100% response rates were observed from pharmacists and nurses and 75% response rates from doctors, practice management and clerical employees. Analysis of staff responses highlighted greater job satisfaction across all service delivery arms and an overall improvement in private cancer services in a regional setting.

### **Conclusion**

This review offered the opportunity for all staff to reflect on the challenges and rewards experienced with the opening of the new cancer care service. Overall, the survey demonstrated that all staff were highly satisfied with their new roles and the ease of transition into these roles. In addition, the survey highlighted greater access to professional development, support and career opportunities that were previously not available in North Queensland. Cancer patients living in regional North Queensland now have access to a purpose designed, modern, specialised cancer service that is consistent with cancer service offered in the metropolitan areas.

## **188. The JumpStart Pilot Program- Overcoming the challenges of re-engaging in everyday life after a blood cancer diagnosis: Using a Self-Health Management Approach**

**Smith A**

*Leukaemia Foundation of Australia*

### **Aim**

Re-engaging in everyday life after a blood cancer diagnosis can be filled with challenges including complex fatigue, lack of energy and decreased cardiovascular fitness. The JumpStart program was designed to empower people to re-engage in their everyday lives using principles of self-health management.

### **Methods**

Participants in Victoria and Tasmania (n=18) responded to expressions of interest advertised at the Leukaemia Foundation. Parameters included 18+ years of age, disease remission and written medical clearance from a GP. Those having active treatment or unstable disease were excluded.

Participants outlined their everyday goals using the Canadian Occupational Performance Measure (COPM) and received individualised and tailored intervention from a supportive care coordinator, occupational therapist, exercise physiologist and clinical dietician over a four month period. This informed the community based individualised self-health-management programs. Quantitative data collected included physiological measurements (VO2 Max, BMI) COPM performance, satisfaction scores and satisfaction surveys.

### **Results**

Results included average increases in COPM Performance scores of 3.28 and COPM Satisfaction scores of 3.92 (both clinically significant). Physiological changes included an average increase of 14% in VO2 max, no significant change in BMI and weight. The quantitative data supports the aim of using a Self-Health Management Approach in overcoming complex blood cancer fatigue allowing; participants to increase performance and satisfaction in their everyday lives.

### **Conclusion**

Programs that address the needs of the increasing blood cancer survivorship population need to be client-centred, sustainable and individually tailored. The JumpStart Program outcomes were achieved by using the existing roles of the Leukaemia Foundation support services coordinators and linking blood cancer survivors with appropriate community based health professionals and Medicare primary care schemes, which are currently available to the blood cancer population across Australia.

## **189. Patterns of initiation of broad-spectrum antifungal prophylaxis in patients with newly diagnosed acute myeloid leukaemia undergoing induction chemotherapy**

**Haywood P**

*Royal Melbourne Hospital, Melbourne, VIC*

### **Aim**

Delay in initiation of broad-spectrum antifungal prophylaxis (BSAP) potentially places patients with newly diagnosed acute myeloid leukaemia (AML) at risk of invasive fungal infections. We sought to examine the frequency of initiation of BSAP and identify any factors resulting in delay of its initiation.

### **Method**

We conducted a retrospective analysis of the medical records of all patients treated with induction chemotherapy for newly diagnosed AML in a single metropolitan haematology department during the past 18 months. Timing of administration of the first dose of BSAP was determined relative to the date of admission and the date of diagnostic bone marrow biopsy. Rates of use of computed tomography (CT), initiation of empirical antifungal treatment and probable or proven invasive fungal disease were also assessed.

### **Result**

The medical records of 32 patients were analysed. One patient with incomplete data was excluded. There was variability in the timing of first administration of BSAP. All patients had BSAP initiated. However, 8/31 (25%) patients had a greater than 5 day period (range -3 to 11) between the diagnosis of AML and the initiation of BSAP. An initial biopsy at another hospital, confirmation of AML diagnosis and decision over fitness to proceed with induction chemotherapy were found to be potential factors delaying initiation of BSAP. There were no identifiable patterns in this small patient cohort of increased fungal infections between patients relative to timing of initiation of BSAP.

### **Conclusion**

For a proportion of patients undergoing AML induction with curative intent, there are correctable factors which result in delayed initiation of broad-spectrum antifungal prophylaxis.

## **190. Severe case of Steven Johnson syndrome caused by co-trimoxazole in an autologous stem cell transplant patient**

**Kenny N, Hollis T**

*The bone marrow transplant unit CDHB Christchurch New Zealand*

### **Background**

Steven Johnston Syndrome (SJS) is a severe allergic drug reaction, caused by any type of drug. Majority of cases proceed with flu like symptoms and high temperatures, eventually affecting the mucous membranes, eyes, skin and genitalia develop erythema, oedema and target lesions.

### **Case**

Mr X is a 61 year old male who presented 28 days post his autologous stem cell transplant for Multiple myeloma. Mr X presented with symptoms of; fever, lethargy, nausea and vomiting, rash his body. A diagnosis of SJS was made in relation to a severe drug reaction to Co-trimoxazole. Mr X developed facial oedema, went into urinary retention, had severe diarrhoea and the rash spread all over his body, with some blistering. An indwelling catheter and a rectal tube were inserted as Mr X had become significantly fatigued and unable to control his bowels. Topical steroids, creams and dressings were applied to various areas on Mr X's rash to keep the skin moist and prevent further blistering. Mr X had no appetite and was commenced on total parental nutrition, nasal gastric tube was inserted and feeding commenced along with intravenous (IV) fluids. IV antibiotics and antifungals were commenced to help with fevers. Eye drops were added to prevent irritation as well as antihistamines. The Intensive care outreach team was involved with Mr X and implemented changes when required. He was also found to have CMV of the gut following a colonoscopy and biopsy, which had caused the severe diarrhoea. Ganciclovir was then commenced. Mr X made slow improvements weekly. The erythema/ rash on Mr X's body improved slowly, but his skin was left dry and fragile.

### **Conclusion**

SJS is a rare disease that can occur due to any reaction to a particular drug. In Mr X's case his was severe but through the use of topical steroids and creams and keeping his skin moist as well as the use of antibiotics, antifungals, fluid replacement and feeding tubes significantly improved Mr X's condition.

## **191. Does parenteral nutrition increase the risk of catheter-related infection? Does the evidence reflect current practice guidelines?**

**Gavin N**<sup>1,2</sup>, Keogh S<sup>1,2</sup>, McMillan D<sup>3</sup>, Rickard C<sup>1,2</sup>

<sup>1</sup> *Royal Brisbane and Women's Hospital*, <sup>2</sup> *Griffith University*, <sup>3</sup> *University of the Sunshine Coast*

### **Introduction**

Parenteral nutrition (PN) is associated with catheter-related infections (CRIs). Clinicians generally refer to clinical guidelines rather than original studies. Our aim was to critique current evidence and assess consistency with practice guidelines.

### **Method**

Journal articles that compared CRIs in (i) central venous access devices (CVADs) for PN and non-PN administration, (ii) single or multiple lumens CVADs, (iii) the configuration of intravenous administration sets (IVAS) and (iv) the frequency of IVAS changes for PN administration were reviewed using systematic methodology and compared to practice guidelines.

### **Results**

Twenty four papers met the selection criteria and found (i) CRI was doubled in groups receiving PN (12.8% with PN vs 6.3% without PN), (ii) CRI was 3-fold higher in patients with a multiple lumen CVAD (9.1% multiple lumen vs 3.9% single lumen catheter), (iii) there was a 5-fold increase in CRI with a multiple use multiple lumen CVAD (33.8% multiple use multiple lumen CVAD vs 7.0% multiple use single lumen CVAD or 5.7% dedicated lumen on multiple lumen CVAD) and (iv) there was a 5-fold increase in CRI when IVAS were changed more frequently (33.4% daily replacement vs 16.5% 2 day vs 6.3% 4 day change).

### **Conclusion**

The literature highlights PN infusion as a risk for CRI. Guideline recommendations to use a single lumen CVAD or dedicated lumen for PN administration on a multiple lumen CVAD are consistent with current evidence. Research favours less frequent IVAS changes whereas the guidelines suggest changing IVAS every 24 hours if they contain lipids. It is difficult to draw definitive conclusions as studies did not use a consistent definition of CRI, and some are of retrospective design or use per catheter rather than per patient analyses. Well designed randomised controlled trials are needed to answer many questions regarding PN as a risk factor for CRI.

## **192. A sudden death d+118 allogeneic stem cell transplantation: A palliative care case study**

**Button E**

*Queensland University of Technology / Royal Brisbane & Women's Hospital*

### **Aim**

To critically reflect and evaluate the end-of-life care of AM, a young patient who experienced a sudden death post transplant.

### **Methods**

A case study approach will be used to evaluate the holistic care of AM and his family. The 5 domains of palliative care will be incorporated in the review of this patient and include: 1) pain and symptom management, 2) advance care planning, 3) carer support and bereavement, 4) continuity of care and, 5) terminal care.

### **Case study**

AM had a history of AML, treated with an unrelated allogeneic stem cell transplant. He developed stage IV graft versus host disease of the gut for which he required a lengthy hospital admission.

AM was unmarried, of Indonesian cultural heritage, and had a strong Catholic faith. He lived at home with his parents and his mother was his full time carer.

AM presented to the Department of Emergency via ambulance on the 23/02/10 23:58 in advanced neutropenic sepsis. His mother reported he had spiked a temperature the previous day but had adamantly refused to come into hospital. On presentation he was haemodynamically unstable and was in respiratory distress, for which he was intubated and started on inotropic support. He was transferred to ICU, commenced broad-spectrum antibiotics and transfused with blood products as necessary.

Despite optimal intensive care support and full active medical treatment, AM deteriorated further. His family were informed of his grave prognosis and he died in the intensive care unit on the 24/02/2010 at 18:50.

### **Results**

This case study will discuss the end-of-life care of AM and the circumstances around his decision not to come into hospital. Suggestions will be made on how we as nurses can improve the holistic care for patients that are at high risk of deteriorating and dying.

### **Conclusion**

Early identification of patients at risk of dying may have the potential to improve end-of-life care in the haematology setting.

## **193. Can mindfulness help haematology nurses improve self and patient care? A systematic review of the literature and exploration of the implications for nurses.**

### **Weeks R**

*Leukaemia & Blood Cancer New Zealand*

### **Background**

'Mindfulness' is a learnt technique that encourages people to pay more attention to every moment; both the physical experience and the emotional response. Benefits to cancer patients are well established, with reduced anxiety, depression and stress most commonly reported. Emerging literature suggests 'mindfulness' may be valuable to healthcare professionals in improving self and patient care.

### **Aim**

To identify what is known about the benefits of 'mindfulness' in a nursing population, and to explore its potential for nurses' self-care and improved care for haematology patients.

### **Method**

A literature search of the Cochrane Library and PubMed databases was conducted to identify studies and review articles describing the use of 'mindfulness' in a nursing population. Only articles in English explicitly discussing and defining 'mindfulness' and its application to general nursing or healthcare professionals working within oncology were included.

### **Results**

Ten out of 88 papers met all the criteria for inclusion: 7 randomized controlled trials, 2 reviews, and 1 evaluation study.

Main outcomes impacted by 'mindfulness' were stress, anxiety, burnout, compassion, focus, self-awareness, and relationships. Outcome measures were not consistent across all studies.

Three studies showed improvements for nurses in symptoms of burnout, increased relaxation and well-being, 3 reported reduced stress symptoms, whilst 2 reported no significant improvement in depression. Two reviews showed 'mindfulness' enhancing self-care and equanimity, particularly during highly charged conversations. One study showed improved healthcare professional responses to stressful situations along with a positive impact on patients' quality of life.

### **Conclusion**

'Mindfulness' among a nursing population can reduce stress and anxiety, improve compassion, self-awareness and personal responses to stressful situations. Its potential to improve therapeutic relationships and patient outcomes, although not robustly established, is evident and requires further research.

Implications for practice: This presentation will synthesize available research findings, and provide practical examples as to how 'mindfulness' can be implemented in practice to manage self-care and improve patient care.

## **194. Montelukast as combination therapy for pulmonary GvHD - a case series**

**Panek-Hudson Y**<sup>1</sup>, Lingaratnam S<sup>1</sup>, Ritchie D<sup>1&2</sup>, Manser R<sup>1&2</sup>

<sup>1</sup> Peter MacCallum Cancer Centre, <sup>2</sup> Royal Melbourne Hospital

### **Aim**

To describe a single-centre, case series experience of using Montelukast, a leukotriene receptor antagonist, in combination therapy for pulmonary graft versus host disease (GvHD). Combination therapy includes Fluticasone, Azithromycin, corticosteroids and other immunosuppressive medication.

### **Method**

Using pharmacy records 10 patients treated with Montelukast for pulmonary GvHD for a minimum of 12 months were identified. Clinical records were examined for disease and treatment-related details. Pulmonary function tests (PFTS) including spirometry and DLCO, and average daily dose of immunosuppression were compared at baseline, 3 months, 6 months and 12 months post commencement of Montelukast. Relevant treatment related adverse effects were also identified from clinical records and documented.

### **Results**

The Montelukast audit captured a 12-month time period during which 10 patients were commenced on Montelukast. All patients were prescribed the recommended daily dose of 10mg. All patients were referred to a respiratory physician for review. The median duration of follow-up was 15 months (range 9-36 months). At the end of the audit period all patients remained on Montelukast. In this single centre, case series experience Montelukast was a well-tolerated treatment for pulmonary GvHD as part of combination therapy. In the 10 patients identified there was no change (improvement or deterioration) in PFTS and there was an overall trend towards reduced corticosteroid dosage.

### **Conclusion**

Montelukast as part of combination therapy for pulmonary GvHD, as demonstrated by this case series was well tolerated and potentially contributed to stabilisation of PFTS and a reduction in corticosteroid dose.

## **195. Implementing Paediatric Treatment Protocols in the adult haematology setting - the why & how**

**Plaster, M**

*WA Youth Cancer Service, Perth, WA*

Adolescent and Young Adult (AYA) patients with malignant illnesses continue to demonstrate poorer outcomes compared with paediatric and adult populations. A growing body of evidence demonstrates improved outcomes for AYAs with haematological malignancies treated with protocols utilising paediatric treatment principles (1-4). Despite these findings, however, there continues to be a delay in uptake and implementation of these treatment approaches.

Factors purported to influence implementation of paediatric-type regimens include complexity of protocol design, dose intensity, use of haematopoietic stem cell transplants and awareness of age-specific disease biology. Treatment adherence and access to psychosocial and supportive care for this unique age cohort may also influence outcomes.

This multidisciplinary-themed workshop explores opportunities for improving outcomes for AYA patients through the implementation of paediatric treatment protocols. Presenters will summarise the current evidence pertaining to treatment for AYA haematological malignancies, the roles and benefits of specialised nursing, age-appropriate psychosocial support, expert symptom management and supportive care.

The national network of Youth Cancer Services exist to provide age-appropriate medical, nursing and psychosocial support and to improve outcomes for AYAs with cancer. Their role in supporting young patients and their treating team to provide these more intense regimens is discussed.

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## **196. Implementing patient blood management initiatives**

**Campbell L**

*Sir Charles Gairdner Hospital, Nedlands, WA*

Evidence regarding transfusion costs, efficacy and safety are compelling arguments for change, but without a co-ordinated approach, the implementation of the Patient Blood Management (PBM) program can be fraught with difficulties. To date, much of the focus of PBM programs has been with peri-operative optimisation of surgical patients, but there are notable transfusion reduction gains being made in non-surgical patients too. A hospital wide introduction of a single unit policy coupled with a revision of the Transfusion Prescription form has produced surprising results in all patients including haematology patients. The implementation of a new program requires a collaborative team approach with clear communication supported by education, policies and resources to help guide and embed transfusion practice changes.

## **197. The patient experience with transfusion and the consent process**

**Craven M**

*Royal Perth Hospital, WA*

Blood Transfusion is a medical intervention which requires informed written consent by the patient prior to its administration. This consent process is to convey verbal and written information as to the potential benefits and risks related to transfusion. If there are alternatives to transfusing the patient, these too, are to be discussed.

A report was written following the comparison of seven different hospital sites across the Perth metropolitan area. This comparison related to the obtaining of valid consent and the patient understanding of their transfusion. Method was by way of a survey completed by each patient or by the auditor with the patient. A minimum of ten patients per site were involved.

Results from the report suggested that whilst written and verbal information is made available to the patient, it is not always well understood. Understanding was variable between the chronically transfused and those who had not had a previous transfusion. Comments from some patients demonstrated a lack of awareness as to alternatives to transfusion. There was variation between the seven sites as to the products transfused, adherence to consent compliance and understanding of what transfusion involved.

The report findings indicate that there may be gaps in the patient understanding of the transfusion process. Improvements could be made to ensure that consent is well informed and if possible, alternatives considered. Further surveys to provide greater sample numbers will provide more evidence to clarify these findings.

**198. Investigation of 1 versus 2 units RBCS in a haematology inpatient unit**

**P'ng S**

*Royal Perth Hospital, Perth, WA*

**ABSTRACT NOT SUBMITTED**

## **199. A tale of two marrows - a panel based complex case presentation**

**Sipavicius J<sup>1</sup>, Panizza N<sup>2</sup>, Penhale J<sup>2</sup>, Avery S<sup>3</sup>, Kelly A<sup>4</sup>**

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Allogeneic hematopoietic stem cell transplantation (HSCT) remains the optimal curative therapy for many patients with hematological disorders. In adults, acute myeloid leukaemia (AML) represents the most common indication for allogeneic HSCT. For the majority of patients with AML, the post remission leukaemia-free survival remains poor when chemotherapy is used alone. For eligible patients with a suitable donor, allogeneic HSCT is the most widely used therapy post remission in AML, and remains the optimal curative therapy for this patient group. However the benefit in survival can be offset by the complications associated with the treatment, rendering significant stress and uncertainty in transplant outcomes and the overall success of HSCT. An essential role of the multidisciplinary BMT team is to provide support and assist patients' and their families' biopsychosocial needs during these stressful circumstances.

This case presentation will explore the complex nature of one patients' unique journey with AML undergoing an allogeneic sibling HSCT. The clinical case will be presented alongside the personal experiences of the patient as he undergoes HSCT, and the associated acute and chronic complications. The session will utilize an expert panel of nursing, medical and allied health specialists to help deconstruct and explore the adverse effects, toxicities and psychosocial effects of HSCT whilst proposing examples of best supportive care.

There will be a focus on patient considerations pre HSCT; medication management with an emphasis on immunosuppression and anti-infective therapy; graft versus host disease; psychosocial impact of undergoing HSCT and coping with uncertainty.

Objectives:

It is anticipated that this session will allow for participants to better appreciate and identify the key issues HSCT recipients undergo, and to develop skills and strategies to help assist patients and their families to manage these needs throughout the prolonged trajectory of HSCT.

## **200. The Australian ATHOME[™] infusion service experience**

**Brown M**<sup>1</sup>, Fraser N, Georgiou P, Bischoff C, Agnew R

<sup>1</sup> Royal Melbourne Hospital, Melbourne, VIC, Australia, <sup>2</sup> Ascott Sales Integration, Crows Nest, NSW, Australia, <sup>3</sup> Royal Adelaide Hospital, Adelaide, SA, Australia, <sup>4</sup> Shire Australia, North Ryde, NSW, Australia

### **Aim**

To evaluate the Shire funded ATHOMETM infusion service for eligible Australian patients prescribed intravenous agalsidase alfa ghu (REPLAGAL®) (AAG) or velaglucerase alfa ghu (VPRIV®) (VAG) for Fabry and Gaucher Disease respectively. ATHOME enrolment is organised by treating physicians for patients after a minimum 12 AAG or 3 VAG in hospital infusions.

### **Method**

The ATHOMETM Program Coordinator arranges an IV administration trained registered nurse to deliver, prepare, administer and monitor infusion safety in the home or workplace. Physicians receive written reports after each infusion. Records of infusion timings, retention rates and patient numbers are collated by the nurses and managed by the ATHOME Coordinator.

### **Result**

ATHOMETM commenced in Australia July 2010 for AAG patients. In May 2013 it was extended to VAG patients. Total enrolments to 28 February 2014 are 30 AAG and 12 VAG patients. Patient retention to ATHOME over the length of the program has been 86.7% and 75.0% with an adherence of 97.9% and 98.1% of planned infusions administered, 89.7% and 86.9% delivered within 2 days of due date for AAG and VAG respectively.

### **Conclusion**

ATHOMETM infusion service successfully offered enrolled patients the convenience and flexibility to receive treatment in their home or workplace environment with high adherence.

## **201. There's no place like home! A nurse-lead ambulatory program to manage neutropenic fever**

**Joyce T**<sup>1</sup>, Thursky K<sup>1,2,3</sup>, Teh B<sup>1,2</sup>, Brown C<sup>4</sup>, Byrne J<sup>4</sup>

<sup>1</sup> *Peter MacCallum Cancer Centre*, <sup>2</sup> *Royal Melbourne Hospital*, <sup>3</sup> *University of Melbourne*, <sup>4</sup> *The Western & Central Melbourne Integrated Cancer Service*

### **Aim**

Neutropenic fever (NF) is a significant complication associated with chemotherapy. The Multinational Association of Supportive Care in Cancer (MASCC) risk index is a validated tool that can predict a patient's risk of significant medical complications associated with their NF presentation. Patients screened and identified as 'low-risk' on MASCC can be safely managed in an ambulatory setting. We outline the establishment and progress of a new standard of care in a tertiary cancer centre.

### **Method**

A 'Low-Risk' NF ambulatory program was established at Peter Mac in March 2014. An educational blitz was undertaken to increase the knowledge of staff around the MASCC risk tool, patient eligibility criteria regarding oral antibiotic switch and discharge to ambulatory care. Nurses lead the program and accept referrals directly from medical colleagues. Patients entered into the program have their care delivered by 'hospital-in-the-home' (HITH) nurses. The HITH nurses visit on Day 1 (Day 0=day of discharge) and Day 2 and +/- Day 4 pending neutrophil recovery. Pathology results are reviewed by a nurse consultant and the treating team are kept updated. All patients discharged to the ambulatory program have a face-to-face clinical review with the nurse consultant 5 to 7 days following their episode of NF.

### **Results**

Eight patients have entered the program. The program is in its infancy stages but preliminary data indicates a reduction in median length of stay for 'low-risk' NF patients by approximately 3 days.

### **Conclusion**

The nurse-lead ambulatory program is a new paradigm shifting inpatient care to the comfort of the patient's home. This program utilises the advanced skill set of specialist nurses to facilitate patient centred care in a safe and well supported environment and freeing inpatient beds for patients with more complex needs.

## **202. The role of the nurse practitioner/nurse practitioner candidate for recipients of autologous transplantation**

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<sup>1</sup> Royal Adelaide Hospital, Adelaide, South Australia, Australia, <sup>2</sup> SA Pathology, Adelaide, South Australia, Australia

### **Aim/background**

The haematology nurse practitioner role has a strong focus on supportive care throughout a patient's cancer journey, achieved by providing high quality, patient focused care, in collaboration with the multidisciplinary team. Autologous transplant recipients were identified as a target population for the practice of this role. During the recovery post autologous transplant patients may experience complications including mucositis, febrile neutropenia, nausea and diarrhoea. The emotional impact of an autologous transplant may not end upon count recovery and patients may continue to experience symptoms including lethargy, nausea and anorexia. Patients may also have psychosocial concerns surrounding financial and employment situations.

### **Method**

The nurse practitioner candidate (NPC) is involved with the patient's journey from the day of admission for transplant. The NPC performs weekday reviews for patients who receive an inpatient transplant. The post autologous transplant clinic (PATC) has been led by the NPC since 2012. Patients who receive an autologous transplant are referred to the PATC upon discharge from the ward or home transplant program. The patient is reviewed by the NPC for one month before returning to their primary haematologist.

### **Result**

Since clinic implementation, 120 patients have been reviewed. This has released 120 medical review appointments for other acute care patients by diverting clinic workload. Patients have a central point of contact and continuity of care has increased. Disease reassessments are ordered if required, assisting future treatment decisions to be made. The NPC is able to provide psychosocial support. Patients receive personalised information regarding long term health maintenance and screening practices

### **Conclusion**

The implementation of the autologous NPC role improves continuity of care and addresses the patient's physical and psychosocial needs. The NPC will continue to provide comprehensive care to recipients of autologous transplantation, in collaboration with the haematologists.

## **203. Extended nurse practice roles in Haematology: groundwork for changing practice**

**Taylor S**

*Royal Prince Alfred Hospital*

### **Background**

Advanced practice or extended scope of practice for nurses is now widely adopted in healthcare where there is clear evidence of benefit to patient care. The Haematology Department identified the benefits of nurse performed bone marrow aspirate and trephine (BMAT). The development of evidence based and clearly articulated procedural, educational and clinical assessment guidelines, would be critical prior to implementing a change in scope of practice. This paper will describe the preparation phase for implementing extended scope of practice for Clinical Nurse Specialists (CNS) in BMAT.

### **Aims**

To implement nurse performed BMAT.

To develop an evidence-based education training program to guide the implementation of nurse performed BMAT. (Focus of paper)

To evaluate the acceptance of, and patient outcomes relating to, nurse performed BMAT.

### **Method**

A systematic approach was taken including engagement with key clinical and managerial stakeholders providing expert guidance on content and processes to support practice change. In line with the Cochrane Collaboration Standards, a systematic search strategy was developed and completed using electronic databases (PubMed, Cinahl, Medline). In addition, key grey literature related to BMAT procedures, risk factors and adverse events were examined. Key authors identified in the review were contacted for additional expert input into the findings. Evaluation of clinical indicators such as infection rates, performance measures, quality of slides, patient experience and competency measures were assessed.

Key findings from the review:

Nurse delivered BMAT can improve pain management, patient experience, quality of care and decreased waiting times. A lack of educational training programs exists leading to the development of a local BMAT program.

Implications for practice

With the rising number of advanced practice nurses and therefore the desire to expand the scope of practice, there is a requirement for educational programs to be able to support this expanded practice in order to be able to meet evidence-based practice. It is hoped upon implementation and ongoing evaluation; we will be able to demonstrate the benefit of implementing a robust program supporting expanded practice.

### **Key Words**

Bone marrow biopsy; advanced practice nurse, evidence-based practice

## **204. Matched Unrelated Donor (MUD) selection**

**Jackson, S**

*Bone Marrow Donor Centre, Perth, WA*

Each year, many Australians are diagnosed with leukaemia or other fatal malignancies, with a bone marrow or haemopoietic stem cell (HPC) transplant offering their only chance of a cure. Whilst siblings are the ideal donors for a patient in need of a transplant, only one in three patients will find a matched donor within their family. The remaining patients rely on the Australian Bone Marrow Donor Registry (ABMDR) or other international registries to find a suitable match.

The Bone Marrow Donor Centres (BMDCs), located in 5 separate states across Australia, function as the donor liaison arm of the ABMDR, facilitating the matching of unrelated donors with patients both nationally and internationally. The BMDCs are fully funded by state government to coordinate the recruitment, collection of haemopoietic stem cells and post donation health care of a MUD for a patient in need of a transplant.

This presentation will examine how MUD are selected and the processes through which HPC donation is facilitated, including:

- History of registries;
- Donor recruitment and enrolment: who is the 'ideal' donor, methods of recruitment, donor eligibility and consent
- Verification stage: typing, counselling, assessment, blood sample collection and consent
- Donor Work-Up to Donation: collection plan, information and counselling, assessment, G-CSF administration, consent and clearance
- Collection of HPCs: peripheral blood stem cells and bone marrow
- Follow-Up: donor and patient
- The future of matched unrelated donors
- Questions and open forum

Funding: State governments fund the Australian Red Cross Blood Service for the services provided by the BMDCs nationally

## **205. Around the Apheresis world in 30 days**

**Brehaut E**

*Royal Adelaide Hospital, Adelaide, SA*

Recipient of the South Australian Premiers Nursing and Midwifery Scholarship, Eva Brehaut, shares her observational findings from her recent scholarship tour. Visiting hospitals within Australia, Canada and the United States of America, both clinical procedures and staffing methodologies were examined. This talk will focus on findings related to LDL column apheresis, Photopheresis and Red Cell Depletion Exchange. A brief review of some of the differing staffing models encountered will also be discussed.

## **206. Stem cell therapy into the clinical arena**

**Sturm M**

*Royal Perth Hospital, Perth, WA*

Mesenchymal stromal cells (MSC) are one of the fastest growing areas of stem cell research. MSC are multipotent cells with ability to differentiate into different tissue types. They secrete cytokines, chemokines and growth factors to support haemopoiesis and have immunosuppressive and immunomodulatory activity, homing to affected tissue and controlling inflammatory and immunological reactions locally. Consequently, MSC have broad therapeutic potential, particularly in the immune disorder and transplantation settings and for tissue regeneration. Their hypo-immunogenicity and inability to elicit an immune response means they are universal donor cells and, therefore, MSC therapy can be readily available using allogeneic cells.

Cell & Tissue Therapies WA (CTTWA) at Royal Perth Hospital is a TGA licensed biotherapeutic manufacturing facility which has been developing the clinical manufacture of allogeneic bone marrow derived MSC since 2007. Safety and efficacy of the therapy is being evaluated through multiple clinical trials in various clinical settings. CTTWA obtained a TGA license to manufacture MSC in 2013. Initially MSC manufacture was developed for seriously ill patients with steroid refractory graft versus host disease (GVHD) associated with bone marrow transplantation. Results from the Phase I study were positive, with an improved outcome for patients and no therapy related adverse events observed. The study has progressed to a randomized Phase 2 clinical trial of naïve GVHD. Our MSC therapy is also under evaluation at multiple Australian sites in other medical conditions including refractory Crohn's disease, acute and chronic solid organ rejection, ischaemia-reperfusion injury of kidney transplant and in chronic obstructive pulmonary disease. Generally, cryopreserved cells are thawed and delivered by infusion through peripheral access. Patients receive  $2 \times 10^6$  cells/kg (patient weight) per infusion and, depending on the trial protocol, may receive 2 or four infusions. MSC therapy appears to be safe with over 500 infusions of CTTWA-MSC performed and no serious adverse events observed. Our first foray into tissue regeneration (bone) is for patients undergoing cranial reconstruction, where the allogeneic MSC are attached to tri-calcium phosphate beads and implanted between scaffolds of polyvinyl lactate sheets, custom molded to exactly fit the cranial void.

The accumulating evidence from our studies with allogeneic, bone marrow derived MSC demonstrates that the cell therapy is safe and has the potential to become a therapeutic option in multiple clinical conditions.

## **207. Nurses Workshop 1: Meaningful measures; the why and how of KPI's and clinical indicators**

**Convery A**

*Oars Across the Waters, Nedlands, WA*

Inevitably, we all come into contact with performance measures in the workplace at some time in our career. We may be involved in the development of indicators, or associated with the collection of data, or working at a senior level, using indicators to inform our business decisions. With performance indicators being so common in today's tight budgetary environment, one might think that they are well understood and easy to develop, implement and even collect! Personal experience has shown that this is often not the case, and that the complexities associated with clinical or performance indicators can be significant. In this workshop, we will explore some of these complexities, and discuss some of the practical issues surrounding meaningful measures.

## **208. Nurses Workshop 2: Nurse led clinics - the what, why and how**

**Jagger J**

*Central Coast Local Health District, NSW*

Nurse-led clinics (NLC) are changing the landscape of how healthcare is delivered. In Australia and internationally nurse-led care is now an integral part of healthcare services, which have had to adapt to the growing burden of cancer. A central element of nurse-led care is advanced practice. Though a nebulous term, advanced practice describes a set of specialist skills that nurses must have in combination with a discrete knowledge of the patient group the clinic is set up for.

In haematology we are witnessing the increasing overall survival of many patient groups such as myeloma, myelodysplastic syndrome and chronic myeloid leukaemia, undoubtedly related to the era of novel therapies and better supportive care. With patients living longer they continue to need regular health monitoring and assessment. In addition, there is the epidemiological challenge of Australia's aging population and the unique needs of the older person with cancer. NLCs are ideally placed to meet this challenge providing patient-centred care through health monitoring and assessment, arranging timely referrals to specialist services, and counselling on self-care and symptom management. NLCs create opportunities for further role development as experienced, specialist nurses may wish to extend their skills and scope of practice.

This session aims to explore the development of nurse-led clinics past and present. Australian examples, from a metropolitan and regional perspective, and a step-by-step approach towards setting up a nurse-led clinic will be presented. The discussion will focus on practical issues that need to be considered when setting up nurse-led services including engaging key stakeholders in the initiative, writing a business proposal, funding sources, skill acquisition and the development of a competency framework. Professional development within the NLC, using techniques such as clinical supervision, and reflective practice will be explored. And finally, a discussion of how to evaluate and measure the effectiveness of the NLC, its sustainability and succession planning.

**209. Nurses Workshop 3: Turning ideas into action, understanding audit, research and QI**

**White K**

*The University of Sydney, Sydney, NSW*

**ABSTRACT NOT SUBMITTED**

## **210. Nurses Workshop 4: Engaging nurses in transfusion education**

**Darby S**

*Sir Charles Gairdner Hospital, Nedlands, WA*

Deficiencies in transfusion knowledge can have an adverse affect on patient safety .

So, therefore it is important to ensure that medical and nursing staff who are involved in the transfusion process are provided with the relevant transfusion education.

The aim of this interactive workshop is to expose the participant to some of the different types of teaching methods that can be used to engage nurses in transfusion education instead of the usual didactic lecture.

The following teaching methods will be included in the workshop:

- Low fidelity patient simulation -Management of a transfusion reaction
- Objective structured clinical examination (OSCE) -Sample collection and labelling requirements
- 'Hands on' learning using The Australian and Red Cross Blood Service 'Pack Check" education resource – Checking procedure of the blood product
- Reflective Practice- debriefing

## **211. Making sense of managing myeloma: A pragmatic overview**

**King, T**

*Prince Alfred Hospital, Sydney, NSW*

The management of multiple myeloma (MM) has improved substantially as a result of advances in our understanding of the disease biology and improvements in treatment and supportive care strategies. A substantial number of new drugs and novel drug classes in early clinical development is expanding the treatment options for patients and resulting in improved overall survival (OS) (Ludwig et al 2014). Additionally it is now possible to categorize patients into different risk groups based on disease biology but also comorbidity and frailty allowing for a more tailored approach to therapy. Increased patient longevity is associated with notable symptom burden (Jordan et al 2013) and low health related quality of life (HRQOL) (Mols et al 2012).

The increasingly heterogeneous nature of myeloma the disease and its management has implications for nurses as they deliver care and inform patients and family members of an ever complex and individual treatment pathway, changeable outcomes and with increasing patients expectations.

This session will broadly explore the main focus of research in myeloma disease biology, newer targets for treatment and supportive care needs.

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## **212. What factors would influence whether a person with multiple myeloma would participate in a physical activity program?**

Hose K<sup>1</sup>, Craike M<sup>2</sup>, Courneya K<sup>3</sup>, Harrison S<sup>4,5</sup>, Livingston P<sup>2</sup>

<sup>1</sup> *The Leukaemia Foundation*, <sup>2</sup> *Faculty of Health, Deakin University, Burwood, Australia*, <sup>3</sup> *Behavioural Medicine Laboratory, Faculty of Physical Education and Recreation, E-488 Van Vliet Center, University of Alberta, Canada*, <sup>4</sup> *Cancer Medicine, Peter MacCallum Cancer Centre, East Melbourne, Australia*, <sup>5</sup> *Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia*

### **Background**

Current evidence suggests that exercise is safe and feasible for people with Multiple Myeloma (MM) and has a positive effect in alleviating disease and treatment – related symptoms and improving quality of life.

### **Aim**

The aim of this qualitative study was to gain an insight into what people with MM would prefer in the delivery of an exercise program in regards to structure, timing in the treatment trajectory, location and the integration of any perceived useful resources.

### **Methods**

Semi – structured interviews were conducted with people who were treated for MM in the preceding 2 – 12 months. Interviews were analysed using the constant comparison coding method. This method reduced data down to the main themes which were then explored.

### **Results**

Twenty – four interviews were conducted. The strongest preference for an exercise program was 2 – 8 months following treatment. Participants were interested in individualised programs guided by a health care clinician with knowledge of MM. The preferences for location and mode of delivery varied. Light to moderate exercise was preferred and information about physical activity was highlighted as a requirement but to reduce information overload it was suggested the information is provided following treatment.

### **Conclusion**

The delivery of an exercise program for people with MM needs to take into account the varied preferences in relation to the location, structure and type of activity that is feasible. The findings suggest that a targeted program facilitated by clinicians and organisations that have experience and expertise with MM would be successful. There also needs to be options for home based exercise as treatment for MM is ongoing and regular hospital appointments may make it difficult to commit to a program. A home based program may also help meet the needs of people with myeloma living in remote regions.

## **213. Results of an exploratory study of the care experiences of patients diagnosed with myeloma**

Houdyk K <sup>1</sup>, Prince M <sup>2</sup>

<sup>1</sup> Myeloma Foundation of Australia Richmond, Victoria, Australia, <sup>2</sup> Peter MacCallum Cancer Melbourne, Victoria, Australia

### **Background**

Myeloma is an incurable blood cancer that predominately affects the elderly. Developments in the treatment of myeloma have resulted in better disease outcomes for some groups but survival has not improved for older patients.

### **Aim**

To explore the unmet physical and supportive care needs of non-transplant eligible patients diagnosed with myeloma. Primary objectives are:

To examine patients' experience of care from diagnosis to 6 months post, and

To identify gaps in service provision from the perspective of treating clinicians and GPs.

### **Method**

20- 30 newly diagnosed or relapsed myeloma patients to be recruited. Patients completed a series of validated measures at T1 (3- 6 weeks post commencement of treatment) and T2 (8-12 weeks post T1). Measures report on QOL, emotional wellbeing, medication adherence and supportive care needs. At six months patients participated in an audio tapped interview to explore their experience of living with myeloma. Patients' treating specialist and GPs participated in a tapped telephone interview to explore their perceptions of care provision for patients with myeloma.

### **Results**

Of the 20 patients referred 10 completed the study. 4 declined to participate, 4 passed away and 2 withdrew. Key findings include:

Patients Global Health Status improved over time (T1 m=56.5, T2 m=62.1). Symptomology scores varied between patients.

Anxiety scores drop over time (T1 m=5.2, T2 m=3.9).

High levels of medication adherence reported.

Need for more information on disease and treatment is greatest at T1. There was limited engagement with support care services.

Key findings of audio tapped interviews include:

Patient interview themes include faith in specialist, role adjustment and navigation of the health care system.

Challenges perceived by specialist are co-morbidities, medication adherence, and treatment toxicities.

GPs report poor disease knowledge.

### **Conclusion**

Findings suggest patients and specialist appear to have a dynamic relationship with limited involvement from other health care providers.

## **214. Validation: The why, what and when**

**James R**

*Australian Red Cross Blood Service, Melbourne, VIC*

Validation is a critical step in the introduction of new products, processes and technologies. However, it is often poorly understood leading to poor implementation of the validation program. This typically leads to complex and arduous validation work which often does not provide the intended outcome for either the patient or the organisation.

In this talk Russell will explain the purpose of validation and how it is an integral part of both the product lifecycle and the Quality Management System. He will cover the basic methodology for the validation of processes and test methods as well as the main steps in equipment qualification.

A firm emphasis will be placed on product and process understanding driving the validation effort. This ensures that appropriate control is achieved and maintained which, in turn, ensures a product that is safe and effective for the patient. The use of risk assessment to guide and manage the validation effort will be discussed in this context.

## **215. Microbial contamination testing of cell therapy products**

**Joyce L**

*St Vincent's Hospital, Melbourne, VIC*

Traditional microbial contamination testing methods involve inoculating cell/tissue products into culture media to detect aerobic, anaerobic and fungal organisms. These methods take 14 days and require daily inspection of the cultures. Commercially available automated blood culture systems offer an alternative which continuously monitor growth enabling early detection of microorganisms. These methods must be validated to ensure that microorganisms likely to contaminate the product are detected at low levels (10-100 CFU/ml).

Microbial contamination testing is not sterility testing. For many cell therapy products, the sterility cannot be guaranteed as the source material and final product cannot be sterilised. There are many opportunities for contamination at the time of donation, processing, storage and preparation for infusion

Limitations of testing include: small volumes of material available for testing with the potential for sampling error, extremely short shelf life of the product and the less rigorous screening of donors than for blood donation.

If microbial cultures are positive, investigations to determine the significance and reproducibility of the result are required. Whether to use or discard the product is often a clinical decision.

New technologies such as nucleic acid testing, MALDI-TOF mass spectrometry, flow cytometry and detection of micro colonies offer the potential for more rapid and sensitive detection of contamination in the future.

An overview of the role of the Microbiology laboratory in microbial contamination testing of cell therapy products including case studies will be presented.

## **216. The implementation of Long Range PCR and Ion Torrent Sequencing into the routine diagnostic HLA laboratory**

**Vukovic, I**

The high resolution genotyping of human leukocyte antigen (HLA) class I and II alleles is important for successful organ transplantation and genetic association studies. The high degree of polymorphism at the HLA loci and the inability to sequence each allele independently by current Sanger Sequencing Based Typing (SBT) at a low cost has led to the increase in HLA genotyping ambiguities. The challenge for registries and clinical laboratories is to provide the highest resolution typing results using efficient low-cost workflows. Next Generation Sequencing (NGS) on the Ion Torrent Personal Genome Machine (PGM) provides a low cost alternative to current Sanger SBT overcoming the HLA genotype ambiguity through the combination of i) clonal amplification, which resolves the cis-trans ambiguities, and ii) massively parallel, which enables an expansion of the HLA regions sequenced. We have developed an in-house long range PCR method which amplifies the full gene length of HLA-A, -B and -C and exon 2 through to exon 3 of DRB1/3/4/5, DQB1 and DPB1 in 6 separate PCR reactions. Long range gene-specific amplicons for each individual are then pooled for a single library preparation using a modified version of the Ion Torrent PGM library preparation and 400bp sequencing chemistry protocol. All data generated was analysed with software provided by Conexio Genomics and Life Technologies. There was good concordance with our current Sanger SBT methods and in the majority of cases the typing resolution exceeded that of our first pass Sanger SBT. The implementation of Ion Torrent NGS into the routine laboratory not only relies on accurate genotyping and ambiguity resolution but also the ability to provide an efficient workflow at a low cost. We have achieved this by automation on robotic liquid handling systems at amplicon pooling and library preparation steps, and the development of an in-house sample management database which allows sample tracking through the entire NGS process. Ion Torrent sequencing of long range PCR amplicons together with implementation of automation and a sample management database provides an efficient, low cost, high resolution typing alternative to our current Sanger SBT method.

## **217. Mesenchymal stromal cell manufacture for clinical application**

**Sturm M**

*Royal Perth Hospital, Perth, WA*

In Australia, cell therapies are regulated by the TGA as a Biological under the new Biologics Framework and must comply with the code GMP (2013) and relevant Therapeutic Goods Orders and standards. This includes cell therapies manufactured for clinical trial evaluation, other than first in man application, and so captures some Phase I and all Phase II/III trials. Along with manufacturing other biotherapies, Cell & Tissue Therapies WA (CTTWA) at Royal Perth Hospital has been manufacturing mesenchymal stromal cells (MSC) since 2007 in their licensed manufacturing facility. A product license to manufacture MSC for clinical trial application was finally obtained in 2013, as required by TGA.

CTTWA manufacture MSC from the bone marrow of allogeneic donors. Donors are relatives or friends of clinical trial subjects and are strictly assessed and tested to minimize the risk of infectious disease transmission and in compliance with TGO 88. MSC are isolated from bone marrow by plastic adherence in culture flasks and then culture expanded using fetal calf serum to passage 5. Cells are harvested, cryopreserved in aliquots of 100 and 50 x 10<sup>6</sup> cells and stored at <-150<sup>0</sup>C. Prior to release, the MSC products undergo extensive testing for viability, phenotype, tri-lineage capability and for cytogenetic analysis. There are on-going validations for shelf life of the product.

CTTWA currently has 7 clinical trials underway using MSC in conditions of immune disorder, such as GVHD, organ rejection and autoimmune disease, and for tissue repair. Cells are also provided off trial on compassionate grounds. Patients receive 2 x10<sup>6</sup> MSC/kg per infusion and, depending on the trial protocol, may receive 2 or 4 infusions. More than 500 infusions of CTTWA manufactured MSC have been performed across multiple Australian sites with no adverse related events seen. The majority of MSC therapy has been used for patients with GVHD (54%), followed by Crohn's disease (30%) and solid organ (lung and kidney) rejection (15%).

Trial result outcomes have been extremely encouraging to date, with significant clinical improvement for patients and no adverse MSC infusion related events observed.

## **218. The Rotary WA Cord Blood Bank**

**Lazzaro G**

*Australian Red Cross Blood Service, Perth, WA*

The Rotary WA Cord Blood Bank Project was established in 2005 on the one hundredth anniversary of the founding of Rotary International and is Rotary WA's centennial gift to the people of Western Australia. The project has been generously funded by Rotary, Inner Wheel, Lotterywest, the corporate sector and the local community.

The project has involved the design and construction of a purpose built facility for cord blood processing, cryopreservation, testing and storage and in line with Rotary's project criteria, space for future growth, research and allied therapeutic applications. The project is managed by the Australian Red Cross Blood Service.

The facility has undergone a detailed commissioning and qualification regime and the execution of a rigorous validation plan for all aspects of operations is underway. The technical requirements and approach to commissioning, equipment, test method and process validation will be discussed.

In operation, the Rotary WA Cord Blood Program will collect cord blood donations from Perth's tertiary teaching maternity hospital. Through the diversity of participating mothers, the program expects to contribute to the range of tissue types represented on the national registry.

## **219. Implementation of ISBT 128 at CTTWA**

**Fogarty, J**

*Royal Perth Hospital, Perth, WA*

Cell and Tissue Therapies WA (CTTWA) currently manufactures a number of clinical products including haemopoietic stem cells, heart valves, skin, serum eye drops and mesenchymal stromal cells (MSC). The facility is licensed by the Australian regulator, the Therapeutic Goods Administration (TGA) and is undergoing international accreditation by FACT/JACIE. Labelling of products is performed in accordance with the Australian Code of Good Manufacturing Practice, Therapeutic Goods Order 87, FACT-JACIE International Standards and NPAAC Requirements. Production, storage and distribution of labels by CTTWA are controlled to prevent unauthorised access and use. CTTWA is registered with the International Council for Commonality in Blood Banking Automation (ICCBBA). The purpose of ISBT 128 is to provide standards for the coding and labelling of products of human origin including blood and cellular therapy products. It provides a globally unique donation numbering system, internationally standardized product definitions, and standard data structures for bar coding and electronic data interchange. StemLab is the current database management system that assists with managing CTTWA inventory, generating ISBT 128 labels and reports, including the ability to correlate product manufacture with clinical outcomes. ISBT128 labelling has been implemented for all cell therapy final product labels.

## **220. Implementation of ISBT128 barcode labelling in a cellular therapy laboratory using Hematrax CT**

**Hutchins C**

*Cellular Therapy / Bone Marrow Transplant Laboratory, Royal Brisbane & Women's Hospital, Herston Qld 4029*

ISBT 128 is the global standard for the terminology, identification, coding and labelling of blood, tissue and cellular therapy products with the objective of achieving international consistency in the information provided on component end labels and the placement of such information. The 5<sup>th</sup> Edition of the FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing and Administration required processing facilities to identify cellular therapy products according to the proper name of the product as defined by ISBT 128 standards. In addition, the FACT-JACIE standards required an implementation plan for the usage of ISBT 128 coding and labelling, if ISBT 128 had not already been fully implemented.

In late 2012, the Cellular Therapy Laboratory at the Royal Brisbane & Women's Hospital committed to implementation of ISBT 128 labelling using the Digitrax Hematrax CT system. The laboratory operates a standalone PC version of Hematrax CT and 3 Zebra Barcode printers with Hematrax firmware for the generation of 4 x 4" full ISBT 128 labels, partial labels and cryovial labels. All labels meet the FDA CFR 21, Section 175.105 requirements for food grade adhesives. An additional Zebra printer with Replitrax firmware is used for the generation of donation identification number (DIN) sets. Annual registration of the facility with ICBBA is required for allocation of a facility identification number (FIN). The presentation will focus on a description of the ISBT 128 cellular therapy standard and labelling systems, and installation and operation of the Hematrax system including minor problems experienced with the hardware and the selection of correct product codes from the product code database.

## **221. Managing a multidisciplinary manufacturing facility**

**Sturm, M**

*Royal Perth Hospital, Perth, WA*

Cell & Tissue Therapies WA (CTTWA) at Royal Perth Hospital is unusual in that it manufactures an array of clinical products across different disciplines and regulatory groups, whereas most other manufacturing and processing facilities concentrate on one type or class of product. CTTWA is TGA licensed, having been commissioned and accredited in 2006. With the introduction of the Biologicals Framework, therapeutic goods are regulated as Medicines, Medical Devices or Biologicals, with some exemptions and exceptions. CTTWA manufactures products in the classes of Medical Devices and Biologicals. Manufactured products include human heart valves, haemopoietic progenitor cells, cultured expanded cells, serum eyedrops and pericardial patches. However, the commonality in requirements for the manufacture of any therapeutic good means that generic systems and operations can be implemented.

CTTWA operates a single quality system that encompasses document control, change managements, materials and equipment control, risk assessments and all other aspects of quality management. Many of our documents are generic such as receipt, labeling, distribution etc but there are also product specific documents that relate to the processing or manufacture of particular products. The facility is comprised of 5 clean rooms class C, that are equipped with basic essential equipment. Product manufacture can be interchanged between the rooms, although some rooms may be restricted at certain times to specific product types. The building management system ensures the facility operates within specification and that all parameters and equipment is monitored. Environmental monitoring is conducted to a rigid schedule but may be tailored to suit the particular type of product manufacturing.

Many of the techniques required for processing are common across the different products, for example cryopreservation. Staff are trained in basic skills that apply generally to GMP and then specialize to several different product types. This ensures that there is always a sufficient number of staff to cover the different manufacturing procedures and provide adequate out of hours cover.

The operation of a multidisciplinary manufacturing facility is an efficient use of facility, equipment and staff, can reduce duplication of services and streamlines accreditation requirements.

## **P001. Generation of CD19-specific chimeric antigen receptor T cells that co-express the RQR8 marker/suicide gene using the PiggyBac transposon/transposase system.**

**Bishop D**<sup>1,2,3</sup>, Ramanayake S<sup>1</sup>, Micklethwaite K<sup>1,2,3</sup>

<sup>1</sup> Cellular Therapies, Westmead Millennium Institute, Sydney, NSW, Australia, <sup>2</sup> Department of Haematology, Westmead Hospital, Sydney, NSW, Australia, <sup>3</sup> Sydney Medical School, The University of Sydney, Sydney, NSW, Australia

### **Aim**

B cell malignancies are common and are frequently incurable. Recent publications have demonstrated the efficacy of CD19-specific chimeric antigen receptor T cells (CAR19 T cells) in the treatment of these haematological malignancies. However, this technology is currently limited by the stringent safety regulations regarding the use of retroviral vectors to transduce T cells with the CAR, and the associated expense. Furthermore, there is concern regarding potential long term toxicity that may be associated with the ability of CAR19 T cells to engraft and persist. We aimed to address these issues by using the non-viral PiggyBac transposon/transposase system to introduce both a second generation CD19-specific CAR (CAR19.28z) and the RQR8 selection marker/suicide gene. RQR8 is a highly compact engineered protein expressed on the cell surface that contains a CD34 epitope and two rituximab-binding CD20 mimotopes.

### **Methods**

CAR19.28z and RQR8 were sub-cloned into the PiggyBac transposon/transposase system and transduced into PBMCs using the Neon electroporation system. CAR19 T cells were selectively expanded in culture in the presence of IL-15 and autologous irradiated PBMCs for CD19 stimulation. RQR8<sup>+</sup> CAR19 T cells were selected using Miltenyi CD34 paramagnetic beads. The ability of RQR8<sup>+</sup> CAR19 T cells to be eliminated by varying concentrations of rituximab in the presence of complement was determined.

### **Result**

The PiggyBac system facilitated the stable expression of CAR19.28z and RQR8 on the surface of T cells. RQR8<sup>+</sup> CAR19 T cells could be exponentially expanded and a highly pure population was produced by selection with Miltenyi CD34 paramagnetic beads. Rituximab was able to eliminate RQR8<sup>+</sup> CAR19 T cells at concentrations achieved in standard clinical dosage regimens.

### **Conclusion**

We predict that use of the PiggyBac system for generation of CAR T cells and of RQR8 as a selection/elimination marker will improve the safety and reduce the cost of generating CAR T cells.

## **P002. ALL/High Grade Lymphomas in Adolescents and Young Adults (AYAs) - The RPH Experience & Challenges with Paediatric Chemotherapy Protocols**

**Cooney J, Loo S, Wright M, Tan P, Cannell P, Carnley B, Yee T, O'Reilly J**

*Royal Perth Hospital*

### **Aim**

To investigate the management of adolescents and young adults (AYAs), we report the cases of Acute Lymphoblastic Leukaemia at RPH, including clinical data and cytogenetics, treated on COG-based Paediatric protocol.

### **Method**

We reviewed ALL/High Grade Lymphoma patients at Royal Perth Hospital since 2008 who received Paediatric protocol therapy via the chemotherapy and oncology database from the Department of Pharmacy, with side-effects and toxicities as well as interruption of therapies identified by case file review.

### **Results**

From 2008, ten patients have been treated for ALL at RPH with the Paediatric COG-based protocol. Mean age was 22 (17-29) years, with 6 Males and 4 Females. There are 5 T-cell and 5 B-lineage disorders, with highly varied presenting blast counts (0-207 x 10<sup>9</sup>/l).

One patient is currently admitted for planned sibling allograft, with high risk cytogenetics. Two had Philadelphia positive disease- neither proceeded to allograft to date. One is a Jehovah's Witness declined allograft and continues on TKI and chemotherapy and remains in partial molecular remission, while the other had major treatment related toxicities requiring cessation of the planned therapy, though continues on TKI and maintenance therapy and is in remission on short follow up).

Five patients had significant problems related to Asparaginase therapy, with two patients requiring complete cessation of this agent. There were treatment delays in all cases. Mean follow-up is 36 (range 3-78) months. All patients are currently alive, with nine in complete remission.

### **Conclusion**

ALL remains a challenging disease to manage in AYAs. Promising results has encouraged the adoption of paediatric regimens in this population. Difficulties related to toxicities and deliverability of this therapy are identified. Various psycho-social issues have been considered, and drug toxicities highlighted by Asparaginase. Despite this, all ten patients are currently alive, with nine in remission, at a mean follow up of 36 months.

## **P003. A summary of patients treated with intensive induction chemotherapy for acute myeloid leukaemia at the Royal North Shore Hospital from 2009 to 2014**

**Downe P**

*Royal North Shore Hospital*

### **Aim**

To summarise patient data and treatment outcomes for patients who underwent intensive induction chemotherapy for acute myeloid leukaemia (AML) since 2009.

### **Method**

Patients were identified via a clinical database. Demographics, pathology and treatment data were obtained via electronic and written medical records. Genetic risk groups were defined by the latest National Cancer Care Network guidelines. Survival was calculated by the Kaplan Meier method.

### **Results**

We identified 75 patients who underwent intensive induction chemotherapy between January 2009 and February 2014. The median age was 61 years (range 19-78) with a male to female ratio of 1.6 to 1. Secondary AML accounted for 43% of cases. Genetic risk groups were favourable (7%), intermediate (61%) and unfavourable (28%).

Standard-dose (45-50 mg/m<sup>2</sup>) or high-dose (90 mg/m<sup>2</sup>) daunorubicin was administered to 30 and 31 patients, respectively, along with cytarabine (100 mg/m<sup>2</sup>). The remaining 14 patients received other variants of 7+3. Early death occurred in nine patients (12%).

Complete remission (CR) was achieved in 36 (48%) patients after induction and a further 20 (27%) after salvage therapy. Median survivor follow-up was 33.3 months. Median overall survival (OS) was 20.9 months. OS was longer in primary AML (not reached (NR) vs. 8.1 months,  $p < .001$ ) and for patients with CR after induction (NR vs. 18.0 months,  $p = 0.011$ ). Median relapse-free survival for patients in CR after induction was 23.5 months. Daunorubicin dosage did not correlate with remission nor survival.

Allogeneic transplant was undertaken in 34 patients (45%). Most had reduced-intensity conditioning (71%).

### **Conclusion**

Our cohort is relatively old with a high rate of secondary AML and few favourable risk patients. Overall survival is comparable to other population-based studies.

## **P004. A rare translocation in AML may not be so bad...**

**Fielding K**<sup>1</sup>, Yannakou C<sup>1</sup>, Ramanan R<sup>1</sup>, Campbell L<sup>2</sup>, Juneja S<sup>3</sup>, Mason K<sup>1</sup>

<sup>1</sup> *Department of Clinical Haematology and Bone Marrow Transplant Service, Royal Melbourne Hospital,* <sup>2</sup> *Director of Cytogenetics, St Vincents Hospital,* <sup>3</sup> *Head of Diagnostic Haematology, Royal Melbourne Hospital*

### **Introduction**

We report a 42 year old man diagnosed with minimally differentiated acute myeloid leukaemia (AML-M0) with a rare chromosomal translocation - t(12;22)(p13;q12).

### **Method**

A previously well 42 year old man presented March 2013 with 3 weeks of muco-cutaneous bleeding and was found to have a white cell count of  $60 \times 10^9/L$ , haemoglobin of 121g/L, and a platelet count of  $14 \times 10^9/L$ . The bone marrow revealed 69% blasts with no associated myelodysplastic change. Cytogenetics revealed t(12;22)(p13;q12) and loss of the Y chromosome. Fluorescent in-situ hybridisation analysis revealed ETV6 signal rearrangements in all 34 metaphase spreads scored – the breakpoint on chromosome 22 was clearly distal to the BCR gene.

He subsequently received HiDAC-3 induction treatment and achieved a complete morphological and cytogenetic remission after the first cycle. This was followed by 2 cycles of IcE consolidation chemotherapy.

The intention had been to proceed to stem cell transplantation as soon as possible given the uncertain prognosis of the cytogenetics – however, no appropriate related or unrelated donor was available. He remains in complete remission 14 months post completion of chemotherapy.

### **Discussion**

Translocations involving ETV6/TEL are found in many haematological malignancies, as well as in sarcoma. Approximately 20 different fusion partners have been described for ETV6, suggesting multiple theoretical mechanisms of leukaemogenesis.

t(12;22)(p13;q12) has previously been reported in myelodysplasia and AML, but is too uncommon a lesion to assign a prognostic significance. The only published case report of minimally differentiated AML with t(12;22)(p13;q11), also including trisomy 9, describes primary multi-drug resistance with a poor response to several chemotherapy regimens. The patient described died within 1 year of diagnosis. It was suggested that the t(12;22) translocation may be associated with multidrug resistance. Our patient has had a different course and outcome.

### **Conclusion**

Our patient with AML and t(12;22)(p13;q12) had a favourable outcome with standard induction and consolidation chemotherapy alone, maintaining a complete morphological and cytogenetic remission for a period of 14 months to date.

## **P005. Cytarabine intensification during induction for Core-Binding-Factor Acute Myeloid Leukaemia is tolerable but does not enhance early molecular responses**

**Fleming S**<sup>1</sup>, Strong D<sup>2</sup>, Chee L<sup>3</sup>, Avery S<sup>1</sup>, Tuckfield A<sup>3</sup>, Wei A<sup>1,4</sup>

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### **Aim**

Given the association with improved survival and depth of molecular response (Yin et al, Blood 2012), we sought to determine whether high-dose cytarabine (HiDAC)-based chemotherapy was associated with greater reductions in molecular minimal-residual-disease (MRD) post-induction and the trade-off in terms of treatment-related haematologic toxicity.

### **Methods**

CBF-AML patients (both inv(16) and t(8;21)) were identified from departmental databases at the Alfred and Royal Melbourne hospitals. Secondary CBF-AML was excluded. Morphologic responses graded by Cheson criteria (Cheson et al, JCO 2003); RT-qPCR (performed by single laboratory) was used assessing MRD. Equivocal results taken as 0 for calculation but not MRD-negative. HiDAC defined as  $\geq 1\text{g/m}^2$  daily cytarabine (Ara-C) during induction.

### **Results**

48 cases (22 Alfred, 26 RMH), (25 inv(16), 23 t(8;21)) were identified. 29 HiDAC induction and 19 standard-dose Ara-C. One induction death (at 22 days) occurred in a HiDAC patient. All others attained morphological remission. Time to neutrophil recovery ( $0.5 \times 10^9/\text{L}$ ) was similar for HiDAC and standard-dose (26.5 vs 28.5 days;  $p=1$ ) similarly for platelets ( $50 \times 10^9/\text{L}$ ); (27 vs 31 days  $p=0.6$ ). G-CSF usage was similar (3/12 vs 6/10,  $p=0.1$ ) Survival outcomes were similar; 3-year OS 79% vs 62% ( $p=0.29$ ), RFS 56% vs 56% ( $p=0.9$ ), for HiDAC versus standard-dose, respectively. HiDAC  $24\text{g/m}^2$  at induction showed similar findings.

MRD-negative remissions post-induction were increased in inv(16) (8/16) vs t(8;21)(0/15) ( $p=0.001$ ). No differences seen with induction intensity (5/20 for HiDAC, 3/11 for standard-dose,  $p=0.77$ ). Median baseline transcripts and thus log-reduction was higher for t(8;21) (2.5log) vs inv(16) (1.9log) ( $p=0.004$ ); no difference by induction (2.1 vs 2.4,  $p=0.12$ ).

### **Conclusion**

HiDAC induction for CBF-AML is tolerable with comparable count recovery times and no excess of non-relapse mortality versus standard-dose cytarabine. Molecular responses and survival were however similar. Further investigation of appropriate induction regimen in CBF-AML is required.

## P006. The frequency of Ph-like ALL is high in Australian adults

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### Aim

Acute Lymphoblastic Leukaemia (ALL) remains the leading cause of cancer-related death in children. While survival rates for childhood now exceed 80%, the prognosis remains poor in adults. Recently, a high-risk group of B-progenitor ALL patients has been identified termed Ph-like ALL that are BCR-ABL1 negative but have a range of genetic alterations that activate cytokine receptor and kinase signalling, allowing potential targeting by available tyrosine kinase inhibitors. The frequency of Ph-like ALL is known to increase with age, however the prevalence across the age spectrum of adolescent and adult ALL is unknown. We sought to identify the frequency and mutational spectrum of Ph-like ALL in adults, and determine the *in vitro* sensitivity to kinase inhibitors.

### Method

The Ph-like ALL gene expression profile was determined by Taqman Low Density Array (TLDA). Flow cytometry with intracellular phosphosignalling (phosphoflow) analysis was used to detect pathway activation resulting in phosphorylation of kinase targets (e.g. CRKL and STAT5), and to assess responsiveness to kinase inhibitors. Candidate RT-PCR, sequencing and FISH were used for identification of kinase rearrangements.

### Results

Thirty-four adolescent/young adult (16-39 years, AYA) and 38 adult (>39 years) B-ALL diagnosis cases were analysed. Overall, 19/72 (26%) were Ph-like including 6/34 (18%) AYA and 13/38 (34%) adults, of which 11 have confirmed fusions (Table 1). The remaining 8 were negative by a panel of >25 Ph-like fusions and are being subjected to RNA-seq. Two adult cases that were not Ph-like by TLDA but had high CRLF2 expression by flow cytometry were found to have the *IGH-CRLF2* fusion. In 8 cases where the fusion partner was identified as *CRLF2*, 3 had concomitant *JAK1/2* mutations.

### Conclusion

These data demonstrate a high frequency of Ph-like ALL in adults. Importantly, rapid identification of these patients may guide intervention with targeted therapies matched to the causative genetic lesion in this high-risk cohort.

Table 1: Screening summary of AYA and adult patients for Ph-like disease.

	TLDA			Phosphoflow			Fusions identified to date	In vitro sensitivity to kinase inhibitors	JAK mutations
	n	Ph-like	%	n	Ph-like	%			
AYA (16-39)	34	6	17.6	27	5	18.5	<i>IGH-CRLF2</i> <i>IGH-CRLF2</i> <i>P2RY8-CRLF2</i> <i>P2RY8-CRLF2</i> <i>EBF1-PDGFRB</i> <i>ATF7IP-JAK2</i>	JAK ND JAK JAK TKI ND	R683S JAK syn none D873N - -
Adult (40 and over)	38	13	34.2	29	8	27.5	<i>IGH-CRLF2</i> <i>IGH-CRLF2</i> <i>IGH-CRLF2</i> <i>IGH-CRLF2</i> <i>ETV6-ABL1</i> <i>IGH-CRLF2*</i> <i>IGH-CRLF2*</i>	ND ND JAK JAK TKI JAK JAK	JAK syn JAK syn none T875N - JAK syn none
Total	72	19		56	13				
	15/38 (39%) Adults positive by TLDA and/or Phosphoflow								
							*p-Flow only	ND - not done	syn - synonymous

## **P007. Is Pure Erythroid Leukaemia a unique entity? An analysis of 7 cases and comparison with Acute Myeloid Leukaemia with greater than or equal to 50% erythroblasts**

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<sup>1</sup> Royal Melbourne Hospital, VIC, Australia, <sup>2</sup> Alfred Hospital, VIC, Australia, <sup>3</sup> Department of Pathology, Division of Cancer Medicine, Peter MacCallum Cancer Centre, VIC, Australia, <sup>4</sup> University of Melbourne, VIC, Australia

Pure Erythroid Leukaemia (PEL) is a rare subtype of acute myeloid leukaemia (AML) and its clinicopathological features of PEL are not well defined.

### **Aim**

To describe the immunophenotypic, cytogenetic, and clinical features of morphologically defined PEL and to compare these with AML with  $\geq 50\%$  erythroblasts.

### **Methods**

Cases of PEL according to WHO (2008) criteria at Royal Melbourne Hospital (RMH), Alfred Hospital, and Peter MacCallum Cancer Centre from 1997-2013 were included. A comparison cohort comprised of AML with  $\geq 50\%$  erythroblasts diagnosed at RMH. Clinical data were retrieved from medical records. The histology of all cases was reviewed by a haematopathologist (SJ or DW).

### **Results**

Seven cases of PEL were included. All were male, median age 54years (range 19-78years). The leukaemic erythroblasts were identified by immunohistology targeting glycophorin C. Blasts frequently expressed CD117 (83%), CD13 (100%) and were myeloperoxidase negative (83%). Cytogenetics was available for 5 cases; all demonstrated complex karyotypes (median 13 chromosomal abnormalities).

Three patients (43%) had prior chemotherapy exposure. Two patients (29%) had preceding de-novo MDS. Overall, 71% of cases could be re-classified as AML with multilineage dysplasia or therapy-related AML.

Six patients (86%) were treated with palliative intent (hydroxyurea 1, azacitidine 1, supportive care 4). Median overall survival was 2.9months.

The comparison group comprised 23 cases of AML with  $\geq 50\%$  erythroblasts. Compared with this group, the PEL cohort had a lower incidence of MPO ( $p < 0.01$ ) and CD33 ( $p = 0.04$ ) positivity and a higher incidence of adverse-risk cytogenetics ( $p = 0.01$ ). Patients with PEL were more likely to have had prior chemotherapy ( $p = 0.01$ ) and there was a trend towards a higher incidence of preceding MDS ( $p = 0.07$ ).

### **Conclusion**

PEL appears to be a unique entity that is often secondary or treatment related, commonly features a complex karyotype and has a poor prognosis. It is immunophenotypically and cytogenetically distinct from AML with erythroid hyperplasia.

## P008. Identification and analysis of oncogenic pathways in deletion 20q acute myeloid leukaemia

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<sup>1</sup> St Vincent's Hospital, <sup>2</sup> Victorian Cancer Cytogenetics Services, <sup>3</sup> St Vincent's Institute of Medical Research, <sup>4</sup> Bio21 Institute  
Aim

We have shown deletion of 20q in AML may be associated with 20q11.2 amplification. Putative oncogene and tumor suppressor genes (TSG) identified in patients with del(20q)AML, *Haemopoietic cell kinase* (HCK) or its precursor and the TSG *Protein tyrosine phosphatase receptor type T* (PTPRT) were tested in the laboratory to confirm their oncogenic potential.

### Method

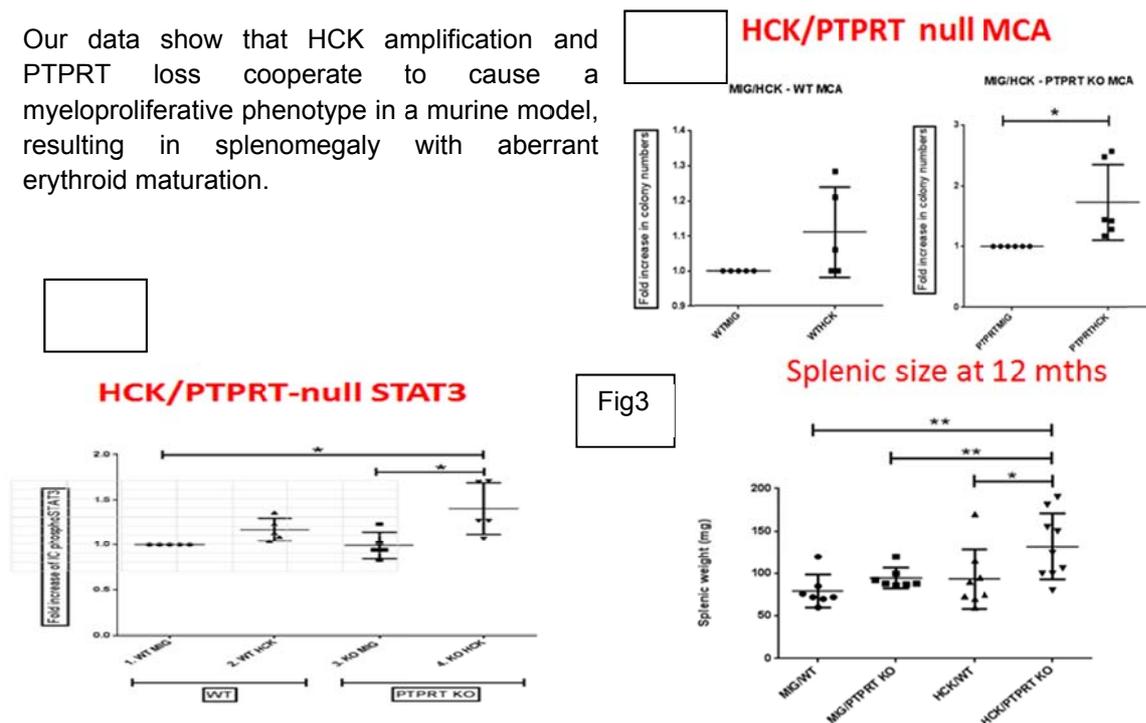
Haemopoietic stem cells (HSC) were isolated from the bone marrows of wild type and PTPRT-null mice by FACS sorting for Lineage negative, C-kit and Sca-1 positive cells (LKS+). Isolated LKS+ HSC were then transduced by either the retroviral construct of HCK or the vector control. These cells were then used in *in vitro* assays such as methylcellulose assay (MCA) and STAT3 antibody assay to assess features of malignancy. The cells were also transplanted into recipient mice to assess outcome.

### Results

1. HCK amplification and PTPRT loss conferred higher methylcellulose colony (MCA) numbers (Fig1).
2. HCK caused STAT3 hyperphosphorylation in the PTPRT-null HSC (Fig2).
3. Direct binding between HCK and PTPRT suggested that they are substrates for each other.
4. Transplantation of PTPRT-null LKS+ cells transduced with HCK produced a myeloproliferative phenotype in 5/9 recipient mice; they developed splenomegaly with excessive nucleated erythroid populations (Fig3).

### Conclusion

Our data show that HCK amplification and PTPRT loss cooperate to cause a myeloproliferative phenotype in a murine model, resulting in splenomegaly with aberrant erythroid maturation.



## **P009. Pilot study of the incidence all invasive fungal infections (IFI) in haematology patients in the Australian Capital Territory (ACT) between 2010-2013**

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### **Aim**

The incidence of invasive fungal infections (IFI) in haematology patients with acute leukaemia and haemopoietic stem cell transplantation (HSCT) ranges from 5% to 40%. Mortality rates from *Aspergillus* species approach 50% in patients with prolonged neutropenia and 86% in HSCT recipients. The study aimed to develop a database to identify the baseline incidence of IFI between 2010-2013, in haematology patients at the Canberra Hospital.

### **Method**

A database was established on all haematology patients with IFI. Details including patient characteristics, diagnoses, treatment regimens, antifungal prophylaxis and an antifungal therapy were data entered retrospectively. The European Organisation for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) guidelines were used for classification of IFI.

### **Result**

85 patients were assessed with a mean age of 54 and a predominance of males (65%). 36% of patients had Acute Myeloblastic Leukaemia (AML), 9% were Acute Lymphoblastic Leukaemia (ALL) and 22% were transplant recipients. 85% received antifungal prophylaxis (posaconazole or fluconazole). There were 13 proven or probable IFI cases over the 4 years. A larger number of non-*Aspergillus* species IFI were seen between 2010-2011 (8 of the 13 cases) which were predominantly *Mucor* species and mostly occurring in patients with AML despite antifungal prophylaxis. The incidence rate of IFI was 28% in 2010, 20% in 2011-2012 and 11% in 2013. This is a much higher incidence than described in the international literature. It was also identified that this period of analysis coincided with external building works and construction at the hospital, which may have increased rates of infection.

### **Conclusion**

This pilot study has revealed a higher rate of IFI and this may have been influenced by building works. This occurred despite antifungal prophylaxis in the majority of patients. Further analysis is required including assessment of the effectiveness of antifungal prophylaxis using therapeutic drug monitoring.

## **P010. Utility of Computed Tomography (CT) abdomen/pelvis in symptomatic haematology patients undergoing intensive myelosuppressive chemotherapy**

Lim H, Williams B, Ashby M, Grigg A

*Department of Clinical Haematology, Austin Health, Heidelberg, Victoria, Australia*

### **Aim**

CT abdomen/pelvis (CTAP) is commonly used for investigation of persistent unexplained febrile neutropenia (FN) and/or abdominal symptoms in haematology patients undergoing profoundly myelosuppressive chemotherapy, despite the paucity of evidence supporting its use. We evaluated the diagnostic utility of such CTs in autologous stem cell transplant (ASCT) recipients and patients receiving chemotherapy for acute myeloid leukemia (AML).

### **Methods**

Retrospective evaluation of eligible patients who had CTAP in this context from January 2010 to April 2014 at Austin Health.

### **Results**

Of the 124 ASCT recipients (53% myeloma autografts), 22 (17%) underwent 25 CTAP, a median of 9 days (5-23) from the day of stem cell infusion. 20% were done for persistent FN and the remaining 80% for investigation of abdominal symptoms, mainly for suspected neutropenic enterocolitis. Sixteen (64%) had positive findings, most commonly neutropenic enterocolitis (n=13), although only 3 patients had therapy change attributable to the CT result – addition of anaerobic antibiotic coverage (n=1) and bowel rest (n=2).

In the 122 admissions of 93 patients with AML cohort, 60 CTAP were performed at a median of 8 days (0-20) from the first FN episode. 35% were done for persistent FN and the remaining 65% (n=39) for investigation of abdominal symptoms. Nineteen (32%) had abnormalities (enterocolitis in 14, other 5) with 4 subsequently leading to therapy change – bowel rest for all (including addition of anaerobic coverage in 2). Combining the two groups, only 8% of the CT scans led to therapy change (arguably some of which may have been instituted anyway) with no patient undergoing surgical intervention based on CT findings.

### **Conclusion**

CTAP in haematology patients with FN and/or abdominal symptoms rarely provides useful information unsuspected clinically or results in therapeutic changes which would not be otherwise be made on clinical grounds.

## **P011. Response to prior therapy determines outcome of salvage therapy in acute myeloid leukaemia resistant to first-line therapy.**

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### **Aim**

To identify determinants of outcome in AML resistant to first-line therapy.

### **Method**

A retrospective database analysis of AML resistant to chemotherapy (failing to attain complete remission (CR) after first-line therapy) between 2004-2013 at the Alfred Hospital, Melbourne, was conducted, correlating clinical and laboratory parameters with survival outcome. Partial remission (PR) to first-line therapy was defined as at least 50% reduction in blast count to between 5-25% bone marrow blasts.

### **Results**

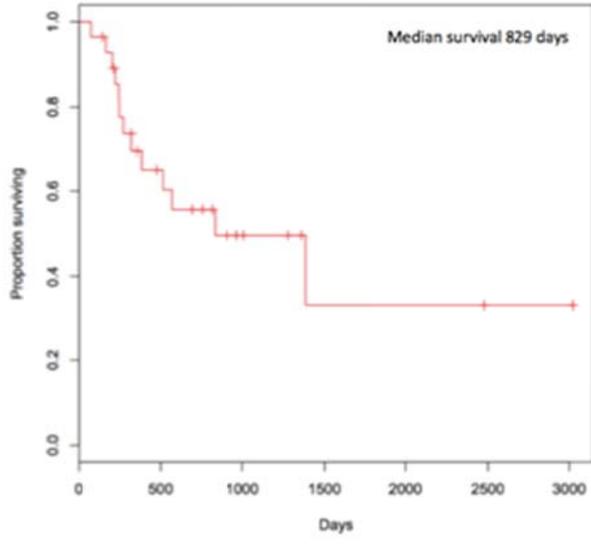
Among 40 patients with resistant AML, median overall survival (OS) for those proceeding to salvage therapy was 829 days, compared to only 174 days for those who did not ( $p=0.002$ ). Of those fit enough to receive salvage chemotherapy ( $n=28$ ), the effect of the following factors in relation to survival outcome was assessed: prior response to first-line therapy (PR vs resistant), age ( $<50$  vs  $\geq 50$ ), AML type (*de novo* vs secondary), cytogenetic risk (adverse vs non-adverse), prior chemotherapy (HiDAC vs non-HiDAC induction), or FLT3-ITD (present vs absent). Univariate analysis revealed that OS was higher amongst those with prior PR to chemotherapy (1385 vs 260 days,  $p=0.005$ ). Age, AML type, karyotype, FLT3-ITD, or prior chemotherapy intensity were not predictive of OS. Prior PR was more likely if first-line therapy included HiDAC (89% vs 43%,  $p=0.02$ ). Expected 3-year OS for patients salvaged after achieving PR to first-line therapy was 73% vs 13%. In contrast, salvage therapy did not improve OS in those failing to achieve a PR to first-line therapy ( $n=18$ ).

### **Conclusion.**

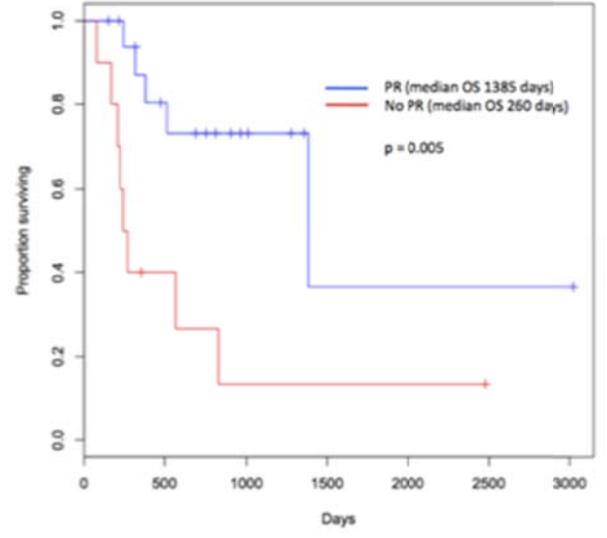
While outcomes in resistant AML are poor, those who attain a PR to first-line therapy have better survival prospects. Patients failing to attain a PR are unlikely to benefit from subsequent salvage therapy and investigational approaches should be considered.

Overall survival after salvage therapy for (A) all resistant AML cases and (B) according to first-line response.

(A)



(B)



## **P012. New molecular methods to detect mutations within the tyrosine kinase domain of the FLT3 gene**

**Osborn A, Hughes Q, Romeo G**

*PathWest LMWA*

Somatic mutations within the tyrosine kinase domain (TKD) of the FMS-like tyrosine kinase 3 (FLT3) gene are seen in haematological malignancies including a small subgroup of acute myeloid leukaemia (AML) patients. The debate of their prognostic significance in normal karyotype AML is on-going. However, new evidence proposes a role of FLT3-TKD mutations in drug resistance. The screening of these mutations by the molecular haematology laboratory, PathWest, RPH is performed using the polymerase chain reaction (PCR) followed by restriction enzyme digestion and capillary electrophoresis. This method has a number of known limitations. This study investigated alternative methods with the aim of improved FLT3-TKD mutation detection, High Resolution Melt (HRM) as a screening test and Competitive Allele Specific Taqman PCR (CAST-PCR) for mutation confirmation. HRM proved to be a reliable, sensitive technique with a similar sensitivity to the current method but able to be performed in a fraction of the time. CAST-PCR, while being very sensitive was found to have cross reactivity issues which could limit its introduction to a diagnostic laboratory. A new workflow is proposed using HRM as a screen followed by Sanger sequencing or CAST-PCR. This workflow would increase the efficiency of FLT3-TKD detection at PathWest.

## **P013. Granular Acute Lymphoblastic Leukaemia with a rare recurrent abnormality in a young adult - a case report**

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<sup>1</sup> *Department of Haematology, ICPMR, Pathology West, Westmead Hospital, Sydney Australia,* <sup>2</sup> *Flow Cytometry Unit, ICPMR, Pathology West, Westmead Hospital, Sydney Australia,* <sup>3</sup> *Department of Cytogenetics, Children's Hospital at Westmead, Sydney, Australia*

### **Introduction**

Despite recent advances and improved access to immunophenotyping and molecular diagnostics, provisional diagnosis of acute leukaemia remains dependent on morphologic examination of blood film and bone marrow aspirate. Cytoplasmic granules, a classical marker of myeloid differentiation, can rarely be found in significant numbers in lymphoid blasts potentially leading to misdiagnosis of AML.

### **Case Report**

An 18 year old boy presented for bone marrow biopsy as part of investigation of pyrexia of unknown origin on the background of a 6 month history of almost daily fevers and night sweats associated with abdominal pain, unintentional weight loss, and progressive anaemia. Previously, extensive investigations including an exploratory laparoscopy with mesenteric lymph node and liver biopsy were non-diagnostic. His peripheral blood count and film on presentation revealed microcytic anaemia (Hb 93 g/L; MCV 66 fL) with reduced transferrin saturation (7%) and elevated ferritin (479 ug/L), thrombocytosis ( $594 \times 10^9/L$ ), and normal white cell count and differential.

Bone marrow aspirate showed a markedly hypercellular marrow with 60% blasts. The pleomorphic blasts featured prominent nucleoli and large abnormal inclusions/granules and vacuolation. The immunophenotype was CD19+, CD20+, CD10+, CD34-, and slg light chain negative. Based on these features a diagnosis of precursor B-cell ALL was made. Subsequently, his karyotype revealed a dicentric chromosome consisting of the long arm of chromosome 7 and 9 [dic (7;9)].

### **Conclusion**

Although very rare in adults, granular ALL should be kept in mind as it may easily lead to diagnostic confusion with AML during morphologic evaluation. Here, we describe a case of granular ALL associated with dic (7;9). To our knowledge, there have been just 19 reported cases of this recurrent abnormality in ALL, and only one other case reported in granular ALL. Furthermore, this case report highlights the importance of immunophenotyping and cytogenetic analysis in characterization of acute leukaemias.

## **P014. The effect of food on the posaconazole pharmacokinetics investigated during the development of a new tablet formulation**

**Rapajic-Moran I**<sup>1</sup>, Dogterom P<sup>2</sup>, van Iersel M<sup>3</sup>, Xu J<sup>4</sup>, Waskin H<sup>4</sup>, Kersemaekers W<sup>2</sup>

<sup>1</sup> Merck Sharp Dohme Australia, <sup>2</sup> MSD Oss, Netherlands, <sup>3</sup> Merck & Co., Inc., Upper Gwynedd, PA, <sup>4</sup> Merck & Co., Inc., Kenilworth, NJ

### **Aim**

To evaluate the pharmacokinetics (PK), safety, and tolerability of posaconazole oral suspension in children 3 months to <18 years with neutropenia or anticipated neutropenia ( $ANC \leq 500/mm^3$ ) expected to last  $\geq 7$  days.

### **Method**

This is a Phase 1B, nonrandomised, multicentre, open-label, sequential dose-escalation study. Enrolled children are divided into 3 age groups (AG): AG1, 2-<7 years; AG2, 7-<18 years; AG3, 3 months-<2 years. AG1 and AG2 are divided into dosage groups (DG): DG1, 12 mg/kg/day divided bd; DG2, 18 mg/kg/day divided bd. Patients received 7-28 days of posaconazole. Extensive PK samples were collected at Days 1 and 7 and trough samples were collected on Days 3, 5, 8, 14 and 28. Primary outcome measure was  $C_{avg}$  at day 7.

### **Result**

Preliminary PK results in AG1 and AG2 (n=43) showed that the target exposure (~90% of subjects with  $C_{avg}$  500 to 2,500 ng/mL) was achieved in 52% of subjects in DG1 (n=25; AG combined) and 56% of subjects in DG2 (n=18; AG combined). High variability was observed among exposures within each age and dosage cohort; the range in  $C_{avg}$  for DG1 was 34.6-3,350 ng/mL and 48.3-4,660 ng/mL for DG2. The median  $C_{avg}$  increased by 28% in AG1 but did not increase in AG2. Posaconazole was well tolerated in DG1 and DG2 with adverse events generally related to underlying diseases and concomitant therapies.

### **Conclusion**

The study suggests that posaconazole 12 and 18 mg/kg/d divided bd failed to achieve the PK exposure target. Observed variability in exposure is likely due to the effect of food intake on posaconazole. Dividing the daily dose tds may enhance PK exposure. We plan to evaluate 18 mg/kg/day divided tds in AG1 and AG2. Two tds dosage groups are planned for AG3 (12 and 18 mg/kg/day).

## **P015. Effect of concomitant medications affecting gastric pH and motility on posaconazole tablet pharmacokinetics**

**Rapajic-Moran I**<sup>1</sup>, Kraft W<sup>2</sup>, Chang P<sup>2</sup>, Van Iersel M<sup>3</sup>, Waskin H<sup>4</sup>, Krishna G<sup>4</sup>, Kersemaekers W<sup>2</sup>

<sup>1</sup> Merck Sharp Dohme Australia, <sup>2</sup> Thomas Jefferson University, Philadelphia PA USA, <sup>3</sup> MSD, Oss Netherlands, <sup>4</sup> Merck & Co, Whitehouse Station, NJ USA, <sup>5</sup> Merck & Co Whitehouse Station, NJ USA

### **Aim**

To evaluate the effect of concomitant medications altering gastric pH (antacid, ranitidine, and esomeprazole) and gastric motility (metoclopramide) on the pharmacokinetics (PK) and safety of posaconazole tablets.

### **Method**

This was a randomised, prospective, open-label, 5-way crossover study in 20 healthy volunteers. In each treatment period, a single 400 mg (100 mg x 4 tablets) dose of posaconazole was administered in the fasting state alone or with 20 mL antacid (aluminium hydroxide 2 g/magnesium hydroxide 2 g), ranitidine (150 mg bd), esomeprazole (40 mg once in the morning for 5 days), or metoclopramide (15 mg qid for 2 days). There was ≥10 day washout between treatment periods. Blood samples for PK evaluation of posaconazole were collected at pre-dose (0 hours) and at 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 120 and 168 hours post-dose.

### **Result**

Posaconazole AUC<sub>0-last</sub>, AUC<sub>0-inf</sub>, C<sub>max</sub>, T<sub>max</sub>, and t<sub>1/2</sub> were similar when administered alone or with medications affecting gastric pH and motility. Geometric mean ratios (90% CI) of AUC<sub>0-last</sub> compared with those of posaconazole alone were antacid, 1.04 (0.90-1.20); ranitidine, 0.97 (0.84-1.12); esomeprazole, 1.02 (0.88-1.17); and metoclopramide, 0.93 (0.80-1.07). Geometric mean ratios (90% CI) of C<sub>max</sub> compared with those of posaconazole alone were antacid, 1.06 (0.90-1.26); ranitidine, 1.04 (0.88-1.23); esomeprazole, 1.05 (0.89-1.24); and metoclopramide, 0.86 (0.73-1.02). Overall, 19/21 subjects reported ≥1 treatment-related adverse event (AE); all AEs were mild to moderate in severity. Most frequent treatment-related AEs were somnolence, diarrhoea, contusion and flatulence.

### **Conclusion**

In healthy volunteers, the PK of a single dose of posaconazole 400 mg tablets was not altered to a clinically meaningful extent when posaconazole was administered alone or with medications affecting gastric pH (antacid, ranitidine, and esomeprazole) or gastric motility (metoclopramide) and was generally well tolerated.

## **P016. Safety, tolerability, and pharmacokinetics of posaconazole oral suspension in neutropenic children**

**Rapajic-Moran I**<sup>1</sup>, Arrieta A<sup>2</sup>, Sung L<sup>2</sup>, Groll A<sup>2</sup>, Lehrnbecher T<sup>2</sup>, Bradley J<sup>2</sup>, Zwaan C<sup>2</sup>, Lee J<sup>2,3</sup>, Waskin H<sup>2,3</sup>, Walker M<sup>2,3</sup>, Kartsonis N<sup>2</sup>, Paschke A<sup>2</sup>

<sup>1</sup> Merck Sharp Dohme Australia, <sup>2</sup> Pediatric Posaconazole Study Group, <sup>3</sup> Whitehouse Station, New Jersey, USA

### **Aim**

To evaluate the pharmacokinetics (PK), safety, and tolerability of posaconazole oral suspension in children 3 months to <18 years with neutropenia or anticipated neutropenia ( $ANC \leq 500/mm^3$ ) expected to last  $\geq 7$  days.

### **Method**

This is a Phase 1B, nonrandomised, multicentre, open-label, sequential dose-escalation study. Enrolled children are divided into 3 age groups (AG): AG1, 2-<7 years; AG2, 7-<18 years; AG3, 3 months-<2 years. AG1 and AG2 are divided into dosage groups (DG): DG1, 12 mg/kg/day divided bd; DG2, 18 mg/kg/day divided bd. Patients received 7-28 days of posaconazole. Extensive PK samples were collected at Days 1 and 7 and trough samples were collected on Days 3, 5, 8, 14 and 28. Primary outcome measure was  $C_{avg}$  at day 7.

### **Result**

Preliminary PK results in AG1 and AG2 (n=43) showed that the target exposure (~90% of subjects with  $C_{avg}$  500 to 2,500 ng/mL) was achieved in 52% of subjects in DG1 (n=25; AG combined) and 56% of subjects in DG2 (n=18; AG combined). High variability was observed among exposures within each age and dosage cohort; the range in  $C_{avg}$  for DG1 was 34.6-3,350 ng/mL and 48.3-4,660 ng/mL for DG2. The median  $C_{avg}$  increased by 28% in AG1 but did not increase in AG2. Posaconazole was well tolerated in DG1 and DG2 with adverse events generally related to underlying diseases and concomitant therapies.

### **Conclusion**

The study suggests that posaconazole 12 and 18 mg/kg/d divided bd failed to achieve the PK exposure target. Observed variability in exposure is likely due to the effect of food intake on posaconazole. Dividing the daily dose tds may enhance PK exposure. We plan to evaluate 18 mg/kg/day divided tds in AG1 and AG2. Two tds dosage groups are planned for AG3 (12 and 18 mg/kg/day).

## P017. Modern educational methods in haematology

Roehrig K<sup>1</sup>, Grey D<sup>2</sup>, Fong E<sup>2</sup>, Erber W<sup>1</sup>

<sup>1</sup> School of Pathology and Laboratory Medicine, University of Western Australia, <sup>2</sup> Department of Haematology, PathWest Laboratory Medicine WA

### Aim

Graduates in science and medicine need to be able to analyse and interpret complex numerical and morphological haematological data. We developed novel eLearning approaches to teach and assess the learning of undergraduate students in transfusion, flow cytometry and morphology.

### Methods

Second year undergraduate science students worked in groups of six within a large class of up to 150 students, with 2-3 tutors in the classroom to assist. Students worked at their own pace through cases by answering questions with feedback in class. Their interpretive skills were tested by an online quiz featuring a new case not previously seen.

### Results

Achievement rates of learning outcomes for haematology educational activities.

Subject	Learning outcome By the end of this class, students able to:	Achievement (% answered correctly)
Transfusion	Correctly interpret ABO blood type	98
	Select appropriate blood in hypothetical transfusion settings	96
Full blood counts	Interpret blood count results, including anaemia, erythrocytosis, neutropenia, neutrophilia, thrombocytopenia, thrombocytosis, pancytopenia	87
Morphology	Identify bone marrow cellularity in morphological images	88
	Identify adequacy of erythropoiesis and granulopoiesis	63
	Identify cells present in images bone marrow	92
Flow cytometry	Interpret dot plots to identify antigen expression	76
Cytogenetics	Identify ploidy from a leukaemia karyotype	99
Diagnosis	Integrate blood count, morphology and flow cytometry to distinguish acute lymphoblastic from acute myeloid leukaemia	75
Prognosis	Combine the diagnosis with cytogenetics to determine likely prognosis	89

**Conclusion** This novel eLearning approach in a large class, small group style was successful in developing and assessing skills in interpretation of a range of haematology tests. The complexity of cases can be adjusted to suit undergraduates, postgraduate medical students or haematology registrars.

## **P018. Acute promyelocytic leukaemia in pregnancy - a case report**

**Sharma A, Sadawarte S, Johnston A, Harrup R, Prasad R**

*Royal Hobart Hospital*

**Introduction:** Acute promyelocytic leukaemia (APML) is an oncologic emergency and its management in pregnancy is particularly challenging. It is a rare malignancy in pregnancy. We report findings in a 25 year old female who presented at 28 weeks gestation with antenatal blood test showing neutropenia and thrombocytopenia. She was asymptomatic.

**Results:** Full blood count showed haemoglobin of 120 g/L, WCC  $1.3 \times 10^9/L$ , neutrophils  $0.4 \times 10^9/L$  and platelets  $75 \times 10^9/L$ . Bone marrow examination revealed a hypercellular marrow. Normal myelopoiesis was markedly reduced with presence of 46% abnormal promyelocytes and 11% myeloblasts. Erythropoiesis and megakaryopoiesis was reduced. Flow cytometry analysis on bone marrow aspirate showed a population of cells in the blast region that were negative for HLA-Dr and were expressing CD117, CD13 and CD33. A proportion of these cells were also positive for CD34. Conventional cytogenetic and FISH detected the pathognomic t(15;17) translocation and PML/RARA rearrangement. Quantitation of PML-RARA transcript by RQ-PCR was 110.6%.

**Management:** Induction with ATRA and prednisolone was initiated immediately. Consolidation was commenced after delivery of a healthy baby with ATRA and arsenic trioxide. Maintenance therapy included ATRA, 6-mercaptopurine and methotrexate. The patient continues to be in complete molecular remission and is on regular follow up. The patient did not receive any anthracycline treatment.

**Conclusion :** The case demonstrates that it is possible to manage low acute promyelocytic leukaemia in pregnancy without intensive anthracycline therapy.

## **P019. Sarcoidosis following chemotherapy for T-ALL**

**Wang J, Chan K, Filshie R, Quach H**

*St Vincen'ts Hospital*

The association between sarcoidosis and malignancy has been described. The existence of a sarcoidosis-lymphoma syndrome is controversial, but the term typically refers to sarcoidosis preceding lymphoproliferative disease (LPD), in particular Hodgkins lymphoma. Here we present a case of rampant sarcoidosis mimicking relapse soon after chemotherapy for T-cell acute lymphoblastic lymphoma (T-ALL).

### **Case 1**

A 40-year-old man presented with a bulky anterior mediastinal mass. Biopsy confirmed T-ALL (CD3+, CD4+, CD8+, TdT+). There was no baseline bone marrow involvement. He received 4 cycles of Hyper-CVAD, with complete metabolic response on PET after 2 cycles.

5 weeks post treatment, enlarging, FDG-avid mediastinal and hilar lymphadenopathy was noted on repeat imaging, prior to POMP maintenance. Intense bone marrow uptake was also seen. Mediastinal lymph node and bone marrow biopsies showed florid involvement with non-necrotising granulomas with no evidence of residual T-ALL on morphology, flow-cytometry or cytogenetics. Other causes of granulomas were excluded, including tuberculosis and fungi. As the patient was asymptomatic, conservative management was chosen and the patient proceeded with POMP maintenance.

### **Discussion**

This is the first time a case of rampant sarcoidosis mimicking relapsed T-ALL is reported in the literature to our knowledge. This is in contrast to the previously described sarcoidosis-lymphoma syndrome in which sarcoidosis usually precedes mature lymphoma. It highlights the importance of a tissue diagnosis upon apparent overt relapsed/refractory LPD. The causal relationship is unknown but may relate to underlying immunologic abnormalities in patients with LPD.

## **P020. Management and outcomes of elderly acute promyelocytic leukaemia (APML)**

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### **Aim/Background**

Elderly patients generally do not tolerate intensive chemotherapy well. The Australian Leukaemia & Lymphoma Group (ALLG) published the APML4 protocol which incorporated All Trans-Retinoic Acid (ATRA), Arsenic (ATO) and Idarubicin in a schedule intended to minimise cumulative anthracycline exposure. The APML4 has shown excellent outcomes compared to previous ATRA/Idarubicin-based protocol. We share our experience of managing patients age >65 with APML at the Gold Coast University Hospital (GCUH).

### **Methods**

Retrospective audit was performed between January 2008 and June 2014. 18 patients were diagnosed with APML of which 8 patients were age > 65. Chemotherapy was based on the APML4 protocol with dose adjustments made accordingly to comorbidities and toxicities. We report the patient characteristics, outcomes and complications.

### **Results**

Median age of the 8 patients was 73 years (range 69-84). All patients received dose-adjusted induction based on the APML4 protocol except in one patient whom Idarubicin was omitted due to pre-existing cardiac failure (baseline ejection fraction 22%). Dose reduction of ATO only was required for 3 patients (prolonged QTc (n=2), deranged liver function (n=1)). Both ATRA/ATO were dose-reduced in 1 patient due to cardiotoxicity. The dosing of Idarubicin were made based on cardiac comorbidities. In 1 patient, 2 doses were omitted while another patient had 50% dose reduction.

During maintenance, 6-Mercaptopurine was dose-reduced in 1 patient due to neutropenia. Methotrexate ceased for restrictive lung disease in 1 patient.

Of the 8 patients, 7 were inducted into complete remission (CR) after 1 cycle while 1 patient died from differentiation syndrome on day 2. At median follow up of 17 months (range 2 days – 40 months), all 7 patients remained in CR, although two patients had died at 16 and 22.5 months respectively while in CR

### **Conclusion**

In elderly patients with APML, treatment is reasonably tolerated but dose adjustments may be necessary. Despite dose reductions, and the lower cumulative anthracycline exposure in APML4, excellent response rates are still achievable.

## **P021. Survival benefit of treatment with hypomethylating agents compared with best supportive care in elderly patients aged 70 and above with acute myeloid leukaemia**

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### **Aim**

Elderly patients with acute myeloid leukaemia (AML) are often not eligible for intensive chemotherapy and thus offered best supportive care (BSC) only. Hypomethylating agents (HMAs) are now available for the treatment of myelodysplastic syndromes (MDS) and proven to prolong survival in MDS patients. HMAs' clinical activity has been demonstrated in AML too. We aim to evaluate survival outcome of patients aged 70 and above with AML in our institution, particularly comparing those who received HMAs to those on BSC only.

### **Methods**

We performed retrospective analysis on 89 patients, aged 70 and above, who were diagnosed to have AML between January 2003 and August 2013. Baseline clinical, haematologic and cytogenetic data were collected from our registry database. Sixty-three, 3 and 23 patients received BSC, induction chemotherapy and HMAs respectively as upfront treatment. The 3 patients who received induction chemotherapy were excluded from further survival analysis. Survival data was analyzed using SPSS version 21.

### **Results**

Median age of the cohort (N=89) was 77 years (range 70-90). Cytogenetic risk categories were favourable (n=2), intermediate (n=61), adverse (n=22) and unknown (n=5). There were 84 de novo AML and 5 therapy-related AML. Majority of the patients (87%) had good performance status (ECOG 0-1). Median follow-up duration was 29.3 months. The median overall survival (OS) was 4.3 months for the whole cohort. OS were 10.9 months for HMAs and 2.2 months for BSC ( $p < 0.0001$ ). Other factors such as age, gender, cytogenetic risk category and the presence of antecedent haematological disorder were all not significant predictor of survival in this cohort.

### **Conclusion**

There is definite survival benefit treating elderly patients with AML with HMAs compared with BSC alone. For elderly patients who are deemed not fit for standard induction chemotherapy, HMAs should be considered as an option of treatment.

## **P022. Impact of JAK inhibitor pre allogeneic transplant on transplant outcome in myelofibrosis**

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### **Aim**

The JAK inhibitor ruxolitinib decreases symptomatic splenomegaly and symptoms related to myelofibrosis (MF). Allogeneic stem cell transplant (alloSCT) is the only curative therapy for myelofibrosis. We evaluated the impact of pre alloSCT ruxolitinib on transplant outcome in myelofibrosis.

### **Methods**

A retrospective review of myelofibrosis patients who were treated with ruxolitinib pre alloSCT was undertaken at two Australian Institutions. Data analysed included recipient demographics, donor/graft characteristics, conditioning, graft versus host disease (GVHD), overall survival (OS), relapse and non-relapse mortality (NRM).

### **Results**

Between September 2011 and August 2013, 7 patients with primary MF (n=6) or post ET MF (n=1) and median age of 44 years (range 28-59) were treated with ruxolitinib pre alloSCT. According to the Dynamic International Prognostic Scoring System (DIPSS) patients were classified as low risk (n= 2), Intermediate-1 (n=1), Intermediate-2 (n=4). The median duration of treatment with ruxolitinib pre alloSCT was 4.5 months (range 2-16). 4 of the 7 patients had progressive symptoms or splenomegaly on ruxolitinib pre transplant. Conditioning was myeloablative in 5 (71%) transplants. 6(85%) patients received a peripheral blood stem cell graft while 4 (57%) patients received cells from matched sibling donors. Median follow up post transplant was 9 months (range 2-31). Neutrophil and platelet engraftment occurred in 7(100%) and 5(85%) transplants respectively. Cumulative incidence of acute Graft versus host disease was 42% by day 100. OS and NRM of the cohort was 57% and 42% respectively. GVHD was the cause of mortality in all cases. The overall survival of responders to ruxolitinib was 100% as compared to those with progressive symptoms or splenomegaly pre transplant 25%.

### **Conclusion**

Treatment with JAK inhibitors pre alloSCT is feasible and experience in this setting is expanding. Loss of response or disease progression on JAK inhibitors is likely to be associated with significant post transplant toxicities and inferior survival.

## **P023. Therapeutic infusion of partially HLA-matched third-party virus-specific T cells (VST) in HSCT patients with refractory viral infection**

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### **Introduction**

VST can be efficacious for prophylaxis and treatment of viral infections post-HSCT. Use of the HSCT donor for generation of VST can have limitations: the donor's cells must be accessible and virus seropositive. Due to manufacturing time VST are not available in urgent clinical scenarios. Alternate sources of VST are desirable.

### **Aim**

To assess the safety of treatment with partially HLA-matched VST infusions derived from third-party donors, for refractory CMV, EBV, or adenoviral infection in allogeneic HSCT patients.

### **Method**

Peripheral blood or PBSC donor monocyte-derived dendritic cells were pulsed with CMV, EBV or adenovirus peptide mixes and used to stimulate donor mononuclear cells. Resultant activated T cells were expanded over 2 weeks. Patients with persistent viral reactivation/infection after 2 weeks of standard therapy were infused up to four doses of  $2 \times 10^7/m^2$  cryopreserved CMV, EBV, or adenovirus VST. Post-infusion assessment includes: infusion safety, GVHD, and virus-related activity, immune reconstitution, and therapy.

### **Result**

Preliminary results are available for 6 patients treated at Westmead Hospital for persistent CMV reactivation/infection (2 with CMV colitis) after a median of 26 days (19-55) anti-viral therapy. Patients received 1-4 infusions (median 1.5) of 2-4/6 HLA match, and have been followed for a median of 4.4 mths (0.8-10.5). No immediate infusion toxicity occurred. One patient with chronic hepatitis C developed abnormal liver function tests 3 mths post-infusion. One patient died from presumed progressive CMV disease. Four patients achieved a best response of CMV PCR negativity (2 with complete resolution of CMV-colitis). The most recently enrolled patient has shown >50% reduction in CMV copy number over 3 weeks. Median time since cessation of anti-CMV therapy in survivors is 3 mths (0.7-6.6).

### **Conclusion**

Third party VST have effectively cleared CMV infection in some patients, and no major safety concerns have arisen in any treated patient. Recruitment is ongoing.

## **P024. A retrospective analysis of allogeneic stem cell transplantation for chronic lymphocytic leukaemia at a single centre.**

**Das R**<sup>1</sup>, Blyth E<sup>1-3</sup>, Gottlieb D<sup>1-3</sup>

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### **Aim**

Allogeneic stem cell transplantation represents the only curative option for patients with high risk chronic lymphocytic leukaemia (CLL) including those who have relapsed soon after chemoimmunotherapy or who have active 17p deleted or p53 mutated disease. We reviewed our experience with allogeneic transplantation for CLL to consider how best to improve outcomes for patients requiring transplantation.

### **Methods**

We performed a single-centre retrospective study of all patients who underwent HSCT for relapsed CLL from 1998 to 2013. From 2011 onwards, minimal residual disease (MRD) was assessed with flow cytometry of the blood and bone marrow using a single tube 10-colour technique.

### **Results**

Between 1998 and 2013, 23 patients with CLL underwent HSCT reduced intensity conditioning (19 patients) and myeloablative conditioning (4 patients). Donors were HLA- matched related (n=13), haploidentical (n=1), and matched unrelated (n=9). All patients received unmanipulated peripheral blood stem cells. The median follow-up was 24 months. Fifteen patients (65%) suffered from acute graft-versus-host-disease (GVHD). Seven of 15 patients had grade 2, and 8 of 15 patients had grade 3 to 4 disease. Fourteen patients (60%) developed chronic GVHD; 6/23 (26%) limited, 8/23 (34%) extensive. Four patients experienced clinical progression of their disease at a median of 30 months post-transplantation. Non-relapse mortality was 43% at 3 years. MRD was assessed in 10 patients between 2011 and 2013; 5 of these attained negative MRD status post-transplantation at a median of 5.2 months. Overall survival (OS) at one year was 69%, at 3-years was 48% and 3-year progression free survival was 40%.

### **Conclusion**

The OS and PFS in this small patient cohort are similar to previously published data. NRM is a greater problem than disease progression. Improvements are likely to stem from further reducing transplant conditioning intensity, strategies to improve infection control and the use of kinase inhibitors, BH3 mimetics or chimeric antigen receptor-modified T cells to control residual disease detected 6-12 months after transplantation.

## **P025. G-CSF vs Plerixafor in stem cell mobilization and stem cell viability: a single centre audit**

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<sup>1</sup> Pathwest, Nedlands, <sup>2</sup> Sir Charles Gairdner Hospital

### **Background**

Up to 35% of patients fail to mobilise haematopoietic progenitor cells (HPC) post granulocyte colony stimulating factor (G-CSF)+/-chemotherapy. Plerixafor use improves HPC mobilisation success. Review of HPC mobilisation following Plerixafor use compared HPC yields and viability of patients undergoing G-CSF+/-chemotherapy with G-CSF+Plerixafor+/-chemotherapy HPC mobilisation.

### **Methods**

This single institution retrospective review of HPC mobilisation identified patients using stem cell laboratory records. Patient demographics, diagnosis, HPC yields (CD34/kg) and viability were collected on patients who had HPC collection (HPCC) between January 2012 and June 2014.

### **Results**

150 patients underwent HPCC (G-CSF+/-chemotherapy 136, Plerixafor+G-CSF+/-chemotherapy 26, both 2). Plerixafor was used according to hospital HPC mobilisation policies; with G-CSF alone in 7 cases and with G-CSF+chemotherapy in 19. Haematological diagnoses were similar between groups; G-CSF+/-chemotherapy: MM 67, lymphoma 57, other 12 and Plerixafor+G-CSF+/-chemotherapy: MM 13, lymphoma 12, other 1. 65.4% of G-CSF+/-chemotherapy and 42.3% of G-CSF+Plerixafor+/-chemotherapy patients required one apheresis. G-CSF+/-chemotherapy day 1 HPC yields were  $7.5 \times 10^6$ /kg(0.7-104.9) and  $5.4(0.5-88.7)$  (n=136) for pre and post thaw samples respectively, falling to  $4(1.2-15.7)$  and  $3.0(0.7-11.7)$  for day 2 HPCC (n=41) and then  $1.8(0.7-3.2)$  and  $1.5(0.5-3.1)$  for day 3 HPCC (n=6), with no difference in HPC losses between HPCC days. For Plerixafor+G-CSF+/-chemotherapy mobilisation, day 1 HPCC CD34 yields were  $3.4 \times 10^6$ /kg(0.7-12) and  $2.5(0.3-7.2)$  (n=26) compared to  $1.7(0.5-3.2)$  and  $1.3(0.5-2.4)$  on day 2(n=13) for pre and post thaw samples respectively. There was no difference in HPC attrition post processing between mobilisation groups.

### **Conclusion**

Plerixafor use is restricted to patients proven difficult to mobilise HPC when other options for autologous HPCC are limited. We have demonstrated that although CD34 yields are lower in Plerixafor-treated patients, HPC viability and rates of HPC attrition during HPC processing are comparable to those mobilised with G-CSF+/-chemotherapy.

## P026. Successful application of algorithms to guide plerixafor use in the hospital setting

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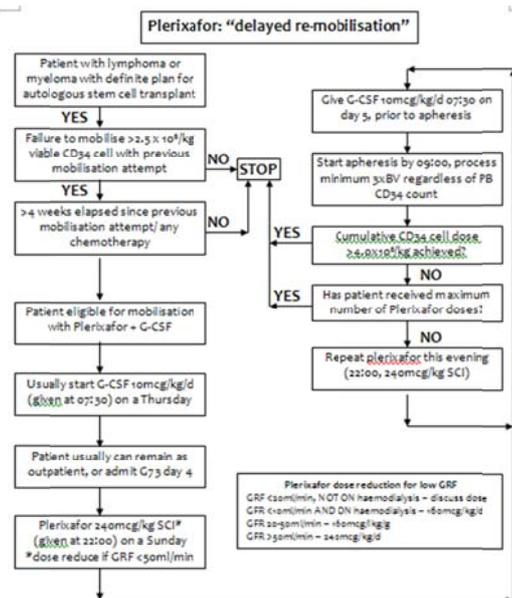
<sup>1</sup> Sir Charles Gairdner Hospital, <sup>2</sup> Pathwest, Nedlands

Plerixafor has been available in Australia for over 2 years for use with granulocyte colony stimulating factor (G-CSF) to mobilise haematopoietic progenitor cells (HPC) into the peripheral circulation but has only recently been PBS listed. The SCGH drug and therapeutic committee supported the supervised use of Plerixafor for HPC collection (delayed remobilisation (DR) and immediate rescue (IR) settings) using Departmental HPC collection algorithms.

**Methods:** Data on Plerixafor use for HPC mobilisation was collected using pharmacy records (April 2011 to May 2014). HPC were collected using a Terumo Optia with a collection threshold of  $20 \times 10^6$  CD34 cells/L; processing up total blood volumes. Patient records and apheresis datasheets were reviewed.

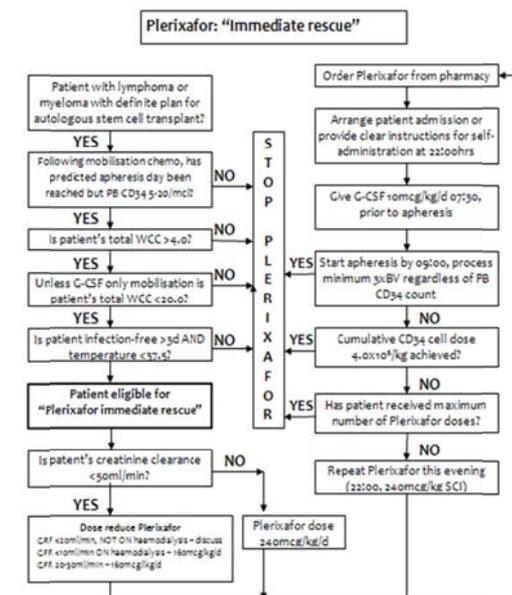
**Results** Plerixafor was used on 32 mobilisation cycles (n=30:15 males, 5 females) in DR (n=7)

IR (n=21) settings. Patient diagnoses were multiple myeloma (n=13), lymphoma (n=13), other (n=2). One patient failed to mobilise HPC following use in the IR setting twice. Another patient required a second mobilisation cycle due to low viability of initial HPC collection. Mobilisation success was achieved with Plerixafor in 30/32 cycles (93.8%): DR 7/7(100%) and IR 19/21(90.5%). One Plerixafor dose(240mcg/kg) was sufficient in 11 cases; two doses were used on 21 occasions. Median CD34 yields were  $3.8 \times 10^6$ /kg for those requiring one dose. For those requiring 2 doses, yields were  $2.6 \times 10^6$ /kg (n=18) and  $1.9 \times 10^6$ /kg (n=19) following 1st and 2nd doses respectively. Venous access issues prevented day 1 HPC collection in 1 patient. 23 of 29 patients have undergone autologous HPC transplant. Reasons for no transplant are progressive disease(n=2), 'rainy day' HPC collect(n=3), allogeneic HPC transplant(n=1).



to 3

and



**Conclusion** Plerixafor can be reliably used to augment HPC collection in the hospital setting. The challenge will be to modify current practice to work within PBS restrictions.

## P027. Post thaw viable CD34 cell count: Influence on engraftment after peripheral blood stem cell transplant

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**Aim** Haematopoietic progenitor cells (HPC) are cryopreserved prior to autologous peripheral blood progenitor cell transplantation. Loss of viable CD34 cells during the freeze thaw process is inevitable. Most laboratories perform a pre-cryopreservation CD34 cell count, which is used for the target cell dose required for the transplant, and post-thaw viable (PTV) CD34 cell counts on HPC product. However the value of the post-thaw viable CD34 cell count is not established. The aim of our study was to assess the effect of the PTV CD34 cell count on the rate of haematopoietic engraftment following autologous peripheral blood stem cell transplantation.

**Method** Sixty eight patients (21Female, 47 Male) underwent autologous peripheral stem cell transplantation between January 2011 and May 2014. PTV CD34 cell count (n=68) was performed using a single platform flow cytometry method, with incorporation of 7AAD dye to assess viability. Following reinfusion of HPC's the time to engraftment of neutrophils and platelets was assessed by blood counts performed daily. Two patients who never had platelets below  $20 \times 10^9/L$  and a patient who did not engraft platelets were excluded from platelet engraftment analysis as was a patient who died on day +20, who engrafted neutrophils though not platelets. Statistical methods used the STATA statistical programme.

**Results** Patients were divided into 3 groups based on the PTV CD34 cells infused. Results of univariate analysis (table) showed neutrophil engraftment (p=0.1) and platelet transfusions (p=0.18) being non-significant, though platelet engraftment (p=0.018) was significantly associated with the number of PTV CD34 cells infused. Multivariate analysis (which included sex, age, platelet transfusions, days to neutrophil engraftment, days to platelet engraftment, diagnosis and time cells stored as covariates) showed only days to platelet engraftment as being significant (p=0.018).

	All patients (n=68)	PTV CD34 $\leq 3 \times 10^6/Kg$ (n=7)	PTV CD34 $3-5 \times 10^6/Kg$ (n=34)	PTV CD34 $\geq 5 \times 10^6/Kg$ (n=27)
PTV CD34 cells infused ( $\times 10^6/Kg$ ) Median (range)	4.65 (1.4-44.44)	2.63 (1.4-2.96)	4.3 (3.07-4.98)	5.78 (5.1-44.44)
Days to neutrophil $>0.5 \times 10^9/L$ Median(range) p=0.10	11 (8-13)	11 (10-12)	11 (9-12)	10 (8-13)
Days to platelets $20 \times 10^9/L$ Median(range) p=0.018	16 (14-43)	19 (15-43)	16 (14-36)	16 (13-21)
No. of Platelet transfusion (pool platelet bags) Median(range) p=0.18	2 (0-22)	3 (1-22)	2 (0-8)	2 (1-8)

**Conclusion** In the current study post-thaw viable CD34 cells infused was found to be predictive of platelet engraftment, though not neutrophil engraftment.

## **P028. A retrospective study of chimaerism after reduced-intensity conditioned allogeneic transplantation for haematological malignancy at a single centre**

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### **Aim**

Establishment of full donor chimaerism (FDC) has been associated with increased survival after reduced-intensity conditioning (RIC) before allogeneic hematopoietic cell transplant (HCT). The aim of the study was to assess chimaerism before and after donor lymphocyte infusion (DLI) and correlate with overall survival.

### **Method**

Patients aged  $\geq 18$  years ( $n=62$ ) undergoing allogeneic HCT after a RIC regimen for a haematological malignancy at our institution between 2000-2012 were identified from a database and results of chimaerism studies and outcome were recorded. FDC was defined as  $>95\%$  donor T-cells. The time from infusion of stem cells to FDC was recorded according to variables including diagnosis, donor source and conditioning regimen. The response to DLI in patients with mixed donor chimaerism (MDC) was recorded.

### **Results**

62 patients met the inclusion criteria. The indications for HCT were myeloma (23), acute myeloid leukaemia (13), non Hodgkin lymphoma (9), myelofibrosis (5), chronic lymphocytic leukaemia (5), myelodysplastic syndrome (3), chronic myelomonocytic leukaemia (3) and chronic myeloid leukaemia (1). The conditioning regimen was fludarabine and total body irradiation (Flu-TBI) (28), Alemtuzumab Fludarabine and Melphalan (19), Fludarabine and Melphalan (6), Fludarabine Busulphan-Anti Thymocyte Globulin (ATG) (2), Fludarabine Cyclophosphamide Rituximab (2), TBI (2), Fludarabine Cyclophosphamide-TBI (1) and Fludarabine Melphalan-ATG (1). One patient was transplanted during aplasia following FLAG-IDA chemotherapy and received no further conditioning. Chimaerism was measured in 59/62 patients at least once (median of 4 measurements; range 1-9). One patient who died on day 42 with acute Graft versus Host Disease (GVHD) did not have chimaerism measured. 38/62 (61%) reached donor T-cells  $>95\%$  without DLI. The number of patients achieving FDC on day 28, 56 and 90 post HCT was 10, 17 and 21 respectively. 37 patients developed either acute or chronic GVHD following HCT. 21 patients received DLI for mixed donor chimaerism (11), disease relapse (9) and post transplant lymphoproliferative disease (1). 9/11 patients with MDC converted to FDC after DLI bringing the rate of T-cell chimaerism  $>95\%$  to 47/62 (76%). 21/47 patients (45%) in the FDC group died. The most common cause of death was relapsed disease. 15 patients did not achieve FDC. 3/15 achieved donor T-cells  $>90\%$  but  $<95\%$  and all survived. All 12 patients who did not achieve donor T-cells  $>90\%$  died. The cause of death in those patients not achieving FDC was disease relapse (4), infection (2), veno occlusive disease of the liver (1), cardiac (1) and other malignancy (1). 3 of the patients were lost to follow up.

### **Conclusion**

RIC allogeneic HCT was successful in achieving FDC in our institution. The majority of patients developed GVHD following HCT. DLI was successful in converting MDC to FDC. The prognosis of patients with persisting MDC was dismal – all died. DLI should be considered in patients failing to reach FDC.

## **P029. Local inflammatory cues permit and augment immunotherapy mediated by central memory T cells**

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<sup>1</sup> Royal Melbourne Hospital, <sup>2</sup> The University of Queensland Diamantina Institute, <sup>3</sup> University of Adelaide

### **Aim**

Adoptive T cell therapy utilises the exquisite specificity of the adaptive immune system to target cancer. Yet the mechanism and means by which to enhance T cell function are incompletely described. We aimed to describe an optimised regime of cell-mediated immunity in epithelium. In particular, this study investigated the interactions between adoptively transferred CD8<sup>+</sup> T cells and the innate immune system.

### **Method**

To optimise immunotherapy, the immunobiology of adoptively transferred T cells in a murine model of cutaneous immunity was examined. In this model, skin bearing a foreign antigen was grafted onto recipient mice. The donor skin or the host animal then received augmented T cell immunotherapeutic regimes with the kinetics of skin graft rejection used as a marker of adaptive immune response.

### **Results**

We show that *in vitro* derived central but not effector memory T cells bring about rapid regression of skin expressing foreign antigen. Local inflammation induced by the TLR7 receptor agonist, imiquimod, decreases time to skin graft rejection elicited by central but not effector memory T cells in an immunodeficient mouse model. In this model, IL-2 facilitates the development of *in vivo* of effector function from central memory but not effector memory T cells. In a model of T cell tolerogenesis, we further show that adoptively transferred central but not effector memory T cells permit successful cutaneous immunity that is dependent on a local inflammatory cue in the target tissue at the time of adoptive T cell transfer.

### **Conclusion**

Adoptive T cell therapy efficacy can be enhanced if CD8<sup>+</sup> T cells with a central memory T cell phenotype are transferred and IL-2 is present with contemporaneous local inflammation. These findings have significant implications for adoptive immunotherapy for malignancy.

## P030. A retrospective comparison of non-myeloablative allogeneic haemopoietic stem cell transplant versus chemotherapy-only in older patients with Acute Myeloid Leukaemia

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**Aim** To investigate the safety and efficacy of non-myeloablative allogeneic haemopoietic-stem-cell transplantation (NMA) for Acute Myeloid Leukaemia (AML) patients aged >55 years in first remission (CR1).

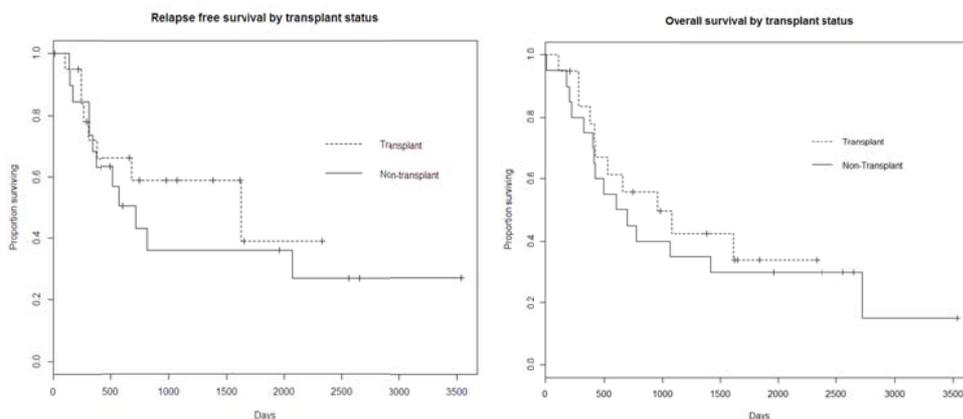
**Method** Two cohorts of AML patients identified from departmental databases. Patients receiving NMA conditioning with Fludarabine 45mg/m<sup>2</sup> days -4 - -2 and low-dose total-body-irradiation (TBI 2Gy) from January 2008 - December 2013 compared to a similar cohort preceding the NMA program (January 2004 - December 2008). Patients were eligible based upon intermediate-risk based upon cytogenetics (revised MRC) irrespective of FLT3-ITD achieving CR1 following 7+3 or HiDAC.

**Results** 19 NMA (9 siblings, 10 unrelated) were identified fulfilling eligibility. Median time from CR1 to transplant was 148 (9-486) days. Eighteen performed as outpatients; three requiring admission before day 30. Median duration of neutrophils <0.5x10<sup>9</sup>/L was 5 days (0-32). 10/17 achieved >95% CD3+ and CD33+ donor-chimerism by D+180. 4 patients had DLI before D+180 for falling chimerism. Twenty chemo-only patients identified fulfilling eligibility. NMA were younger than chemo-only patients (median 61yrs vs 68yrs; p=0.06). FLT3-ITD+ was similar (3/15 in NMA, 3/8 in chemo-only). Median follow-up was shorter for NMA (53 vs 85 months, p=0.0007).

Median overall survival (OS) and event free survival for NMA and chemo-only was 31 vs 21 months (p=0.57) and 17 vs 22 months (p=0.5). 3-year OS and relapse free survival was 42% vs 35% (p = 0.32) and 59% vs 36% (p=0.11). 32% of NMA patients developed ≥ grade 2 aGVHD, 47% extensive cGVHD and 26% treatment-related-mortality (TRM, all in context of GVHD).

**Conclusion** NMA is deliverable in older AML patients as outpatient therapy with successful engraftment and low early morbidity. Patient numbers limit conclusions on OS however it appears at least equivalent to a non-transplant approach, which provides a backbone for further investigation of the approach utilising methods to reduce GVHD and relapse.

*Kaplan-Meier plots of Relapse-free (left) and Overall (right) survival by transplant status*



## P031. Evaluation of plerixafor to rescue G-CSF-primed chemo-mobilisation of peripheral blood stem cells using an algorithm-based approach

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**AIM** Plerixafor augments stem cell mobilisation via disruption of the CXCR4 receptor and stromal derived factor-1 interaction. The benefits of rescuing a failing G-CSF-primed stem cell mobilisation with this agent are well documented. However, limited data exists on its use to salvage failing G-CSF-primed chemo-mobilisation. We evaluate an algorithm-based approach using plerixafor in this setting.

**METHOD** The algorithm uses peripheral blood CD34 count (PBCD34) and leucocyte count to advise the use of plerixafor when there is either:

- 1) Failure to achieve PBCD34 of  $1 \times 10^4$ /mL with a rising leucocyte count ( $\geq 4 \times 10^9$ /L) by the anticipated day of collection. **or**
- 2) Suboptimal first day collection ( $CD34 \leq 1 \times 10^6$ /kg) despite an adequate PBCD34 ( $\geq 1 \times 10^4$ /mL).

The target cell dose was  $\geq 2 \times 10^6$ CD34/kg. Records were retrospectively reviewed. The primary endpoint was efficacy of the approach. Secondary endpoints were number of procedures required, the number of plerixafor doses and the CD34+ cell dose of apheresis product.

**RESULTS** A total of 226 autologous mobilisations occurred at our institution between Jan 2009 – June 2014. Plerixafor was used on 22 occasions. 5 were for remobilisation after a previous failed attempt, 1 was for non algorithm-based rescue and 16 followed the algorithm.

N= 16	Median (range)
Age yrs	56(27-68)
Male	10
Diagnosis	
MM	2
NHL	12
HL	2
No of plerixafor doses	1 (1-2)
No of apheresis procedures	2 (1-2)
Pre Plerixafor PB Leucocyte count ( $\times 10^9$ /L)	5.3 (3.2-19.5)
Pre Plerixafor PBCD34 ( $\times 10^4$ /mL)	0.5(0.1-1)
Post Plerixafor PBCD34 ( $\times 10^4$ /mL)	2.1(0.1-9.5)
Total no of CD34 cells mobilized ( $10^6$ /kg)	2.8 (0-6.8)
Target Cell dose achieved	13 (81.3%)*

\* 3 Failures: CD34 dose ( $\times 10^6$ /kg) of collection – 0,0.4,1.4 respectively. Plerixafor doses used 2,1,2 respectively.

**CONCLUSION** An algorithm-based approach with plerixafor can be successfully employed to rescue a failing G-CSF-primed chemo-mobilisation.

## **P032. Real-time quantitative PCR of insertion/deletion polymorphisms is a precise and sensitive test for measuring mixed chimerism post allogeneic HSCT**

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### **Aim**

Quantitation of short tandem repeats (STR) post DNA amplification is the routine method for measuring mixed chimerism post allogeneic haematopoietic stem cell transplantation (HSCT) in Australia. This method has limited sensitivity to approximately 5% cell chimerism. We have examined the use of insertion/deletion-specific hybridisation probes with real-time quantitative PCR (RT-qPCR) to determine if this alternate method is more sensitive and precise.

### **Method**

We have examined 10 primer sets designed in-house and the AlleleSEQR Chimerism Assay with 34 markers provided by a commercial supplier. Primer sets were examined across a range of DNA samples to determine polymorphism frequency, titration experiments were performed to determine sensitivity and precision at the 1% and 5% mixed chimerism level and archived patient DNA samples used to compare RT-qPCR to the routine STR methodology.

### **Result**

Genetic polymorphisms were studied across 19 individuals from diverse racial backgrounds. All individuals could be differentiated from one another on the basis of this panel of markers with most individuals being polymorphic at multiple markers. Seven commercial primer sets were selected for further analysis and 30 replicate assays performed at the 1% and 5% chimerism levels. Mean quantitative values across the seven markers were  $1.28 \pm 0.52$  and  $5.9 \pm 1.1$  at the 1% and 5% level, respectively, indicating a high level of precision. Sensitivity of the insertion/deletion markers was typically below 0.1% mixed chimerism. Both RT-qPCR and STR analysis performed on archived patient material provided similar chimerism results with the RT-qPCR methodology identifying small amounts ( $\leq 2\%$ ) of recipient DNA at early time points post-transplant that were not identified by the STR method.

### **Conclusion**

Measurement of mixed chimerism by RT-qPCR is a more precise and sensitive test for low level mixed chimerism than STR testing. Experiments are now prospectively examining new onset of low level mixed chimerism as a marker for early disease relapse.

### **P033. Novel Gene Regulatory Network in diabetic bone marrow-derived endothelial progenitor cells**

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Endothelial progenitor cells (EPCs) are a group of rare cells that originate from bone marrow (BM) or the wall of blood vessels. They are believed to play an important role in the repair of injured vascular endothelial cells and assisting in reperfusion of ischemic tissue. Decreased production and/or loss of function of EPCs are associated with diabetic vascular complications such as diabetic retinopathy, nephropathy and cardiovascular disease. However, the molecular mechanisms by which diabetes impairs EPCs remain unclear. In this study we conducted microarray analysis of the differential gene expression between Akita diabetic mice and age-matched non-diabetic controls in BM-derived Lin<sup>+</sup> cells and Lin<sup>-</sup>/VEGF-R2<sup>+</sup> EPCs isolated from animals 18 weeks after diabetes. EPCs were isolated using MACS technology based on hematopoietic lineage depletion followed by enrichment for VEGF-R2<sup>+</sup> cells to produce Lin<sup>-</sup>/VEGF-R2<sup>+</sup> EPCs. Lin<sup>+</sup> fractions were kept and used as non-hematopoietic cells for analysis. RNA was extracted, processed and then hybridized to mouse WG-6 V2 beadchips, followed by data analysis. In total, 11 differentially expressed genes were identified as specific to BM EPCs including 3 genes (*CLCNKA*, *PIK3C2A*, *PTF1A*) with known association with diabetic complications and 8 genes classified as transcription factors (*PPARG*, *PPARA*, *VDR*, *FOXO1*, *AR*, *NFKB1*, *HNF4A*, *SREBF1*). Further analysis led to establishing a novel gene regulatory network specific to diabetic EPCs, which includes 11 main well documented diabetic genes and 47 genes and transcription factors regulating/regulated directly by those genes. Our results suggest that diabetes may influence specific signature genes in BM EPCs altering their capacity to proliferate and differentiate.

## **P034. Outcome of patients with lymphoma following autologous peripheral stem cell transplant with BEAM conditioning at the single tertiary centre**

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### **Objectives**

Outcomes following autologous peripheral stem cell transplant (AuSCT) with BEAM conditioning in patients with lymphoid malignancy is quite variable. In this heterogeneous disease group with variable indications for AuSCT it is important to ascertain long-term outcome.

### **Methods**

This is a retrospective analysis on outcome of lymphoma patients undergoing AuSCT with BEAM conditioning, treated at our Institute between 2004 and 2013. Data was extracted from the in-house transplant database and e-medical records and key outcome variables analysed retrospectively using SPSS and Graphpad prism software.

### **Results**

Total of 88 patients with lymphoma underwent 89 AuSCTs with BEAM conditioning. Median age was 57 years (23-74 years) and M:F was 62:27. In regard to the diagnosis and disease status at transplant, Diffuse large B cell lymphoma (DLBCL) was the most common indication requiring AuSCT which was in 31 cases (CR1-13, CR2-16, CR3 and beyond-2) whereas nine patients with Follicular cell lymphoma underwent this intervention (CR 1 and 2 – 4, CR>2 – 5). The remainder of 30 patients had the following diagnosis: Hodgkin disease – 6, mantle cell lymphoma - 7, T cell NHL – 4 and other lymphoproliferative disorders - 9. Median time to AuSCT from time of diagnosis was 24 months (5-300 months). Mean CD34 dose was  $3.26 \times 10^6 / \text{kg}$  (1.34 - 7.07). The median follow up duration was 64 months (range 6-124). Full haematopoietic engraftment was achieved in 98.9% of patients. Median time to leukocyte and platelet engraftment was 10 days (7-49 days) and 15 days (9-71 days) respectively. Overall survival (OS) and disease free survival (DFS) after BEAM autologous peripheral stem cell transplant were 86.25% and 66.2% respectively whereas non-relapse mortality rate was 1.25%.

### **Conclusion**

This study provides long-term follow up data of patients treated at our institution with BEAM AuSCT and supports the observation that long-term lymphoma free survival is achievable in about 2/3 of patients with an array of lymphoma types.

## **P035. A phase I clinical trial of peptide-pulsed monocyte-derived dendritic cell vaccination to expand adoptively transferred CMV-specific cytotoxic T lymphocytes after allogeneic haematopoietic stem cell transplantation (HSCT)**

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### **Aim**

We present preliminary data on a phase I clinical trial of adjuvant dendritic cell (DC) vaccination given with CMV specific T cells to patients who have undergone allogeneic HSCT.

### **Methods**

CMV specific T cells and monocyte derived dendritic cells (DC) were generated according to established protocols, using an HLA-A2 restricted epitope of CMV pp65 (NLVPMVATV) as antigen. HLA-A2 positive patients with CMV seropositive donors were recruited. Transplant recipients received a CMV specific T cell infusion from day 28 and two intradermal CMV peptide-pulsed DC vaccinations, one week apart. Patients were monitored for adverse events, efficacy and CMV specific immune reconstitution.

### **Results**

4 patients received CMV specific T cells and DC vaccines 32-93 days post HSCT and have been followed for 5 to 12 months. No immediate infusion or vaccination related adverse events were noted. In 1 patient, the second DC vaccine was omitted due to development of acute graft versus host disease (GVHD) in the week after T cell infusion and initial DC vaccination. The patient went on to develop grade III skin and gut GVHD and CMV colitis. Three patients have developed chronic (c)GVHD, 2 extensive, 1 limited. No other patients have developed CMV reactivation to date. Increased immune reconstitution against CMV was demonstrated in 3 patients tested. ELISPOT analysis on pre- and post- infusion samples showed a mean of 210 SFU/10<sup>5</sup> cells and 1395 SFU/10<sup>5</sup> cells before and within 100 days post infusion respectively, and NLV-tetramer analysis revealed a mean of 2.1 fold increase in tetramer specific T cells post infusion.

### **Conclusion**

Adoptive immunotherapy with CMV specific T cells and adjuvant DC vaccination enhanced CMV specific immune reconstitution above baseline. The small patient numbers do not permit conclusion about GVHD risk. Adjuvant DC vaccination may be of value in expanding antigen specific T cells following adoptive immunotherapy.

## **P036. Establishment of an Australian bank of third party antiviral T lymphocytes**

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### **Introduction**

Adoptive transfer of donor derived virus specific T cells can be effective therapy for infections in allogeneic HSCT recipients. However, this is not a practical strategy to treat acute infections due to the time required to prepare products and potential donor unavailability. To overcome this, treatment with cryopreserved partially HLA matched T cells from third party donors is being investigated. Recent reports describe disease resolution using cells matched at only one or two HLA alleles. This less stringent requirement for matching would allow a small bank of cells to provide most patients with a therapeutic product. We describe the establishment of a T cell bank to treat patients who have failed antiviral pharmacotherapy.

### **Methods**

Products were generated by co-culturing peripheral blood mononuclear cells with dendritic cells loaded with overlapping peptides covering cytomegalovirus pp65, adenovirus hexon or Epstein barr virus BZLF1, LMP2A and EBNA1 proteins. Cultures were re-stimulated once with peptide loaded dendritic cells and cultured for 14 days with interleukin-2.

### **Results**

T cell products have been expanded from 25 donors to create a bank of 177 bags of virus specific T cells (75 CMV, 47 AdV and 55 EBV). CMV specific products were predominantly T cells (mean 97.4±2.9%) with variable CD8<sup>+</sup> (mean 45.5±23.3%) and CD4<sup>+</sup> (41.2±23.3%) composition. Specificity could be mapped to epitopes restricted to HLA-A\*0101, A\*0201, A\*2402, B\*0702 and B\*3501. Eight of 12 adenovirus specific cultures had a higher proportion of CD4<sup>+</sup> (mean 54.3±22%) than CD8<sup>+</sup> (mean 34.4±21%) T cells. Specificity was mapped to epitopes restricted to HLA-A\*0101 and multiple MHC class II alleles (HLA-DRB1 \*0101, DRB1\*0301, DRB1\*0701, DRB1\*1501). Specificity of EBV products to the three antigens was variable. Based on HLA frequency analysis we estimate 94%, 89% and 74% of patients would have access to a CMV, AdV and EBV specific product respectively with the current bank.

## **P037. Factors influencing graft versus host disease in recipients of blood stem cell transplants.**

**Panicker S, Blyth E**

*Westmead Hospital*

### **Aim**

To assess the incidence and risk factors for acute and chronic GVHD, overall survival (OS) and progression free survival (PFS).

### **Method**

The study population consisted of adults  $\geq 18$  years of age who underwent an allogeneic haematopoietic stem cell transplantation between the 1<sup>st</sup> of January 2012 and the 1<sup>st</sup> January 2014, at the Blood and Marrow Transplant Unit at Westmead Hospital.

The OS and PFS were estimated using the Kaplan and Meier Method. A univariate analysis of the impact of donor source, type of conditioning, T-cell depletion and GVHD prophylaxis on acute or chronic graft versus host disease was performed. These variables were tested for statistical significance using the log rank test (Mantel-Cox). The impact of age and the percentage of apoptotic cells in the stem cell infusion (7AAD%) on acute and chronic GVHD were determined by Cox-Regression analysis.

### **Results**

121 patients were included in the study. The median follow up of the entire patient cohort after Allo-HSCT was 12.6 months (range 1-27.8m). The median OS and PFS after Allo-HSCT was not reached in our patients. The estimated PFS and OS at 18 months were 70% and 75% respectively.

Though there was no statistically significant influence of the recipient age on acute or chronic GVHD there was a trend for increasing risk of aGVHD with increasing age of the patient ( $p=0.07$ ). No impact of the donor source ( $p=0.63$ ), type of conditioning ( $p=0.23$ ) or T-cell depletion ( $p=0.63$ ) on acute GVHD could be demonstrated. The presence of 7AAD positive cells  $\geq 2.5\%$  also did not have any influence on the outcome of acute GVHD.

Those patients who had T cell depleted grafts had significantly low incidence of chronic GVHD ( $p=0.04$ ) where as those patients who received higher amount of apoptotic cells developed significantly low levels of chronic GVHD ( $p=0.05$ ).

### **Conclusion**

The impact of apoptotic cells (7AAD positive) on chronic GVHD requires further study in a larger population cohort as it may have therapeutic implications in minimising this devastating complication.

## **P038. Conditioning chemotherapy dose adjustment in obese individuals: The Royal Melbourne Hospital experience with Etoposide**

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*The Royal Melbourne Hospital, Clinical Haematology and BMT Service*

### **Background/Aim**

Chemotherapy dosing in obese patients undergoing haematopoietic stem cell transplantation has largely been based on empiric data due to a paucity of evidence in transplant populations. Traditionally at the Royal Melbourne Hospital (RMH), patients with a Body Mass Index (BMI)  $>27\text{kg/m}^2$  have had an idealised body weight calculated, adjusting their weight to provide a BMI equal to  $27\text{kg/m}^2$ . We look at how this approach compares with recent American Society for Blood and Marrow Transplantation (ASBMT) dose adjustment guidelines, using Etoposide as an example.

### **Methods**

We extracted data for 50 overweight/obese (BMI  $>27\text{kg/m}^2$ ) patients who had previously received dose adjusted Etoposide at RMH, 25 receiving Etoposide/TBI (mg/kg directed dosing) and 25 receiving BEAM (BSA directed dosing). ASBMT recommended doses were calculated for each and compared with the actual dose given. A subset analysis was performed, isolating patients with a BMI  $>30\text{kg/m}^2$ .

### **Results**

For the Etoposide/TBI cohort, the RMH dose adjustment showed a mean dose increase of 9.7% ( $p < 0.001$ ) compared with the ASBMT schedule. This difference decreased when isolating the 8/25 patients with a BMI  $>30\text{kg/m}^2$ , with only a 6.9% increase noted ( $p = 0.004$ ).

Conversely, the BEAM cohort had a mean dose reduction of 8.7% ( $p = 0.001$ ) compared with the ASBMT dose. This difference was exaggerated in the BMI  $>30\text{kg/m}^2$  subgroup (16/25) who had a mean dose decrease of 12.2% ( $p < 0.001$ ).

### **Conclusion**

The RMH dosing adjustment showed little difference from the ASBMT recommendation, with less than a 10% difference being seen overall in Etoposide dosing. Differences became more apparent for BSA based dosing in patients with a BMI  $>30\text{kg/m}^2$ . There remains a paucity of evidence on conditioning chemotherapy dosing in obese individuals. Further trials are required in this area to build a solid evidence base for future recommendations.

## **P039. The relationship between pre-transplant 25-hydroxy-vitamin D levels, survival and graft-versus-host disease, in allogeneic haematopoietic stem cell transplantation**

Perera T, Lim A, Mason K, Szer J, Ritchie D

*The Royal Melbourne Hospital, Clinical Haematology and BMT Service, Melbourne, Australia*

### **Background/Aim**

Low serum vitamin D levels are becoming increasingly implicated in infections, pulmonary disorders, cancer incidence and autoimmune conditions. Their role in allogeneic haematopoietic stem cell transplantation (alloHSCT) remains unclear, with some studies showing deficiency to be associated with lower overall survival (OS) and increased graft versus host disease (GVHD) rates, while other studies show no differences in OS or GVHD rates. We investigated the relationship between low vitamin D levels pre-alloHSCT and post-transplant outcomes (OS, progression-free survival [PFS], non-relapse mortality [NRM], relapse and acute and chronic GVHD).

### **Methods**

We reviewed 492 alloHSCT recipients who had pre-transplant vitamin D results available. Data on dates of death, last follow-up, disease progression/relapse and acute and chronic GVHD status were collected. Patients were categorised as replete (25-OH-Vit D  $\geq$  50nmol/L or on replacement therapy) or deficient (25-OH-Vit D < 50nmol/L). Subgroup analysis was performed on B-cell non-Hodgkin lymphoma patients (B-NHL).

### **Results**

The vitamin D-deficient cohort had a higher mortality rate compared to the replete group. This reduction in survival was maintained in the multivariate analysis (HR 1.5, 95% CI 1.1-2.0, P=.013). There were no significant differences in NRM, PFS, acute/chronic GVHD, or relapse rates between the two groups. No significant differences were noted with any of these outcomes in the 123 B-NHL patients.

### **Conclusion**

Vitamin D deficiency appears associated with increased mortality in alloHSCT recipients. The mechanisms of this finding remain unclear. GVHD rates did not appear to be affected by deficiency. Further research looking at whether immunomodulatory effects of vitamin D are responsible for the survival differences noted in our study, and previously reported studies, is required.

## **P040. Pre-screening for capacity to consent as a potential family bone marrow donor**

**Presta M**, O'Flaherty E, Lim A, Dowsing C, Sipavicius J, Ritchie D

*Royal Melbourne Hospital*

### **Aim**

When bone marrow transplantation (BMT) is indicated, identification of a suitable donor is often a matter of urgency. However, donation by a patient's family member can present substantial psychosocial, ethical, medical and medico-legal challenges different from those presented by volunteer unrelated donors. The World Marrow Donor Association (WMDA) has standards for the care of matched family donors once identified, however no guidelines exist for the pre-test counselling and consent processes particularly in those potential donors who have impaired capacity.

### **Methods**

We assessed the incidence of family donors searches for transplants undertaken at RMH or one of our major referring institutions, over an 18 month period where there was identified impaired capacity for consent in the potential donors.

### **Results**

A total of 301 individual family member tests were undertaken in the specified time period. From these we identified 6 family members who had impaired legal capacity to consent to HLA testing due to intellectual impairment (2), below the adult age of consent (3), psychiatric illness (1).

In the cases of intellectual impairment there were significant medico-legal implications that delayed HLA-testing and release of the results.

At the Royal Melbourne Hospital we have initiated a policy for the pre-screening and education of family members, obtaining informed written consent prior to undertaking tissue typing, in addition to consent for infectious disease testing, collection, storage and discard of products and recruitment to research studies. This process has helped address family concerns, enhance administrative processes and mitigate potential delay in the timing of donor identification.

### **Conclusions**

All potential family donors should be pre-screened for potential impaired capacity to consent prior to HLA blood testing. All potential donors should have access to an independent advocate to provide counselling regarding the implications of HLA-typing and provide instructions regarding the release of results.

## **P041. Severe anaphylactic reaction with Haemopoietic stem cell infusion from a sibling donor HLA- & blood group matched**

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### **Aim**

To report the case of a severe anaphylactic reaction during intravenous infusion of HLA-matched peripheral blood stem cells from a sibling donor. The patient was transplanted for AML in second remission, and was blood group and sex-matched with the donor, with the cells being infused shortly after collection. We review available literature and management options.

### **Method**

Reviewing clinical and laboratory information, as well as published data via PubMed, Haemopoietic stem cell (HSC) transplantation reactions have been identified during or after infusion usually attributed to the presence of cryoprotectant, associated volume, Human Leucocyte Antigen (HLA) or blood group incompatibility. It is uncommon for an anaphylactic reaction to occur in a donor receiving stem cells from a HLA matched and ABO/Rhesus compatible sibling donor without the presence of cryoprotectant.

### **Results**

A 52 year old female with Acute Myeloid Leukaemia (AML) was admitted for a sibling HSC transplant with cyclophosphamide and busulphan conditioning. There were a total of  $5.1 \times 10^6$  stem cells in a volume of 560mls. The recipient had previous allergies to Vancomycin, Penicillin and was noted to have a rash to a platelet transfusion once previously, and documented anti-platelet antibodies.

Thirty minutes from the commencement of the infusion, the recipient had a marked facial urticarial rash followed by dyspnoea associated with laryngeal swelling and angioedema. A total of 278mls had been infused prior to cessation of the initial infusion. Her symptoms resolved after the administration of intravenous anti-histamines, steroids and subcutaneous adrenaline. The remainder of the cells were stored overnight at 4°C and then processed to remove platelets and plasma. Following pre-medication, the processed cells were slowly infused the next day without any complications.

### **Conclusions**

While uncommon, we report a severe anaphylactic reaction during infusion of HLA-matched peripheral blood stem cells from a sibling donor. Awareness in this clinical setting of patients with allergies and anti-platelet antibodies is suggested, together with considering graft manipulation (such as removal of donor platelets and plasma).

## **P042. Cotransplantation of Haploidentical related peripheral blood stem cells and umbilical cord for severe aplastic anaemia**

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### **Aim**

We report the case of co-transplantation of haploidentical CD34+ enriched peripheral blood and unrelated umbilical cord stem cells in a patient with severe refractory aplastic anaemia (SAA). The best chance of cure for young patients with SAA is allogeneic stem cell transplantation (SCT). However, only approximately 30% of patients will have a HLA matched sibling donor. A recent case series has reported early engraftment and encouraging survival with the novel approach of cotransplanted haploidentical and cord blood stem cells.

### **Method**

We electively admitted a 22 year old female with relapsed/ refractory SAA. After diagnosis at age 12 she achieved remission following ATG based immunosuppression. Her subsequent course was marked by relapse and refractoriness to multiple therapies including double cord transplant and Eltrombopag. At the time of this transplant she had severe pancytopenia with significant bleeding and infection complications.

### **Results**

The conditioning regimen consisted of ATG, Fludarabine, Cyclophosphamide and low dose total body irradiation (TBI). Immunosuppression was with Tacrolimus and Mycophenolate Mofetil. Neutrophil engraftment occurred at day +10 and platelets after day +18. We will present data on KIR matching, Graft versus Host Disease (GVHD) and chimerism.

### **Conclusion**

This case supports the emerging literature indicating that co-transplantation of haploidentical haematopoietic and umbilical cord stem cells is a promising approach for patients with SAA and no well matched donors

## **P043. Choice of conditioning regimen influences risk of Thymoglobulin infusion reactions in allogeneic haematopoietic cell transplantation**

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*The Royal Melbourne Hospital, Melbourne, VIC, Australia*

### **Background**

Thymoglobulin (Genzyme, Mass., USA) is used in allogeneic haematopoietic cell transplantation (alloHCT) for graft-versus-host disease (GVHD) prophylaxis. Infusion-related reactions are common and challenging to manage. We explored characteristics of Thymoglobulin infusion reactions (TIRs), predictors of TIR and the relevance of TIR to post-alloHCT outcome.

### **Method**

We reviewed records of 113 patients who received Thymoglobulin prior to alloHCT for haematologic malignancy. We defined TIR as fever (temperature  $\geq 38^{\circ}\text{C}$ ); rigors; or two or more episodes of heart rate  $> 120$ , respiratory rate  $> 26$ , oxygen saturation  $< 92\%$ , systolic blood pressure  $< 90$  mmHg, occurring within 24 hours of commencement of Thymoglobulin infusion, without bacteraemia.

### **Results**

Fifty-one patients (45%) experienced TIR. Of these, 88% first developed features of TIR during the infusion. Features of TIR were fever in 90%, rigors in 58%, tachycardia in 34%, hypotension in 18%, tachypnoea in 16% and hypoxia in 10%. No patients required intensive care transfer. On univariate analysis (Fisher exact test), age, conditioning regimen, and alloHCT for chronic lymphoproliferative disorder, were significantly associated with TIR. On multivariate analysis (logistic regression), only choice of conditioning remained significant. The influence of conditioning remained significant after adjustment for age and disease type. Incidences of TIR were 13% (2/16) for total body irradiation (TBI)/etoposide (VP16), 19% (5/26) for busulfan/cyclophosphamide (BuCy), 60% (18/30) for fludarabine-based reduced intensity regimens, and 63% (26/41) for cyclophosphamide/TBI (CyTBI). Compared to CyTBI, BuCy (odds ratio 0.1, 95% CI 0.0-0.4,  $P=.001$ ) and TBI-VP16 (odds ratio 0.1, 95% CI 0.0-0.3,  $P=.002$ ) were associated with markedly reduced incidence of TIR. The presence of TIR did not significantly influence survival, non-relapse mortality, relapse or acute or chronic GVHD.

### **Conclusion**

We report for the first time detailed information regarding TIR in alloHCT, highlighting its prevalence and an intriguing relationship with particular conditioning agents.

## **P044. Effect of prolonged transportation on haemopoietic progenitor cells for CD34 positive selected allogeneic transplants in paediatrics: Evaluation of cell viability and transplant outcomes**

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### **Introduction**

Haematopoietic progenitor cells (HPC) from matched unrelated donors (MUD) frequently require transportation from their site of collection, often internationally. Due to geographical isolation, allogeneic transplant recipients in Australia often receive cells from international donors with transit times in excess of 24 hours.

### **Methods**

We performed a retrospective analysis using an institutional database to evaluate the effect of prolonged transportation on haematopoietic progenitor cells for CD34 positive selected allogeneic transplants.

### **Results**

We were unable to show a significant difference in neutrophil or platelet recovery, CD34% recovery and purity, 100 day survival or overall survival resulting from retrieval of HPC (either bone marrow or peripheral blood stem cell) between local collection centres and those with transit times in excess of 24 hours.

### **Conclusion**

We found that increased transportation times did not adversely affect graft outcomes in MUD transplants, and that it is safe to receive HPC from international collection centres and have them processed in the laboratory for a CD34+ selection procedure prior to infusion. We feel that this adds to the current literature, particularly as there is little paediatric data, or data for patients undergoing allogeneic transplantation for non-malignant causes.

## **P045. The burden of cardiovascular risk factors in individuals receiving stem cell transplantation as a child or young adult.**

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### **Aim**

Survivors of stem cell transplantation (SCT) are at high risk for a range of late effects. Recipients of SCT in childhood or young adulthood are unique as survival extends over many years. Cardiovascular disease occurs with increased frequency following SCT with typically long latency. We conducted a cross-sectional study to establish the prevalence of cardiovascular risk factors in individuals who received SCT in childhood or young adulthood attending a specialised adult SCT late effects clinic (LEC) and compared this to the age-matched general population.

### **Method**

Consecutive patients transplanted aged  $\leq 25$  years attending their initial adult LEC had weight, abdominal circumference, blood pressure, fasting glucose and lipids measured. Comparative Australian Bureau of Statistics data was used.

### **Results**

From October 2008 to June 2014, 30 individuals (53% male) were assessed. 93% underwent allogeneic SCT predominantly for acute leukaemia (82%). Median age at SCT was 17 (1.5-24) and at study enrolment 23 (19-37) years. Median time since transplant was 7.9 (2-18.9) years. Cardiovascular risk factor prevalence is shown below.

Risk factor	n (%)
Increased abdominal circumference (men $\geq 94$ cm; female $\geq 80$ cm)	18 (60%)
Glucose $\geq 5.6$ mmol/L	15 (50%)
Hypertension (systolic $\geq 130$ , diastolic $\geq 85$ mmHg)	11 (37%)
HDL-cholesterol (men $< 1.03$ , women $< 1.29$ mmol/L)	8 (27%)
Triglycerides $\geq 1.7$ mmol/L	9 (33%)

Compared with the general Australian population aged 18-24 years, a significantly higher proportion of both males and females have increased abdominal circumference (27.9% vs 56%, OR 5.4 [95% CI:1.8-15.9],  $p=0.001$  and 34.3% vs 64%, OR 3.4 [95% CI:1.1-11.8],  $p=0.04$ ) indicating visceral adiposity and therefore risk of developing chronic disease.

### **Conclusion**

SCT survivors who received their transplant in childhood or young adulthood represent a unique and vulnerable population. We demonstrate a high prevalence of cardiovascular risk factors compared to an age-matched general population. These data should encourage systematic screening in long-term survivors and the institution of appropriate preventative measures when identified.

**P046. An economic analysis of total hospital costs comparing a reduced intensity (fludarabine, single fraction TBI) to a non-myeloablative (fludarabine-melphalan-alemtuzumab) conditioning regimen in patients with acute myeloid leukaemia undergoing allogeneic bone marrow transplantation**

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**Aim**

To perform an economic analysis from a public hospital perspective on the difference in total costs at one year between a reduced intensity and non-myeloablative conditioning regimen for allogeneic hematopoietic cell transplantation in patients with acute myeloid leukaemia (AML).

**Methods**

A retrospective analysis was performed on all AML patients conditioned with oral-fluTBI (oral fludarabine 48mg/m<sup>2</sup>, total body irradiation 2 Gy) or flu-mel-campath (intravenous fludarabine 25mg/m<sup>2</sup>, melphalan 140mg/m<sup>2</sup>, alemtuzumab 10mg) between January 2008 to May 2013. Statistical significance in survival was calculated using the log rank test. Total costs included in the evaluation were calculated using hospital pharmacy drug dispensing data and hospitalisation costs based on inpatient bed hours associated with initial and subsequent inpatient admissions up to one year post transplant. Government revenue, as well as community and patient costs were excluded.

**Results**

Eight patients conditioned with oral-fluTBI and fourteen patients with flu-mel-campath were identified during the study period. This was associated with an overall survival rate of 63% and 86% respectively at one year post transplant (p=0.285). The average total costs per patient at one year was \$23,379 for oral -fluTBI (range \$3,497 to \$72,386) and \$52,798 for flu-mel-campath (range \$17,072 to \$208,565). There was approximately a ten-fold difference in average costs per patient with respect to initial conditioning costs (oral-fluTBI \$2,411 versus flu-mel-campath \$29,166) and total costs of antifungal use at one year (oral-fluTBI \$2,822 versus flu-mel-campath \$23,890). This may reflect internal hospital guidelines which require patients conditioned with a non-myeloablative regimen to be admitted as an inpatient prior to transplant, as well as routine use of antifungal prophylaxis until D+100.

**Conclusion**

In patients with AML, conditioning with a reduced intensity regimen prior to allogeneic stem cell transplant is associated with lower overall treatment costs at one year compared to those conditioned with a non-myeloablative regimen.

## **P047. A case series of BK virus haemorrhagic cystitis in CLL patients treated with FCR chemotherapy**

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### **Introduction**

Haemorrhagic cystitis, secondary to BK virus, is a well described complication post bone marrow transplant but is rarely seen with less intensive chemotherapy regimens. Asymptomatic infections are thought to occur in childhood with serological evidence of past infection seen in approximately 80% of healthy adults. After primary infection the virus can remain in a latent phase in the kidney and reactivation can occur during periods of immunosuppression. We describe three cases of BK induced haemorrhagic cystitis occurring in the setting of fludarabine, cyclophosphamide and rituximab (FCR) chemotherapy for chronic lymphocytic leukaemia (CLL).

### **Case Histories**

54 year old male with widespread lymphadenopathy and splenomegaly was commenced on FCR chemotherapy. The patient developed dysuria and frank haematuria post 4<sup>th</sup> and final cycle. Bladder irrigation was commenced and haematuria resolved. Cystoscopy revealed erythema and mucosal oedema consistent with cystitis. Urine JC virus PCR was positive. There was no recurrence of symptoms.

60 year old male was commenced on FCR chemotherapy for treatment of significant drenching sweats, fevers and weight loss associated with CLL. Post 5<sup>th</sup> cycle he developed urinary frequency, urgency and haemorrhagic cystitis. Urine JC virus PCR was noted to be positive. The patient required intensive care admission with cystectomy and ileal conduit formation for management.

81 year old male with multiply relapsed CLL was commenced FCR chemotherapy for progressive lymphadenopathy and weight loss. He incorrectly took a higher dose of fludarabine than prescribed resulting in significant immunosuppression. He developed dysuria and urinary frequency post 1<sup>st</sup> cycle and was found to have a bacterial cystitis. His symptoms remained despite antibiotic treatment. His urinary JC virus PCR was positive. He was put on an attenuated regimen of FCR on subsequent cycles and his urinary symptoms improved.

### **Conclusion**

BK virus can cause haemorrhagic cystitis in CLL patients treated with FCR chemotherapy. It should be suspected and screened for in this patient group.

## **P048. Efficacy of idelalisib in CLL subpopulations harbouring del(17p) and other adverse prognostic factors: Results from a phase 3, randomized, double-blind, placebo-controlled trial**

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### **Aims**

Idelalisib (IDELA) is a potent and selective inhibitor of PI3K $\delta$ , which is critical for activation, proliferation and survival of B cells and their homing and retention in lymphoid tissues. An unmet need exists for effective therapies in patients with CLL positive for del(17p) and other adverse prognostic factors. This report describes the efficacy of IDELA in combination with rituximab (R) in patients with high-risk relapsed CLL.

### **Methods**

Samples for del(17p), del(11q), TP53mut, IGHVmut, ZAP70 and CD38 expression, and  $\beta$ 2-microglobulin were collected prospectively and tested using standard methods. Patients were stratified based on presence of del(17p) and/or TP53mut, and on IGHV mutational status. The endpoints evaluated in the high-risk subpopulations in the preplanned 1<sup>st</sup> interim analysis include progression-free-survival (PFS) and overall response rate (ORR). The primary study analysis was reported in NEJM 2014.

### **Results**

IDELA+R retained robust efficacy across all high-risk subpopulations (see Table). Importantly, IDELA+R achieved 76.5% ORR and PFS HR 0.13 in the highest risk patients who were positive for both del(17p) and TP53mut, compared to 80.4% ORR and PFS HR 0.17 in those who had neither present.

	PFS		ORR			
	IDELA+R		IDELA+R		Placebo+R	
	HR	95%CI	<i>n</i>	%ORR	<i>n</i>	%ORR
Overall	0.15	0.08,0.28	88	80.7	88	12.5
Rai stage III or IV	0.12	0.05,0.27	52	75.0	60	11.7
Binet Stage C	0.13	0.06,0.30	47	74.5	51	13.7
Del(17p)	0.14	0.04,0.47	20	80.0	24	0.0
TP53mut	0.11	0.04,0.31	34	79.4	30	10.0
Del(17p) and/or TP53mut						
: Both	0.13	0.04,0.47	17	76.5	15	0.0
: Either one alone	0.09	0.02,0.42	20	85.0	24	12.5
: Neither	0.17	0.07,0.43	51	80.4	49	16.3
Del(11q)	0.10	0.02,0.46	28	82.1	29	6.9
IGHV unmutated	0.13	0.06,0.27	71	78.9	72	12.5
ZAP70+	0.13	0.06,0.26	77	79.2	74	12.2
CD38+	0.13	0.05,0.34	43	83.7	34	17.6
β2-microglobulin > 4mg/L	0.14	0.07,0.27	75	77.3	68	10.3

### Conclusion

These results confirm the retained robust efficacy of IDELA in high-risk CLL subpopulations and support IDELA as a potentially important novel treatment for patients with CLL positive for del(17p) and other adverse prognostic factors.

## **P049. Health-related quality of life impact of idelalisib (idela) in patients with relapsed chronic lymphocytic leukemia (CLL): Phase 3 results**

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### **Aims**

Patient-reported outcomes (PROs), including health-related quality of life (HRQL), from randomized clinical trials may be used to inform clinical decision making and reimbursement decisions. Idelalisib (IDELA), an oral inhibitor of PI3K $\delta$ , is highly active in frail, heavily pretreated patients with CLL as single agent or combined with rituximab (R). The aim of this study was to use PROs to evaluate HRQL among patients with relapsed CLL being treated with idelalisib in Study 116, a double-blind, placebo-controlled phase 3 trial (Furman et al, NEJM, 2014).

### **Methods**

Patients were randomized to IDELA + rituximab (R) (n=110) vs. placebo + R (n=110). The 44-item Functional Assessment of Cancer Therapy–Leukemia (FACT-Leu) scale was used to measure physical (PWB), functional (FWB), social (SWB) and emotional (EWB) well-being and leukemia-specific concerns (LeuS). The FACT-Leu total score is the sum of subscales; Trial outcome index (TOI) is the sum of PWB, FWB and LeuS. Higher scores reflect better HRQL. Repeated measures mixed-effects models assessed change from baseline within and between arms.

### **Results**

IDELA+R was superior for OS: HR=0.28 (0.09, 0.86), p=0.018. In the mixed-effects model analysis, PWB (p=0.015), FWB (p= 0.014), LeuS (p=0.001), TOI (p=0.002), and FACT-Leu total (p=0.006) scores were significantly higher for IDELA+R. EWB/SWB scores did not change significantly over time. Repeated measure mixed-effects model results are shown in the table.

Week	PWB	FWB	LeuS	TOI	FACT-Leu Total
2	-0.1 (0.65)	0.6 (0.80)	0.4 (1.31)	1.3 (2.38)	1.1 (2.96)
4	0.8 (0.66)	1.0 (0.81)	2.5 (1.33)	4.0 (2.41)	4.0 (3.01)
6	0.1 (0.68)	1.0 (0.84)	2.2 (1.37)	2.9 (2.48)	3.9 (3.09)
8	0.6 (0.71)	0.7 (0.87)	3.5 (1.43)*	4.6 (2.57)	5.2 (3.2)
12	1.1 (0.75)	1.5 (0.92)	4.7 (1.51)**	7.0 (2.72)**	6.5 (3.39)
16	1.9 (0.83)**	1.3 (1.01)	5.3 (1.66)**	8.4 (2.99)**	9.2 (3.72)**
20	1.6 (0.91)	1.4 (1.13)	5.4 (1.85)**	9.0 (3.33)**	9.0 (4.14)**
24	1.8 (1.02)	1.9 (1.26)	5.0 (2.06)**	9.1 (3.69)**	10.0 (4.58)**
30	2.1 (1.14)	2.6 (1.41)	3.0 (2.32)	7.7 (4.13)	9.6 (5.13)
36	1.5 (1.26)	2.8 (1.56)	5.1 (2.54)**	8.2 (4.59)	9.1 (5.69)
42	2.1 (1.57)	2.8 (1.93)	3.9 (3.16)	8.1 (5.57)	9.1 (6.92)
48	3.6 (1.79)**	3.6 (2.20)	5.5 (3.60)	12.4 (6.32)**	13.1 (7.85)

\*  $p < 0.05$ ; \*\*  $p < 0.05$  and exceeded established minimally important difference (MID) change scores of 2, 4, 5 and 6 points for PWB, LeuS, TOI and FACT-Leu total, respectively, between arms

### Conclusion

In this frail CLL population, IDELA+R had superior efficacy, clinically significant improvements in HRQL, and superior symptom control occurring by 8 weeks compared to R+placebo.

## **P050. Investigating a new methodology for the detection of low frequency 17p deleted clones in Chronic Lymphocytic Leukaemia (CLL): Impact on prognostic and therapeutic resistance**

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Cytogenetic analysis by standard FISH of malignant CLL lymphocytes has been identified as predictive of disease outcome. However CLL is likely to be a disease of many sub-clones which either evolve post chemo-immunotherapy or are present in very low frequency at the outset. In the contemporary therapeutic environment greater understanding of these drug resistant sub-clones is required to prevent inappropriate treatment options being employed to treatment naive patients. However, this can only be achieved by meaningful and reliable analysis of low frequency clones.

CLL patients with 17p (TP53 gene) deletion, have the poorest outcome with an overall survival of 2-3 years due to chemo-refractoriness and early relapse. Although TP53 gene deletions are infrequent in CLL at initial diagnosis (5%-10%), their frequency increases to 40%-50% in the relapsed refractory patients. Importantly, CLL patients having less than 5% 17p deleted cells by standard FISH are considered normal with good prognosis, while patients with high frequency 17p deleted nuclei have the worst outcomes. A significant number of patients have TP53 deleted clones of the order of 5-40% of total cell number in which we suspect chemotherapy will have a disadvantageous effect on the genetic profile but whom currently receive this treatment.

### **Aims/Methods**

We are therefore exploring techniques to reliably quantify low frequency clones carrying 17p and other critical gene deletions using Flow FISH a flow cytometry-based method (Amnis Corp) capturing multiple images by Image Stream X (ISX). Extended Depth of Field (EDF) technology expands the depth of focus over the entire cell to enumerate nuclear FISH probe "spots" accurately. The "spot count/cell" program allows to stratify the number of "spots" which based on the intensity and size parameters. Our current project requires development of unique probe sets with sufficient signal to allow reliable detection of single gene deletions with this technology. Patient samples pre and post therapy are being evaluated before a larger pretreatment cohort is evaluated.

## **P051. Defining the genetic region of the 11q deletions in chronic lymphocytic leukaemia**

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Chronic lymphocytic leukaemia (CLL) is a malignancy of B lymphocytes, with a median age at diagnosis of 72 years. It is the most common form of leukaemia in the Western world and is currently incurable. It has a variable disease course, but patients with a homozygous deletion on the long arm of chromosome 11 (del(11q)) have a poor prognosis. The complete loss of function of the tumour suppressor gene Ataxia Telangiectasia Deleted (ATM), which is located at 11q22.3, has been identified in many CLL patients with del(11q)3. However there are two lines of evidence that suggest that the complete loss of function of ATM is not the cause of the poor prognosis associated with del(11q) in all instances. This is evidenced by the rare CLL cases which have an atypical 11q deletion that does not include ATM and in CLL with an ATM-inclusive 11q deletion, an inactivating mutation in the remaining ATM allele has only been detected in 30-40% of cases.

### **Aims/Methods**

We aim to identify patients with del11q in which the ATM gene is not deleted and analyse these patients by SNP array to identify the minimally deleted region for this cohort. The genes included in this region will be analysed using current gene maps and the expression profiling is being performed. Candidate genes are being further analysed for loss or significant gains in expression from that region.

Findings of this work will be applied to the CLL5 and CLL6 cohorts of the CLL Australian Research Consortium and ALLG to validate the relevance of the identified genes in a uniformly treated disease cohort.

**P052. Dose reduced fludarabine, cyclophosphamide and rituximab (FCR) is well tolerated in older patients with chronic lymphocytic leukaemia (CLL) and has preserved therapeutic efficacy**

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**Aim:** FCR is the standard of care for CLL but is often poorly tolerated in elderly patients at full doses. Therefore, we investigated the safety and efficacy of dose reduced FCR in elderly patients.

**Methods:** We performed a retrospective analysis of 43 patients with CLL aged >65 years who received FCR at Peter MacCallum Cancer Centre and St Vincent's hospital. We collected baseline characteristics, dosing regimens and survival outcomes. Kaplan-Meier analysis was used to correlate dose reductions and survival.

**Results:** The cohort's median age was 72 (range 65 – 87) years. Only one patient received full dose. The median cumulative fludarabine dose was 217mg/m<sup>2</sup> or 48.2% of maximum. Despite virtually universal dose reductions, response rates and progression free survival (PFS) remained comparable to the equivalent outcomes in young patients in clinical trials receiving maximal doses (see table). We found no survival disadvantage in receiving one, two or three days of chemotherapy per cycle (mPFS 19.4 v 21.9 v 24.2 months, respectively; p=0.8318) and no significant difference between 2-3, 4-5 or 6 total cycles (mPFS 23.3 v 20.1 v 37.2, p=0.3903). Eastern Cooperative Oncology Group Performance Status (ECOG-PS) ≥2 significantly predicted poor survival compared to patients with ECOG-PS ≤1 (mPFS 12.4 v 27.1, p=0.0097).

Rx status	n=	Median F Dose (% of max)	mPFS (months)	ORR (CRR)	Equivalent clinical trial data	
					mPFS (months)	ORR (CRR)
Overall	43	48.2	22.3	84% (35%)		
First line	17	66.7	Not reached (57% at 3 years)	94% (53%)	65% at 3 years	90% (44%)
Previously treated	26	39.4	20.1	77% (23%)	21	74% (30%)

**Conclusion:** Elderly patients receiving dose attenuated FCR appear able to achieve favorable clinical outcomes equivalent to young fit patients receiving standard doses in clinical trials. Patients with an ECOG-PS of ≥2 have poor responses to FCR and should be considered for alternative therapies.

## **P053. Skin cancers are common in patients with Chronic Lymphocytic Leukemia treated with Fludarabine, Cyclophosphamide and Rituximab and result in significant comorbidity**

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**Aim:** We describe the epidemiology and clinical outcomes of skin cancers occurring in patients with CLL treated with FCR. FC impairs DNA repair and causes prolonged T-lymphopenia; thus, FCR has the potential to induce mutagenesis and compound CLL-associated immunosuppression, increasing the risk of second malignancies.

**Methods:** Single-center, retrospective study of patients treated with FCR for CLL between January 2001 and September 2008. We documented the occurrence and histology of skin lesions following FCR treatment until death or last follow-up, irrespective of relapses or subsequent therapies.

**Results:** The median age of the 67 study patients (44 male, 23 female) was 58 years and 48% were in Rai stages 3 or 4. Prior to FCR, 8 (12%) patients had an antecedent diagnosis of skin cancer, including 4 squamous cell carcinomas (SCC), 1 basal cell carcinoma (BCC) and 3 malignant melanomas. Following FCR, 18 (27%) patients developed one or more skin cancer, with the first skin cancer being an SCC in 11 (16%), BCC in 5 (8%), and melanoma in 2 (both with antecedent history of melanoma and metastatic recurrence at 36 and 89 months post FCR). 44% of patients with skin cancer had more than one histological subtype, with the second subtype occurring a median of 18 months after the first. The clinical consequences of SCC or BCC development were local excision or cryotherapy in 47%, extensive excision under general anaesthetic and/or requiring skin flaps in 33%, and metastatic disease in 20%.

**Conclusions:** Skin cancers are common following FCR chemotherapy for CLL. The clinical consequences of skin cancers in this population can be serious, including requirement for major surgery and/or the development of metastatic disease in over 50%. Clinicians should remain vigilant for the emergence of skin cancers in patients following FCR treatment for CLL.

## **P054. Minimal residual disease monitoring by flow cytometry in CLL post allogeneic stem cell transplant (alloHSCT)**

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### **Aim**

Recent studies have demonstrated improved outcomes in patients with CLL who achieve MRD negativity within 12 months of alloHSCT. We aimed to quantitate residual CLL after alloHSCT for the purposes of directing post-transplant chemo-immunotherapy to optimise outcomes.

### **Methods**

We recently published a method for a single tube 10 colour flow cytometry assay to detect MRD in CLL and have used it for the quantitation of MRD post alloHSCT in a cohort of 10 patients transplanted for CLL between 2011 and 2013 at our institution.

### **Results**

56 MRD assessments were performed (median 5 per patient, range 1-13), the majority on PB (80%). Three patients died within the first 12 months post-transplant due to transplant related complications. Five patients attained MRD negative status very early post-transplant (range 1-6 months) and remain MRD negative and in remission at a median follow-up of 24 months (range 19-39 months). Two patients with persistent MRD at all times up to 12 months post-transplant were treated pre-emptively in response to rising MRD by tapering immunosuppression and administering escalating doses of DLIs, both without and on the final occasion with a preceding cycle of lympho-depleting chemotherapy. One patient has achieved MRD levels below 0.01% in PB within 22 months post alloHSCT. The other patient (17p- disease) has responded to treatment and has stable residual disease 19 months post alloHSCT.

### **Conclusion**

Monitoring MRD at regular intervals gives a dynamic assessment of disease trends that is more meaningful than a single MRD assessment at 12 months post-transplant and may be a better indicator of disease trajectory. The application of MRD monitoring to guide pre-emptive immune interventions or targeted therapies shows promise in preventing clinical relapse post alloHSCT and warrants further investigation.

## P055. Obinutuzumab (GA101) is highly effective as monotherapy for patients with relapsed/refractory 17p- deletion CLL

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Patients with 17p-deletion (17p-) CLL have limited treatment options. Obinutuzumab (GA101) is a glycoengineered type II anti-CD20 with increased direct cytotoxicity and enhanced ADCC. We reviewed our institutional experiences with Obinutuzumab, provided through compassionate access, as monotherapy in patients with 17p- CLL.

**METHODS:** Six patients with relapsed/refractory 17p- CLL were treated with GA101 as monotherapy. As per infusional guidelines, each dose was 1000mg weekly x 3, then monthly with the intent to treat with a further 5 doses. However, 3 patients received 2-6 doses as a bridge to other therapies.

**RESULTS:** Median age of patients was 75 (range 68-85); 50% were male. Median lines of prior therapy was 4 (range 3-5), with 50% demonstrating fludarabine-refractory disease. All patients had demonstrable 17p- by FISH (median 56% deleted interphase cells, range 17-96.7%). At commencement of GA101, 16.7% were Rai stage 0-II, and 83.3% Rai III-IV. Significant cytopenias were present in 66.7%. Median peripheral blood lymphocyte count was  $20.5 \times 10^9/L$  (range  $0.77-170 \times 10^9/L$ ). Results of therapy are presented in the table below.

Pt	GA101 doses	Lymphocytes	Nodes and spleen**	Marrow response	IWCLL response	Hb rise	Plt rise	Best response and Current status
HJ	3	↓95%	↓30% (CT)	↓24%	SD	N	N	Successful bridge to ABT-199
MB	2	*	*	↓100% (MRD+)	CR (MRD+)	*	*	Ongoing remission at 4 months
SD	3	↓86%	*	↓45%	PR	*	*	Still on therapy, in remission
FD	4	↓98%	*	N/A	PR	Y	Y	Still on therapy, in remission
DW	8	*	↓61% (CT)	N/A	Nodular PR	Y	Y	Ongoing remission at 6 months
MM	6	↓99%	Complete resolution (E)	Small residual nodules	Nodular PR	Y	Y	Ongoing remission at 5 months

Grade 3 or 4 infections occurred in 66.7% and exacerbation of ITP in 1 patient. At a median of 4 months post initiation GA101, 1 patient was successfully bridged to a clinical trial, 3 sustained an ongoing clinical response (of which 2 were intended to enter a novel agent study, but due to the depth of response to GA101, no longer qualified), and 2 patients have demonstrated a partial response and are on ongoing therapy.

**CONCLUSIONS:** Obinutuzumab is well tolerated and demonstrates substantial clinical activity, as a single agent, in heavily pre-treated and refractory patients with 17p- CLL.

## **P056. Rare skin manifestations successfully treated with primary B-CLL treatment**

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### **Aim**

Skin manifestations in B-chronic lymphocytic leukaemia (B-CLL) occur in approximately 25% of patients; these are commonly basal/squamous cell carcinomas and viral infections. Here, we describe 2 rare conditions associated with underlying B-CLL in which specific anti-leukaemia treatment successfully eradicated the skin lesions.

### **Case Study 1**

Mr JU, a 46-year-old patient, presented in 2008 with generalised painful urticarial lesions. A biopsy confirmed leukocytoclastic vasculitis consistent with hypocomplementemic urticarial vasculitis (HUV). His Stage A B-CLL with del(13q) was diagnosed in February 2012 with no indications for treatment. However, his HUV was difficult to manage with multiple immunosuppressive agents. As single agent Rituximab has been shown to improve refractory HUV in a small number of cases, Mr JU was treated with two doses of Rituximab 500mg/m<sup>2</sup> in September 2013. This resulted in normalisation of his lymphocyte count within days and an almost complete resolution of his skin lesions by 4 months sustained off any immunosuppressive therapy.

### **Case Study 2**

Mr GK, a 69-year-old patient, presented with an ulcerated lesion in his right lower leg in 2005. A biopsy confirmed the diagnosis of pyoderma gangrenosum (PG). Multiple immunosuppressants were trialled with no significant improvement. His Stage A B-CLL with del(13q)-Y was diagnosed in 2007 with no indications for treatment. Multi-agent chemotherapy with Fludarabine, Rituximab and Cyclophosphamide (FCR) was commenced in September 2012 primarily to treat his refractory PG. He achieved minimal residual disease status and his leg ulcers are almost completely resolved 16 months post-completion of FCR without further immunosuppressants.

### **Conclusion**

HUV is usually associated with solid organ malignancies with only rare cases reported with lymphoid malignancies. Similarly, PG is more commonly associated with myeloid malignancies and plasma cell dyscrasias. These 2 cases illustrate rare skin conditions predating the diagnosis B-CLL, in which definitive treatment of the primary haematological condition resulted in resolution of these skin manifestations refractory to multiple treatments.

## **P057. Central nervous system relapse in Philadelphia positive acute lymphoblastic leukaemia (Ph+ ALL) monitored with the GeneXpert**

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### **Aim**

Detection of BCR-ABL transcripts in leukocytes present in cerebrospinal fluid (CSF) using conventional quantitative PCR is technically challenging. The Cepheid Xpert BCR-ABL Monitor (GX) is a cartridge based automated quantitative PCR system for determination of BCR-ABL transcript levels in whole blood. Two patients with Ph+ ALL who were treated with hyperCVAD plus dasatinib according to the ALLG ALL5 trial relapsed with central nervous system involvement. We tested if BCR-ABL could be detected and/or quantified in serial CSF specimens from the two patients.

### **Method**

The GX method was adapted in our laboratory to enable monitoring of BCR-ABL in bone marrow (BM) and CSF. For BM monitoring, a reduced volume of 20 $\mu$ L is loaded onto the cartridge compared to the standard volume of 200 $\mu$ L used for blood. Analysis of CSF was performed either by centrifugation of 2mL of CSF and resuspending the leukocytes in 200 $\mu$ L of phosphate buffered saline or by using up to 200 $\mu$ L of whole CSF. Where possible, the BCR-ABL level on the International Standard (IS) scale as reported by the GX software was used. In other cases, the threshold cycle (Ct) number for BCR-ABL and the ABL reference gene were used to estimate the BCR-ABL IS using the standard curve for the GX batch.

### **Result**

Three of seven CSF specimens were directly measurable, whereas the remainders were estimated from the Ct difference. At relapse, the first case had a loss of MR4.5 with IS=0.28% in blood, 0.51% in BM and 29% in CSF, whereas the second case had a loss of MR4.5 with IS=0.086% in blood and an estimated 47% in CSF. In each case, serial CSF samples taken during intrathecal therapy had progressively reduced BCR-ABL.

### **Conclusion**

BCR-ABL is quantifiable in CSF with minimal processing and clinically applicable to monitoring the success of intrathecal chemotherapy.

## **P058. Investigation of BCR-ABL1 and BCR degradation in response to time variation in chronic myeloid leukaemia.**

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### **Aim**

To determine if the level of BCR-ABL1 and BCR mRNA transcript degradation affects the International Scale (IS) ratio in blood samples stored at room temperature from 0-72 hours from patients with CML presenting to Royal Perth and Fremantle Hospitals.

### **Method**

Ethics approval was granted to request an additional blood sample from patients newly diagnosed with CML or with their routine blood samples for monitoring response to treatment for CML. Blood samples from 28 patients were stored for 24, 48, 60 or 72 hours prior to RNA extraction. Reverse transcription was performed, followed by real time quantitative Taqman PCR (QPCR) to measure e13a2 and e14a2 BCR-ABL1 and BCR transcript levels. The results from stored samples were compared to the results from fresh samples to calculate the significance of mRNA degradation.

### **Results**

Results were statistically analysed from 20 patients. We observed an increase in IS over storage time, though this was not statistically significantly different after 24, 48, 60 and 72 hours compared with the zero time point. Additionally we observed variation in e13a2 and e14a2 BCR-ABL1 transcript levels after storage in "dual breakpoint" patients. 4/5 patients that were tested for both breakpoints demonstrated an increase in e13a2 breakpoint levels after 24 hours storage that was still prominent after 72 hours storage.

### **Conclusion**

We have demonstrated that IS results from blood samples stored for up to 72 hours at room temperature prior to BCR-ABL1 QPCR are not statistically significantly different to results from fresh samples, however due to the individual variation that was seen in some patients' results over time, we would recommend processing blood samples as soon as possible after collection. Further testing is warranted to determine if the changes in e13a2 and e14a2 BCR-ABL1 transcript levels after storage affect the IS.

## **P059. Successful maintenance of molecular remission in CML through pregnancy with transition from imatinib to pegylated interferon**

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### **Background**

We present the case of a 30 year old female with Chronic Myelogenous Leukemia (CML) who successfully maintained a Major Molecular Response (MMR) during pregnancy after transition from imatinib mesylate to pegylated interferon (PEG-IFN).

### **Case Report**

Our patient was originally diagnosed with chronic phase CML in October 2009. She was enrolled on the ALLG CML9 trial and initially commenced on imatinib at 400mg daily. At her three month assessment, her quantitative BCR-ABL qPCR was 13% on the international scale (IS), a marker of sub-optimal response and her dose was increased to 400mg twice daily, with minimal toxicity. At six months, she was in Major Molecular Response (MMR) with 10% BCR-ABL and remained on this dose of imatinib in sustained MMR for the next 36 months, achieving MR<sup>4</sup> (BCR-ABL <0.01% IS) at 27 months but never MR<sup>4.5</sup> (BCR-ABL <0.0032% IS).

We advised deferring pregnancy until maximal response to imatinib. At 41 months her response plateaued and it appeared unlikely she would achieve sustained MR<sup>4.5</sup>, to allow a trial of imatinib cessation, within a period acceptable to her. We sought further opinions and elected to cease imatinib and commence PEG-IFN. Pregnancy was confirmed 4 months after ceasing imatinib.

Throughout her pregnancy she maintained MMR, initially with 180mcg weekly however required dose reductions in the second half of pregnancy to 90mcg/week due to anaemia and thrombocytopenia. She had no significant non-haematological toxicity and successfully delivered a healthy 2559g term infant. As she remained in MMR, she continued on PEG-IFN for two months post-partum to allow breast-feeding. She was then transitioned from PEG-IFN to nilotinib with the goal of achieving a deeper molecular response with view to eventual drug cessation.

### **Conclusion**

We consider this approach of transition from TKI to (pegylated) interferon alpha is a safe and reasonable option in women wishing to become pregnant who are yet to achieve MR<sup>4.5</sup>.

## **P060. Quantative PCR for bcr-abl in central nervous system relapse of acute lymphoblastic leukaemia**

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### **Background/Aim**

Quantitative polymerase chain reaction (qPCR) of the bcr-abl fusion product allows highly accurate quantitation of disease burden in chronic myeloid leukaemia (CML) and Philadelphia chromosome positive (Ph+) acute lymphoblastic leukaemia (ALL). Mutations in the tyrosine kinase domain, conferring resistance to the tyrosine kinase inhibitors, may be demonstrated by Sanger sequencing. These tests are typically performed on peripheral blood (PB) and bone marrow (BM) specimens.

We report our experience in performing bcr-abl qPCR and kinase domain mutation studies in cerebrospinal fluid (CSF) specimens from a patient with isolated central nervous system (CNS) relapse of Ph+ ALL.

### **Method**

CSF, PB and BM (performed to exclude systemic relapse) were tested by qPCR. Briefly, RNA was extracted and reverse transcribed prior to performing qPCR specific for the e13a2 breakpoint. The control gene used was BCR.

### **Result**

qPCR was successful in PB, BM and CSF. The bcr-abl/bcr (IS%) levels in these three specimens were 0.009%, 0.043% and 196% respectively. Kinase domain mutation studies on BM and CSF specimens demonstrated a F317L variant (conferring resistance to imatinib and dasatinib) in all transcripts. This variant had also been identified approximately 2 years prior in a BM specimen.

qPCR on subsequently submitted CSF specimens proved unsuccessful due to insufficient starting RNA.

### **Conclusion**

Successful quantitation of the bcr-abl transcript and sequencing of the kinase domain in the initial CSF specimen demonstrates that performing these studies on samples other than PB and BM is technically feasible. Insufficient RNA quantity during follow-up may reflect a reduction in blast count during therapy or inadequate CSF sampling. This testing method may allow for an alternative, highly sensitive method for demonstrating CNS involvement by Ph+ ALL in patients with identified transcripts.

## **P061. Adrenal function after pulsed high-dose glucocorticoid-containing cytotoxic chemotherapy regimens**

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### **Aim**

The aim of this study was to evaluate whether cytotoxic chemotherapy with an element of pulsed high-dose glucocorticoid is associated with biochemically evident hypoadrenalism post-therapy.

### **Method**

This was a single-arm, prospective observational pilot study. 23 patients from the Haematology Department of the Princess Alexandra Hospital who had been diagnosed with a haematological malignancy were recruited. Pre- and post-therapy early-morning cortisol and ACTH levels were assayed using LC-MS/MS methodology. Student's paired *t*-test was performed to identify whether there was a statistically significant difference in adrenal function post-therapy.

### **Result**

No statistically significant difference in adrenal function was identified when the pre- and post- therapy samples were compared.

### **Conclusion**

There was no evidence of a statistically significant difference in pre- and post-therapy adrenal function. There was no evidence of hypothalamic-pituitary-adrenal axis suppression, despite exposure to repeated pulsed high-dose glucocorticoid given with cytotoxic chemotherapy. This result goes towards validating current clinical practice, where weaning doses of glucocorticoids are not generally considered necessary in glucocorticoid-containing chemotherapy regimens for lymphoma.

## **P062. DLBCL – a single institution audit of management including outcomes, CNS prophylaxis and the use of a new live-input questionnaire.**

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### **Aim**

To document details of patients with a new diagnosis of DLBCL that were managed at St George Hospital in 2009 and 2012, including demographics, prognostic scores, first line therapy, CNS prophylaxis use and remission rates.

To assess the use of and efficacy of the live-input questionnaire being completed at lymphoma MDTs to capture data, and identify ways to improve data collection.

### **Method**

The local haematology/oncology patient database was interrogated for all patients with DLBCL with a diagnosis date in 2009 or 2012. Additionally a list of which of these patients had completed Lymphoma MDT questionnaires was generated. Basic demographics, IPI scores, first line therapy received, CR rates, and proportion of patients receiving CNS prophylaxis were recorded. Qualitative analysis was undertaken.

### **Result**

29 newly diagnosed patients were managed in 2009 and 26 in 2012, with slight male predominance, and average age in low 60s in both cohorts. There was a greater variation in first line treatment in 2012 and CNS prophylaxis increased from 10% to 29%, with a shift from intrathecal to intravenous methotrexate observed. CR rates (excluding palliative patients) were higher in 2012 (83% vs 74%). Utilisation of the questionnaire increased from 38% to 96%, and correlated with simpler data extraction for the 2012 cohort. ECOG was the most poorly recorded parameter.

### **Conclusion**

First-line treatment, including prescription of CNS prophylaxis, changed between 2009 and 2012 despite similar patient demographics. A trend towards better CR rates perhaps validates these changes. Increased use of the questionnaire has improved data collection. These findings have prompted additional consultant led quality improvement initiatives. Hopefully this will enable more streamlined auditing in the future, which will guide ongoing improvement in patient care.

## **P063. A novel simultaneous copy number variation and telomere length assay on the Nanostring nCounter platform and assessment of its utility in chronic lymphocytic leukaemia**

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### **Background**

Abnormalities of telomere length are important in numerous congenital and acquired haematological disorders including congenital bone marrow failure, acquired aplastic anaemia, myeloproliferative disorders and lymphoproliferative disorders. Acquired short telomere length in CLL has been shown to be associated with other high-risk genetic features and a decreased survival.

### **Aims**

To investigate the performance of a novel combined copy number variation (CNV) and telomere length assay using the Nanostring nCounter platform and to investigate its application in diagnostic samples from patients with chronic lymphocytic leukaemia (CLL)

### **Method**

A CNV codeset with a spiked-in beta test telomere probe was obtained from Nanostring technologies. DNA was extracted from banked cord blood samples with known telomere length (measured by terminal restriction fragment analysis and RT-PCR). The cord blood samples were assayed to create a standard curve, perform regression analysis and determine inter-run variability. The assay was then used to assess telomere length and CNV in peripheral blood and bone marrow samples from patients with CLL.

### **Results**

48 samples were analysed. Regression analysis showed a coefficient of determination ( $R^2$ ) of 0.69 between the reference methods and the Nanostring assay. Removal of one outlier yielded an  $R^2$  value of 0.89. The inter-run assay variability was 18.12% (over 4 runs). Results from CLL samples confirmed numerous cases with acquired telomere length abnormalities. Simultaneously acquired CNV data on CLL samples confirmed abnormalities previously detected in these cases by fluorescence *in situ* hybridisation (FISH) as well as other previously undetected CNV.

### **Conclusion**

The novel Nanostring CNV and telomere length assay showed satisfactory correlation with reference methodology with performance characteristics comparable to other methodologies in this area. Further optimisation of the assay is ongoing. The combination of simultaneous CNV and telomere length assessment is a potentially useful assay for prognostic assessment in CLL.

## **P064. Overall and Progression-free Survival of relapsed or refractory Hodgkin Lymphoma after IVAC salvage chemotherapy, Autologous or Allogeneic Stem Cell Transplants: A Single Centre Retrospective Audit**

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### **AIMS:**

1. To determine the efficacy (PFS and OS) of IVAC salvage chemotherapy (Ifosfamide, Etoposide, and Cytarabine) followed by ASCT, and 2. Outcomes following allogeneic SCT (alloSCT) in patients with relapsed and/or refractory HL.

### **METHOD:**

Retrospective analysis of relapsed and/or refractory HL patients treated at our institution from 2000 to 2014. Data on age, sex, histology, treatment and outcome were collected from hospital records. OS and PFS were calculated from the date of initiation of salvage treatment.

### **RESULTS:**

Forty-six HL patients underwent an ASCT, 19 of whom received IVAC chemotherapy (primary refractory - IVAC 32%, non-IVAC 9%,  $p=0.07$ ). The overall response rate for IVAC and alternative therapy was 57% and 63%, respectively. Seventy five per cent of the 8 patients who failed IVAC relapsed or progressed after ASCT, similarly of the 10 patients who failed non-IVAC chemotherapy, 80% relapsed or progressed after ASCT. Overall 22 patients relapsed or progressed post-ASCT and 16 subsequently received an alloSCT. After a median follow-up of 20 months, the predicted 5-year OS and PFS for IVAC salvage chemotherapy followed by ASCT were 90% and 49%, respectively, with no non-relapse mortality. Current surviving patients have had either an alloSCT or other novel therapies. Non-IVAC salvage chemotherapy followed by ASCT resulted in 5-year OS and PFS of 75% and 60%, respectively. There was no significant difference in PFS between the IVAC and non-IVAC salvage chemotherapy groups ( $p=0.46$ ). The alloSCT cohort demonstrated a 3-year OS and PFS of 40% and 25%, respectively. Seventy-five per cent of patients who proceeded to alloSCT were primary relapsed HL whereas 25% were primary refractory HL.

### **CONCLUSIONS:**

IVAC salvage chemotherapy followed by ASCT results in comparable rates of PFS when compared to alternative approaches. Patients undergoing alloSCT for post-ASCT relapse remain at high risk of progressive disease but a significant minority is cured.

## **P065. Dose adjusted-EPOCH-R in Primary mediastinal large B cell lymphoma – The Royal Perth Hospital experience**

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### **Aim**

To report outcomes of adult patients treated with the dose adjusted EPOCH-R regimen through Royal Perth Hospital.

### **Background**

Primary mediastinal large B cell lymphoma (PMBL) presents in young adults as a mediastinal mass. Dunleavy et al. (NEJM 2013) reported their case series of 51 patients with PMBL who were treated with dose adjusted EPOCH-R with event free and overall survival in excess of 90% at 63 months follow-up.

### **Method**

We examined the Royal Perth Hospital electronic pharmacy database to identify all cases of PMBL treated with da-EPOCH-R. All cases had pathology confirmed in accordance with WHO 2008 criteria. Data was retrospectively analysed for baseline disease characteristics, interim response, toxicity and treatment outcomes. Results

6 patients with a median age of 35 were identified (3 males, 3 females) who received treatment between 2012 and 2014. All patients completed at least 6 cycles of da-EPOCH-R therapy. 4/6 patients had chemotherapy administered via an ambulatory chemotherapy program. 2 patients had inpatient chemotherapy.

The admission rate for febrile neutropenia was low (1 episode in 38 treatments). Grade 1 peripheral neuropathy was reported in 2 patients with 1 requiring dose modification. 3/6 patients developed DVT, all of whom had line-associated thrombosis identified in the first treatment cycle.

5/6 patients reached CR based on an interim PET scan with the remaining patient having a vgPR. This patient remained in vgPR at the end of therapy and progressed with CNS disease 1 month after completing treatment. At our centre the overall CR rate is 83% with 83% PFS at a median follow-up of 15 months.

### **Conclusion**

This cohort of 6 patients represents 12% of the Dunleavy et al. cohort. Our results are similar to those reported, with acceptable levels of toxicity, though with a high rate of line-associated venous thrombosis. This series demonstrates this regimen is successful in clinical practice and may be safely administered in the home environment.

## **P066. Extensive Focal Paraneoplastic Skin Ulceration associated with Hodgkin Lymphoma**

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### **Aim**

To report the unusual case of extensive localised paraneoplastic skin ulceration associated with Hodgkin Lymphoma (HL), which preceded the diagnosis of malignancy in a 47 year-old man. The skin ulceration recurred in disease relapse but resolved with achievement of both first and second clinical remissions.

### **Method**

We reviewed of clinical and laboratory information including skin biopsies, lymph node biopsies and imaging following the clinical progress of this case. We studied published data via PubMed regarding HL, paraneoplastic disorders and skin lesions.

### **Results**

A 47 yo overweight truck driver noticed gradual progression of the skin ulcer on the right forearm over several months prior to the diagnosis of stage II-A Nodular Sclerosing HL, with extensive bilateral cervical lymphadenopathy and moderate mediastinal lymphadenopathy. Sequential tissue sampling of the skin lesion included a possible suppurative process, pseudoepitheliomatous hyperplasia, and non-specific ulceration and thought to be in keeping with a paraneoplastic effect.

The patient initially responded well to 8 cycles ABVD, including an interim negative PET. The skin lesion healed following twelve weeks of therapy, coinciding with suppression of HL. Three months after completing initial therapy, the skin lesion recurred- follow up imaging and biopsy confirmed relapse of HL. The patient received Moskowitz ICE chemotherapy and the skin lesion again healed after eight weeks. After subsequent autologous transplantation and follow up radiotherapy, the patient remains in remission with no further recurrence of HL or the associated skin lesion.

### **Conclusion**

Skin lesions may be varied and present as a paraneoplastic association in Hodgkin Lymphoma, and precede the initial diagnosis.

## **P067. Patient-reported outcomes data from a phase 2 study of idelalisib in patients with refractory indolent B-cell non-hodgkin lymphoma (iNHL)**

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### **Aims**

Idelalisib, a selective oral inhibitor of PI3K $\delta$ , demonstrated considerable anti-tumor activity in patients with relapsed/refractory iNHL in a phase 2 trial (Gopal, 2014). The purpose of this analysis was to evaluate patient-reported outcomes (PRO) data to determine whether drug treatment was associated with a change in health-related quality of life (HRQL).

### **Methods**

Eligible iNHL patients (pts) were refractory to both rituximab and an alkylating agent. Idelalisib 150 mg PO BID was administered continuously until disease progression. HRQL was measured by the 42-item Functional Assessment of Cancer Therapy-Lymphoma (FACT-Lym), comprising FACT-G subscales: Physical Well-being (PWB), Social/Family Well-being (SWB), Emotional Well-being (EWB), Functional Well-being (FWB), and the Lymphoma Subscale (LymS). Trial Outcome Index (TOI) is PWB + FWB + LymS. Higher scores reflect better HRQL. Minimally important differences (MID) on the FACT-G ranged 3-7 points (2-5 points for subscales). The FACT-Lym was administered every 4 weeks (0-24), then every 6 weeks (30-48), and at week 60. Change from baseline in FACT-Lym was analyzed.

### **Results**

Enrolled pts (N = 125) had a median age of 64 years [range 33-87] and were 64% male. With a median follow up 9.4 months, overall response rate (ORR) is 57% (95% CI = 47.6, 65.6) and median DOR is 12.5 months. Median PFS for all pts is 11.0 months. Improvements were noted in the FACT-G, FACT-Lym, and TOI scores during the study, progressively increasing with time. In FACT-G subscales, improvements were noted by 4 weeks for EWB scores. The median best changes from baseline for the FACT-G, FACT-Lym, and TOI total scores were 5.0, 8.3, and 6.0, respectively. LymS change scores exceeded the MID for  $\geq 90\%$  of pts indicating a clinically significant improvement in lymphoma-related concerns at some point in the study. The median best change from baseline for the LymS was 5.0 and median time to improvement was 1.9 months.

### **Conclusion**

PRO data indicate that clinically significant improvements in HRQL, including lymphoma-related concerns, were noted for most patients.

## **P068. Multiple B-cell clones in the peripheral blood detected at routine flow cytometry**

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### **Aim**

Analysis of patients with multiple B-cell clones identified in the peripheral blood at routine diagnostic flow cytometric phenotyping.

### **Method**

Data from the flow cytometry laboratory was obtained for patients having phenotyping for detection of a possible lymphoproliferative disorder (B-LPD). Patients with two or more clones reported here were recorded manually.

### **Results**

In total, 30 B-LPD cases with 2 or more B-cell clones were recorded from 2000-2014. There were more males (19) than females (10), an M:F ratio of 1.9:1. The median age was 68.8 (range 53-96) years. Full blood count parameters showed Hb 81-158 g/dl, WCC 1.4-51.2, lymphocyte count 0.5-39.6, and platelet count 36-476. Immunoglobulin levels were measured in 15 patients with levels of IgG 3.3-14.01, IgA 0.58-29.83, and IgM 0.07-39.92.

Of the 30 patients with dual clones, 26 had a CLL-type (CLL, MBL or SLL) clone as one of the two clones. The next most common was CD5-negative, B-cell lymphoma in 20 patients, 2 with morphology consistent with splenic lymphoma and 1 CD10-positive consistent with follicular lymphoma. There were 4 Hairy Cell Leukaemia (HCL), 3 Mantle Cell Leukaemia (MCL), and 1 myeloma.

The most common combinations were CLL with NHL accounting for 19 patients while dual CLL clones were seen in 4 patients, all of whom had both a kappa and a lambda clone. One patient had 3 clones with NHL- $\kappa$ , NHL- $\lambda$ , and a tiny CLL clone. The 4/30 patients with an HCL clone appears higher than expected from the incidence of HCL. These were paired with CLL in 2, MCL in 1 and myeloma in 1.

### **Conclusion**

Patients with 2 or more clones are not a rare event in the flow cytometry laboratory. The most common combination is a CLL and NHL clone. HCL clones are seen in combination possibly more common than might be expected.

## **P069. The 2014 Global Lymphoma Patient Survey: An Australian perspective**

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### **Background**

Lymphoma is the most common blood cancer in Australia and other parts of the world, and incidence rates are rising. Therefore, it is essential that we continue to explore how lymphoma is managed and its impact on those affected.

### **Aim**

This paper will discuss the Australian findings of the 2014 Global Lymphoma Patient Survey.

### **Methods**

The Leukaemia Foundation participated in the development and implementation of the 2014 Global Lymphoma Patient Survey, led by the international Lymphoma Coalition. A global committee was established that included representatives from Australia, the U.S., Bulgaria, Canada, India, Japan and Spain. A collaborative approach was used to review and adapt survey questions from the 2012 survey and to develop new questions. Topics covered by the survey's questions included participant demographics, circumstances around diagnosis, clinical trials participation, and symptom management. In Australia, the survey was distributed electronically to all people with a lymphoma diagnosis who were registered on the Leukaemia Foundation database. The survey also was available via a link on the Leukaemia Foundation's website ([www.leukaemia.org.au](http://www.leukaemia.org.au)) and a printed version was distributed upon request. Data analysis was conducted by a researcher from the Lymphoma Coalition and, when released, the results will be reported from both a global perspective and for individual countries.

### **Results**

The survey was completed by 3492 people worldwide who are affected by lymphoma, including 260 people from Australia. The 2014 survey results are pending and will be presented for the first time at HAA 2014.

### **Conclusion**

With the incidence of lymphoma increasing, this disease and how it is managed will continue to affect the lives of an increasing number of people living in Australia. This paper will discuss the results from the latest Global Lymphoma Patient Survey from an Australian perspective.

## **P070. Optimisation of a clinical grade system for the production of CD19 Chimeric Antigen Receptor (CAR) expressing T cells using a SuperPiggyBac transposon/transposase system**

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### **Background**

We have previously shown that CD19 positive CAR T-cells with activity against B cell malignancies including Non-Hodgkin lymphoma, Chronic Lymphocytic Leukaemia and Acute Lymphoblastic Leukaemia can be produced using the SuperPiggyBac transposon/transposase system with the Neon™ (Life Technologies) nucleofector. However, for these cells to be used in human clinical trials, a system with GMP grade reagents is required.

### **Aim**

To optimise a clinical grade system using the Amaxa-4D nucleofector™ (Lonza) for the use of CD19 CAR T cells for B cell haematological malignancies.

### **Methods**

Peripheral blood mononuclear cells (PBMCs) were isolated from venesection units from consented normal healthy donors. PBMCs were rested for 24-48 hours prior to electroporation with the Amaxa-4D™ or Neon™ nucleofector in parallel. 7 Amaxa 4D™ programs were tested in triplicate and compared to our previously optimised Neon™ protocol. After identifying the optimal Amaxa-4D™ program, cell number, plasmid concentration and cytokine cocktails were varied. Outcomes were cell recovery and expansion by trypan blue exclusion and CAR expression by flow cytometry. Statistical analysis included: mean, median, range, t-test and ANOVA analysis where appropriate.

### **Results**

The optimal condition was electroporating  $10 \times 10^6$  PBMCs with the Amaxa-4D™ program EN138 with 5microg/microL of SuperPiggyBac and CAR19.28z plasmids respectively. Pre-electroporation incubation with IL4 and IL7 followed by post-electroporation culture with IL15 produced the best CAR T-cell expansion and expression on D21. However, cell recovery, CAR T cell expansion and expression with the Neon™ system was superior in comparison to the Amaxa-4D™ optimised program ( $p < 0.01$ ;  $p < 0.058$  and  $p < 0.01$  respectively). Calcein cytotoxicity results are pending.

### **Conclusion**

CD19 CAR T-cells were successfully produced using a transposon/transposase system with the Amaxa-4D™ nucleofector, however cell recovery, culture expansion and CAR expression were inferior in comparison to CD19 CAR T-cells produced using the non-GMP grade Neon™ system.

## **P071. Clinical characteristics and factors predicting outcome in relapse after autologous stem cell transplantation for aggressive NHL**

**Gangatharan S**, Kuruvilla J, Keating A, Kukreti V, Tiedemann R, Crump M

*Princess Margaret Cancer Centre, Toronto, ON, Canada*

### **Aim**

Despite high-dose chemotherapy and autologous stem cell transplantation (ASCT) for relapsed/refractory aggressive NHL, 30-50% of patients will still relapse. There is no standard of care for management of relapse post-ASCT, and selected patients may be candidates for investigational agents. We reviewed patterns of relapse post-ASCT and outcome to better define natural history and prognosis in this patient population.

### **Methods**

We identified patients at Princess Margaret Cancer Centre undergoing ASCT for relapsed/refractory aggressive NHL from 2007-2012. Data were collected retrospectively on patients who relapsed post-ASCT with regards to pre- and post-transplant characteristics, therapies and outcome.

### **Results**

Of 126 patients undergoing ASCT, 51 relapsed (34 DLBCL, 11 transformed, 6 T-cell). Prior to ASCT, second-line IPI: 0-1 - 29%, 2 - 21%,  $\geq 3$  - 29%, and 45% relapsed within 12 months of initial therapy.

Median time to relapse after ASCT was 3 months (range 1-52). Stage IV disease in 57%, bone marrow involvement in 33% and extra-nodal disease in 47%. Time to relapse after ASCT was not predicted by pre-ASCT characteristics.

After second relapse, 35 patients (69%) received IV/PO chemotherapy including clinical trial agents in 13 patients. Radiotherapy was incorporated in 26. Management was strictly palliative/unknown in 8.

With median follow-up 28 months from ASCT, median overall survival (OS) after relapse was 7 months; 2-year OS 21%. Predictors of OS were second-line IPI (median 23 v 9 v 4 months;  $p=0.015$ ) and time to relapse after ASCT  $<6$  months (median 4 v 23 months;  $p=0.006$ ). Stage, bone marrow involvement and extra-nodal sites did not predict OS. No specific treatment after relapse significantly predicted OS.

### **Conclusion**

In patients who relapse post-ASCT, unfavourable second-line IPI and early relapse after ASCT predicted short survival. Use of these prognostic factors should help in patient selection and interpretation of results of phase I-II evaluation of novel agents.

## **P072. Baseline lymphocyte count prior to conditioning rather than lymphocyte recovery is a prognostic factor in ASCT for aggressive NHL**

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### **Aim**

There are conflicting studies in the significance of lymphocyte recovery as a prognostic marker for outcome after stem cell transplantation for haematological malignancies. We aim to investigate the prognostic significance of lymphocyte counts pre- and post- autologous stem cell transplantation (ASCT) in the management of relapsed/refractory aggressive NHL.

### **Method**

Patients undergoing ASCT for relapsed/refractory aggressive NHL were identified from the transplant database at Princess Margaret Cancer Centre, 2007-2012. Data were collected retrospectively on patient characteristics and outcomes. Lymphocyte counts were recorded on first day of apheresis, prior to conditioning chemotherapy, day 14, day 21 and day 28 after stem cell infusion. Kaplan-Meier method was used to estimate progression-free survival (PFS) and overall survival (OS). Lymphocytes  $\geq 0.5 \times 10^9/L$  was used to categorize groups at day 14 while lymphocytes  $\geq 1 \times 10^9/L$  was used for the other time-points. Log-rank test was used to compare groups.

### **Results**

126 patients were transplanted; 80 for DLBCL, 30 transformed lymphoma, 12 T-cell, 4 other. Second-line IPI: 0-1 40%, 2 35%, 3-4 – 25%. Median lymphocyte counts (and interquartile range) for time-points: day 1 of apheresis -  $0.76 \times 10^9/L$  (0.45-1.6), baseline pre-conditioning -  $0.6 \times 10^9/L$  (0.4-0.9), day 14 -  $0.41 \times 10^9/L$  (0.2-0.7), day 21 -  $1.0 \times 10^9/L$  (0.7-1.6), day 28 -  $1.5 \times 10^9/L$  (1.0-2.0).

With median follow-up of 33 months, 5-year PFS and OS were 40% and 50% respectively. Baseline lymphocyte count  $\geq 1.0 \times 10^9/L$  was associated with improved PFS (5-year 67% v 33%;  $p=0.022$ ) and OS (2-year 70% v 45%;  $p=0.09$ ). Lymphocyte counts at other time-points were not significant for PFS. Second-line IPI predicted PFS and OS.

### **Conclusion**

Baseline lymphocyte count is associated with improved PFS and OS in ASCT for aggressive NHL. Lymphocyte recovery post-ASCT was not a predictor of outcome in this population. Evaluation of lymphocyte subsets before and after ASCT is necessary to clarify the contribution of immunological recovery to disease control.

## **P073. Alternating R-CHOP with R-DHAC chemotherapy is a highly efficacious and well tolerated regimen for previously untreated Mantle Cell Lymphoma**

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Mantle Cell Lymphoma (MCL) is an aggressive B-cell lymphoma for which standard chemotherapy regimens result in a high response rate, but a large number of early relapses which have led many authorities to recommend aggressive therapy with autologous stem cell transplantation in CR1. The optimal treatment for elderly patients is yet to be determined.

### **Aim:**

To compare the Progression free survival (PFS), Overall survival (OS), overall response rates (ORR) and toxicity of R-CHOP/R-DHAC compared to R-CHOP chemotherapy.

### **Methods:**

From October 2011, 6 newly diagnosed patients with MCL at our institution were treated with alternating R-CHOP/R-DHAC (total of 6 cycles) due to the observed poor rates of durable CR for patients receiving R-CHOP chemotherapy alone. The outcomes of this cohort were compared to a historical cohort of 15 patients treated with R-CHOP at our institution between 2002 and 2011.

### **Results:**

All patients had advanced MCL at diagnosis. The median age 67.9 vs 67.1, LDH, ECOG and MIPI did not differ significantly between groups. A total of 3 patients (50%) in the R-CHOP/R-DHAC cohort and 3 (20%) in the R-CHOP cohorts underwent autologous stem cell transplantation. The median overall response rate with R-CHOP/R-DHAC was 100% vs 87%. The 3 year PFS was 83% vs 61%, with OS of 100% vs 85%. There were no clinically significant toxicities reported in the patients who received R-CHOP/R-DHAC.

### **Conclusion:**

The addition of cytarabine to conventional R-CHOP chemotherapy for patients with advanced stage Mantle Cell lymphoma is well tolerated with high efficacy. This promising regimen is deserving of further clinical trial evaluation including in autologous stem cell transplant ineligible patients.

## **P074. High-dose Methotrexate Consolidation in poor-risk Diffuse Large B-cell lymphoma is associated with Improved Overall Survival**

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Patients with poor risk Diffuse Large B-cell Lymphoma have disappointing outcomes with approximately 50% of patients eventually succumbing to their disease. Dose intense regimens have largely failed to improve this and are associated with increased toxicity.

**Aims:** To examine the effect of HD-MTX on overall survival (OS) and Progression Free Survival (PFS) in patients with poor-risk DLBCL.

**Methods:** A total of 92 patients with newly diagnosed DLBCL and an R-IPI  $\geq 3$  completed R-CHOP-like chemotherapy. A total of 17 patients received HD-MTX 3g/m<sup>2</sup> and an additional 2 cycles of Rituximab. Survival correlates were analysed by Cox regression using SPSS.

**Results:** Patients receiving HD-MTX were younger (median age 69 v. 74), though the proportion over 60 years (HD-MTX 88% v. standard 83%), proportion with advanced stage (100% v. 85%) and raised LDH (82% v. 80%) were similar.

At a median follow-up of surviving patients at 2.5 years, patients who received HD-MTX had improved five year OS (73% v. 44%, HR 0.50, P =0.082) and PFS (65% v. 34%, HR 0.50, P=0.048). This was mainly attributable to a reduction in the five year systemic relapse rate (17.6% v. 38.7% (HR 0.46, P=0.063). No difference in the rate of CNS recurrence was evident. The regimen was well tolerated with no severe toxicity. On multivariate analysis lower IPI and the use of HD-MTX were associated with favourable outcomes.

**Summary/Conclusion:** The use of consolidative HD-MTX is associated with improved OS and PFS in patients with poor-risk DLBCL, including the elderly. The low rates of toxicity observed may be due to sequential rather than concurrent administration of HD-MTX and careful patient selection. Further prospective studies are warranted to validate this approach in poor-risk patients with DLBCL.

## **P075. CODOX-M/IVAC therapy in high-risk Burkitt's lymphoma may be associated with a higher incidence of adverse events in HIV-positive patients compared to HIV-negative patients**

**Goncalves I, Coutsouvelis J, Patil S**

*Alfred Health, Melbourne, VIC, Australia*

### **Aim**

High intensity therapy such as CODOX-M / R-IVAC in high-risk Burkitt's lymphoma (BL) is effective in Human immunodeficiency virus (HIV)-positive patients however severity of therapy-related toxicity in HIV-positive patients is not adequately established. This retrospective study from a tertiary public hospital evaluated the outcome and toxicity of R-CODOX-M/R-IVAC in HIV-positive compared to HIV-negative patients with BL.

### **Method**

Patient medical records were used for retrospective analysis. A total of 20 adult patients (11 HIV-positive and 9 HIV-negative) diagnosed with Burkitt's lymphoma were treated with R-CODOX-M / R-IVAC (stage IV) or R-CODOX-M (Stage I to III) during the period of 2009 to 2014. Toxicity was evaluated according to the National Cancer Institute common toxicity criteria (NCI-CTC), version 4.0. Given the small sample size, the Fisher's exact test was employed for statistical analysis.

### **Results**

The demographics and performance status of the HIV-positive patients were comparable with those who were HIV-negative. Seven (63%) HIV-positive patients received R-CODOX-M / R-IVAC, three patients were unable to tolerate the full protocol and received R-CODOX-M/ CHOP while one patient had stage II disease and received R-CODOX-M. Five (55%) HIV-negative patients received R-CODOX-M / R-IVAC while four (45%) received R-CODOX-M for Stage 1A disease. All patients experienced NCI-CTC Grade 3-4 adverse events, which included neutropenia, mucositis, infection and tumour lysis syndrome. While HIV-positive patients appeared to have more therapy related infection or mucositis (55% and 66% for HIV-negative and 82% and 82% for HIV-positive patients, respectively) this was not statistically significant. Complete remission rates were 73% in HIV-positive and 88% in HIV-negative patients. Three (27%) HIV-positive patients died during induction, two died of severe sepsis and one of progressive disease. No HIV-negative patients died during induction therapy.

### **Conclusion**

From our single-centre cohort, R-CODOX-M / R-IVAC treatment results in equivalent remission rates in HIV-positive and negative patients with high-risk Burkitt's lymphoma, but potentially at an increased risk of chemotherapy induced toxicity in HIV-positive patients.

## **P076. A rare case of primary effusion lymphoma sine effusion in a HIV/HHV8 positive patient**

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### **Aim**

Primary effusion lymphoma (PEL) is a rare Human Immunodeficiency Virus (HIV) related lymphoma (3%) with a poor median survival of 6 months. It is invariably caused by Human Herpes Virus 8 (HHV8) infection. PEL as the name defines, is usually confined to the body cavities. Here we report a case of a solid tumour variant of HHV8 positive PEL at initial diagnosis of HIV.

### **Method**

A 44 year old man was diagnosed with HIV infection when he presented with fever and pancytopenia, generalized lymphadenopathy and 9 cm mass at the anterior abdominal wall. HIV viral load (VL) and CD4 counts were 39, 7000 copies /ml and 99/ul respectively at diagnosis.

### **Results**

Core biopsy of the mass demonstrated medium to large cell infiltrates with positive CD30, CD38 and MUM1 (Multiple Myeloma Oncogene 1) markers but CD20 staining was negative, suggesting non-B non-T phenotype with plasmablastic differentiation. HHV8 immunostaining with anti-latent nuclear antigen (LNA) monoclonal antibody was positive in >95% of the cells. The tumour cells showed high proliferative activity with 100% positivity for ki67 marker. Extra-cavity/solid tumor variant of stage IVB HIV/HHV8 associated PEL was therefore diagnosed.

Patient was commenced on CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) therapy with combined anti-retroviral therapy (CART). Complete metabolic remission was achieved after 6 cycles of CHOP. HIV VL declined significantly while CD4 count remained stable.

### **Conclusion**

In summary, HIV/HHV8 associated PEL constitutes a rare distinct entity of HIV related lymphomas with its unique null type histopathology, pathogenesis and poorer prognosis. The use of molecular staining for HHV8 plays an important role in diagnosing this rare lymphoma in the absence of classical serous effusions. More clinical research studies are required to develop better chemotherapy and targeted treatment regimens to improve the outcome in these patients.

## P077. Time to next treatment in relapsed/ refractory cutaneous T-cell lymphoma treated with histone deacetylase inhibitors is comparable with other systemic therapies

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### Aim:

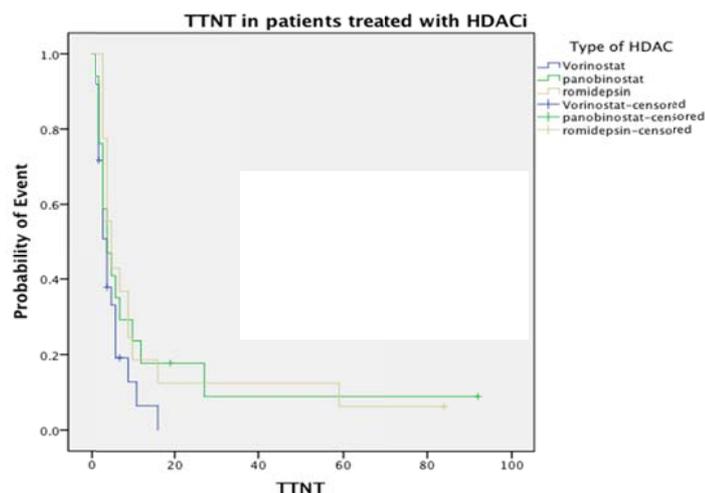
To investigate time to next treatment(TTNT) as a useful, objective and clinically relevant endpoint, for analysis of retrospective data in patients with cutaneous T-cell lymphoma(CTCL) treated with histone deacetylase inhibitors(HDACi) .

### Methods:

Analysis of TTNT, interval between D1 of successive therapies, in consecutive CTCL patients treated with HDACis from a national database between 2000-2013.

### Results

Sixty patients were managed with HDACi in trials or access programmes. Median age: 56 years (range 21-79) at diagnosis, 63 years (range 33-84) at treatment. M:F ratio was 1:1. 41 patients had mycosis fungoides and 19 patients had Sezary syndrome. Most patients had early stage disease: Ia(15%) and Ib(30%) at diagnosis. The median number of prior therapies was 3 (range 1-9). The median overall survival was 123 months(CI, 46-199). The median TTNT for the entire cohort was 4.5 months (range 1-92); subgroup analysis revealed that median There was no difference in TTNT for patients treated with vorinostat (n = 25), panobinostat (n = 17) and romidepsin (n = 18) was 4 months (range 1-16), 4 months (range 1-92), and 5 months (range 3-84), respectively(p=.176), which demonstrated that some patients derived prolonged benefits from HDACi. When compared to other systemic therapies; the median TTNT for high dose methotrexate(n=15) was 1.2 months (range 1-13), interferon alpha (n= 26) 5 months (range 1-50), CHOP chemotherapy(n = 10) 3 months (range 1-10), gemcitabine (n = 10) 3.8 months (range 1-38), and intensive multiagent chemotherapy(n = 52) 3 months (range 1-54).



### Conclusion:

Our data compares with larger studies for vorinostat (Duvic,2007), panobinostat (Duvic,2013) and romidepsin (Whittaker,2010), which report time to progression of 3, 4 and 8 months respectively. Relapsed/ refractory patients with CTCL who are treated with HDACi have a median TTNT similar or superior to other systemic therapies with some patients achieving prolonged periods of disease control.

## **P078. Low dose subcutaneous alemtuzumab is effective and well tolerated in patients with heavily treated Sezary Syndrome**

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### **Aim**

Alemtuzumab is a monoclonal antibody directed against surface antigen CD52, expressed on normal and malignant lymphocytes. Response rates of 40-80% are noted in patients with advanced Sezary Syndrome(SS) treated with an intravenous(i.v.) dose of 30 mg TIW for 12 weeks; this regimen has significant toxicities. We investigated the tolerability and efficacy of low dose alemtuzumab 10mg by the subcutaneous route(s.c.) in patients with relapsed/ refractory SS.

### **Method**

Patients with relapsed/refractory SS were treated with with alemtuzumab 10mg s.c. TIW for 4 to 6 weeks; this was followed by either allogeneic haematopoietic progenitor cell transplant or extracorporeal photopheresis(ECP) +/- Interferon $\alpha$ / vorinostat. Co-trimoxazole, posaconazole and valaciclovir prophylaxis was routine. CMV PCR was monitored in seropositive patients. Response rates, time to next treatment(TTNT) and adverse events were compared to a historical cohort of patients treated with i.v alemtuzumab

### **Results**

11 patients have been treated between Mar 2013 -Mar 2014. Median age was 68 years(range 33-83). M:F ratio was 1:1.2. Median prior lines of systemic therapy: 4(1-10). At a median follow up of 10 months (range 3-14) the overall survival was 90%. ORR was 90% including 2 complete responses, 8 partial responses and 1 progressive disease. Median TTNT was not reached. Adverse events included 1 case of acute renal failure and 1 CMV reactivation. No significant cytopenias were seen. The historical cohort (n=7) had a median age of 47 (31-68) with a median of 8 (5-16) lines of therapy. The ORR rate was 38%; median time to progression was 3 months. Grade 3-4 cytopenias were noted in >50% of patients along with CMV, HSV, VZV and parvovirus infections (Kennedy,2003,EJH).

### **Conclusion**

Low dose short duration s.c. alemtuzumab therapy is well tolerated and associated with high ORR in heavily pretreated patients with SS. Responses can be prolonged if followed up by immunomodulatory or maintenance therapy.

## **P079. Iodine-131 rituximab radioimmunotherapy for lymphoplasmacytoid lymphoma**

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### **Aim**

Current treatment options for lymphoplasmacytoid lymphoma (LPL), a bone marrow based B cell malignancy commonly associated with IgM paraprotein, include combination therapy with rituximab, corticosteroids, alkylating agents, and nucleoside analogues. However, relapses are inevitable and exploration of novel agents which confer survival advantage, long periods of disease remission, and minimal toxicity is essential. We have treated patients with LPL with Iodine-131 Rituximab radioimmunotherapy (<sup>131</sup>I-rituximab RIT) and now analyse outcomes.

### **Method**

All patients with LPL who were treated with <sup>131</sup>I-rituximab RIT at Fremantle Hospital and Health Service were identified. After informed consent, a tracer dose of <sup>131</sup>I-rituximab was given according to the standard personalised dosimetry protocol on day -7, predicated upon a whole body radiation absorption dose of 0.75 Gy. Treatment dose <sup>131</sup>I-rituximab RIT was then administered and we followed up patients with regard to response to treatment and toxicity.

### **Result**

Seven patients with LPL were treated with <sup>131</sup>I-rituximab RIT between 2006 and 2013. 4/7 patients were male, median age 60 years (range 51-85). 4/7 patients had <sup>131</sup>I-rituximab RIT as first line therapy, whilst the other 3 had a median of 2 prior lines of therapy. All patients reported resolution of symptoms attributable to LPL post <sup>131</sup>I-rituximab RIT. No cases of sepsis requiring admission to hospital occurred. Hypothyroidism occurred in 2/7 patients who were treated with thyroxine. No other significant toxicity occurred. 5/7 patients remain alive at a median follow up of 51.5 months, only one of whom required further treatment with intravenous immunoglobulin.

### **Conclusion**

These preliminary data suggest that <sup>131</sup>I-rituximab RIT is an appealing treatment for LPL as it is efficacious with minimal toxicity. This modality of treatment has not been reported as yet and more experience is necessary to further elucidate the long term response rate and also its benefits over conventional chemotherapy.

## P080. Chronic renal graft-versus-host disease (GVHD) following reduced intensity conditioning allograft manifested as nephrotic syndrome secondary to minimal change disease on a background of thin basement membrane disease: A case report and literature review

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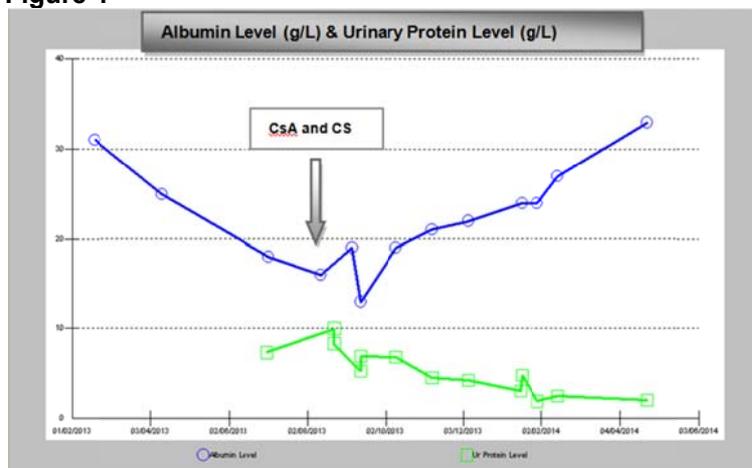
### Case Presentation

A 58 year-old-woman developed progressive peripheral oedema and limited sclerodermatous skin changes 18 months following fludarabine/melphalan sibling peripheral blood allograft for multiply relapsed follicular lymphoma which remained in ongoing remission. All systemic immunosuppression (SI) was withdrawn by 8 months post allograft. Investigations revealed marked hypoalbuminaemia of 16 g/L, mild eosinophilia of  $0.6 \times 10^9$  /L, hypercholesterolaemia (total cholesterol 8.2mmol/L), microscopic haematuria (330 RBC/ul), proteinuria of 10.98 g/24hrs with preserved renal function (serum Cr 47  $\mu$ mol/L) and a positive ANA titre (>1280, homogenous nucleolar). Renal biopsy electron microscopy demonstrated widespread foot process swelling and effacement and also thin basement membranes (mean 163nm). The clinical diagnosis was renal chronic GVHD with a pattern of minimal change disease and the patient was commenced on cyclosporine (CsA) (150 mg bd) and prednisolone (25 mg/d). We observed an excellent response within 10 months with marked reduction in proteinuria to 0.7g/24hrs and normalized albumin (Figure 1) as well as improvement of her skin GVHD. The patient demonstrates an ongoing response to reduced dose of cyclosporin and prednisolone at 12 months.

### Discussion

Nephrotic syndrome due to renal graft versus host disease is a rare complication of allogeneic stem cell transplant with a reported incidence of 1.3% for matched sibling donor. The aetiology is largely heterogenous and remains poorly understood. It is most commonly associated with membranous glomerulonephritis (MGN) and less frequently with minimal change disease (MCD), IgA nephropathy and focal segmental glomerulosclerosis (FSGS). Due to paucity of data, the natural history and the most effective treatment options remain ill defined and are largely based on idiopathic MGN and MCD as well as cGVHD. CsA and corticosteroids (CS) are most commonly used immunosuppressants with variable response rates and underreported long-term outcomes. According to previously published case reviews, MCD appears to have significantly greater response rates than MGN (90% vs 62%).

Figure 1



## **P081. Burkitt-like post-transplant lymphoproliferative disorder (PTLD) presenting with breast mass in a renal transplant recipient: A report of a rare case**

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### **Introduction**

Post-transplant lymphoproliferative disorder (PTLD) is a known complication of both solid organ transplant and stem cell transplant. PTLD has a variety of clinical presentations but it very rarely involves the breasts. We report a rare case of a patient with PTLD who presented with a breast mass and the biopsy of the mass showed Burkitt-like lymphoma.

### **Case Report**

A 55-year-old woman with past history of hypertension, gastric ulcer, and chronic renal failure had cadaveric renal transplant in April, 2001. Her maintenance immunosuppressive therapy was azathioprine and cyclosporin. In 2009, she presented with a mass in her left breast. Physical examination showed a non-tender mass of 5 cm in diameter at the lateral aspect of her left breast. There were no palpable lymph nodes or hepatosplenomegaly. The lactate dehydrogenase level was elevated at 2093 U/L. Biopsy of the left breast mass revealed PTLD, Burkitt-like lymphoma. Bone marrow examination did not show any evidence of lymphoma involvement.

The patient was treated with immunosuppression reduction. There was early initiation of hyper-CVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone) chemotherapy in view of the aggressive nature of Burkitt-like lymphoma. She also received intrathecal methotrexate and cytarabine as central nervous system (CNS) prophylaxis. Further consolidation with high-dose methotrexate (1g/m<sup>2</sup>) and cytarabine (3g/m<sup>2</sup>) were given. She was given six cycles of hyper-CVAD chemotherapy alternating with high-dose methotrexate and cytarabine. Repeated imaging showed that complete remission was achieved after chemotherapy. The patient remained well four years after treatment and there was no evidence of a relapse of the lymphoma.

### **Conclusion**

This is a rare case report of Burkitt-like PTLD involving the breast. PTLD would be considered as a differential diagnosis of breast mass in post-transplant patients. Early recognition of this rare but aggressive entity is important because immunosuppression reduction with concurrent chemotherapy can result in good clinical outcome.

## **P082. HHV-8 Unrelated Primary Effusion Lymphoma**

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*Western Health*

HHV-8 unrelated primary effusion lymphoma (PEL) is a rare but increasingly recognized subtype of PEL not described in the WHO classification. 55 cases have been published in the literature with favourable outcomes following standard chemotherapy<sup>1</sup>. We report 3 cases at our institution.

Three women, aged 67, 76 and 79 years-old respectively, presented with symptomatic pleural effusions. In each case, there was no history of HIV positivity or immunodeficiency. Pleurocentesis was performed and in each case, examination of pleural fluid revealed large atypical cells. Two cases demonstrated expression of pan-B cells markers and light chain restriction, the remaining case demonstrated strong CD20 expression by immunohistochemistry (IHC). Stains for HHV8 and EBV-ISH were negative for all three women. One patient demonstrated activated B cell subtype (Hans classification); another germinal centre B subtype (Hans classification) with Myc and Bcl-2 positivity by IHC, confirming double-hit status. Two patients underwent PET scan, the other CT-staging, confirmed the absence of associated tumour masses. One patient was treated with pleural aspiration alone; another aspiration and VATs pleurodesis. The third patient was treated with VATs pleurodesis and 6 cycles of R-CHOP chemotherapy.

Compared to PEL, HHV8-unrelated PEL typically affects HIV-negative immunocompetent, older patients. These 3 cases add to the literature suggesting the need for expansion of the WHO classification for lymphoma detected only in effusions.

<sup>1</sup> Wu W, Youm W, Rezk SA and Zhao X. Human Herpesvirus 8 – Unrelated Primary Effusion Lymphoma-Like Lymphoma. *Am J Clin Pathol* 2013;140:258-273.

## **P083. CD20 negative relapsed DLBCL in the upfront rituximab era: a single institution experience**

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*Princess Alexandra Hospital, Brisbane, QLD, Australia*

The clinical significance of CD20 negative relapsed Diffuse Large B Cell Non Hodgkin Lymphoma is of interest in the rituximab era. Its true incidence has yet to be elucidated but case reports and small retrospective cohort studies have suggested poor outcomes for CD20 negative relapse, also there is some data suggesting that azacitidine may modulate surface CD20 expression. To further inform our clinical practice, we reviewed our institution's DLBCL database to assess the incidence and outcomes of CD20 negative relapses in an institution with autologous stem cell rescue available.

A prospective lymphoma database collated at the Princess Alexandra Hospital Brisbane was used to identify patients with DLBCL who received upfront immunochemotherapy. This and the electronic radiology and pathology systems were then interrogated to determine the patients who had relapsed, whether they were biopsied and what their CD20 status at relapse was.

A total of 274 patients with CD20 positive newly diagnosed DLBCL, or variants, treated with upfront rituximab containing chemotherapy between April 2003 and June 2013 were identified. Of these 274 patients, 71 either progressed during front-line therapy or relapsed after gaining complete. Forty-one of these patients were re-biopsied to confirm relapsed/refractory lymphoma. Of the 41 biopsies, 36 had CD20 expression assessed by immunohistochemistry and/or flow-cytometry and in five CD20 was unable to be detected. None of the five patients had straight-forward DLBCL. All five had died of disease within 20 months of diagnosis with none having gained meaningful response to salvage therapy precluding a subsequent ASCT.

Our data suggest that CD20 negative DLBCL relapse is rare after first line therapy for de novo DLBCL. Our five CD20 negative relapsed cases had a universally dismal outcome, but it is difficult to conclude any relationship with the CD20 status as all five had independent poor prognostic factors at diagnosis.

## **P084. Australian Governance and Management Framework for Immunoglobulin Products**

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### **Aim**

Australian governments are committed to providing an adequate, safe, secure and affordable supply of blood products, and promoting safe, high quality management and use of those products.

In Australia it is estimated that over 99% of all intravenous immunoglobulin (IVIg) is supplied and publicly funded under national blood arrangements through contracts administered by the National Blood Authority (NBA). Demand continues to rise at a rapid rate.

### **Method**

In 2012, governments commissioned a review of the adequacy of clinical governance and authorisation of IVIg to recommend options for improvement.

The review concluded there were significant variations in management processes and diagnoses nationally, high prescription rates in some conditions compared to international rates of use, and limited transparency of price with no accountability for cost with the prescriber. These conclusions are supported by analysis of data from the current process for authorisation of product requests.

### **Result**

Governments have endorsed a program of measures to improve the governance and management of immunoglobulin products, to ensure:

use and management reflects appropriate clinical practice and is cost effective, in accordance with relevant national safety and quality standards for health care;

access is consistent with the criteria for access determined by governments; and

use and outcomes are captured to inform future changes to the criteria.

### **Conclusion**

This strengthened governance and management framework will be delivered by more integrated and direct partnering of clinical, policy, analysis and health economic perspectives through a number of initiatives that support appropriate and improving clinical practice and effective product management. This will be based on improving evidence derived from a national immunoglobulin ordering and outcomes database, so that product management and clinical practice will best meet patient needs while ensuring effective and sustainable use of immunoglobulin products going forward.

## **P085. The role of gemcitabine based therapy as second-line salvage therapy after failure of platinum-based salvage in aggressive NHL**

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*Princess Alexandra Hospital*

### **Introduction**

Relapsed and refractory aggressive non-Hodgkin lymphoma (NHL) has a poor prognosis. Salvage chemotherapy followed by high dose chemotherapy and stem cell transplantation improves progression free and overall survival. The outcome for patients who do not respond to initial salvage and are treated with an alternative regimen is less clear.

### **Aim**

The aim of this study was to determine whether gemcitabine based salvage chemotherapy is effective in patients who have failed platinum based salvage and whether subsequent stem cell transplantation in these patients is potentially curative.

### **Methods**

An audit of transplant and pharmacy databases at the Princess Alexandra Hospital was conducted to identify patients who had received gemcitabine based therapy following failed platinum based salvage. A retrospective review of patient charts, pathological and radiological databases was conducted.

### **Results**

Twelve cases were identified who were treated with gemcitabine based second-line salvage. Of these patients, eight had refractory disease to first salvage, two had renal toxicity from platinum and two had partial responses to platinum-based therapy. Six received R-VGIF, 2 FGIV, 1 R-VGF, 2 VGF and 1 R-GDP. Four patients (33.3%) achieved a partial remission (PR) or better to second salvage and all of these patients underwent stem cell transplantation. The remaining 8 patients (66.7%) did not respond and died of progressive disease. The 4 patients who were transplanted were alive at day 100 post transplant. The overall survival was poor with a median survival post second salvage of 2.8 months. At the time of audit, 9 patients had died from progressive disease, 1 patient had died from another malignancy and 1 patient was lost to follow-up. Only 1 patient was still alive and in CR, though with a second primary malignancy, at last follow-up 19 months post gemcitabine based salvage.

### **Conclusion**

Gemcitabine based therapy following failed ESHAP-like therapy achieves a response in a third of patients, opening up the potential for subsequent transplant. The overall survival post-transplant remains poor for these initially chemo-refractory patients.

## **P086. Flow cytometric analysis of peripheral blood is of limited value in the investigation of non-Hodgkin lymphoma and lymphoproliferative disorders in the absence of lymphocytosis**

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### **AIM**

To investigate the value of peripheral blood lymphocyte malignancy immunophenotyping for the investigation of non-Hodgkin lymphoma (NHL) and lymphoproliferative disorders (LPD) in our tertiary institution.

### **METHOD**

A retrospective review was conducted of all peripheral blood specimens sent to the Fremantle Hospital Flow Cytometry Laboratory for lymphocyte malignancy immunophenotyping in 2013. Clinical indications were correlated with flow cytometry results and clinical outcome.

### **RESULTS**

241 tests were performed. 93/241 had a clear indication for lymphocyte testing based on the clinical notes provided and/or the presence of lymphocytosis. 55/241 had various clinical details of uncertain relevance to lymphoid malignancy. Of these, one case had an atypical flow cytometry finding of monoclonal B cell lymphocytosis (MBL).

The remaining 93/241 specimens were for the investigation of NHL/LPD in the absence of lymphocytosis. 78 (84%) of these cases showed no evidence of atypical flow cytometry; 16 were found to have an NHL/LPD, 23 had further testing with no NHL/LPD outcome, 27 had no evidence of further testing, and 12 were lost to follow up.

15/93 cases sent for NHL/LPD with no lymphocytosis had atypical flow cytometry results; 7 of these were monoclonal B cell lymphocytosis (MBL), 6 were B-NHL confirmed by concurrent tissue biopsy testing, and 2 were equivocal.

### **CONCLUSION**

We conclude a high number of inappropriate requests for lymphocyte malignancy immunophenotyping on peripheral blood specimens are sent to our laboratory. The majority of these requests appear to be as a screening tool for NHL/LPD. Our results demonstrate flow cytometry of peripheral blood is of limited value to confirm or exclude NHL/LPD in the absence of lymphocytosis. As a result, our laboratory has introduced more stringent testing guidelines and vetting procedures.

## **P087. T-Cell Prolymphocytic Leukaemia: single institution experience with 10 patients**

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*Royal North Shore Hospital*

**Aim:** To review patients treated for T-cell prolymphocytic leukaemia (T-PLL) at our institution over the past seven years. During this period we have adopted the Royal Marsden Hospital's approach of alemtuzumab monotherapy induction followed by haemopoietic stem cell transplantation where possible in eligible patients.

**Methods:** Patients with T-PLL were identified by a database search. Overall survival (OS) was calculated from diagnosis to death. Progression-free survival (PFS) for alemtuzumab patients was calculated from treatment commencement to relapse or death.

**Results:** We identified 10 patients treated for T-PLL between 2007 and 2014. The median age at diagnosis was 67.5 years (range 49-81) with a slight male preponderance (6:4). The immunophenotype 4+8- (6 patients) was more common than 4+8+ (3) and 4-8+ (1). A complex karyotype was seen in eight patients; isolated del(10p) in one; and a normal karyotype in another.

Seven patients received alemtuzumab monotherapy induction as initial therapy. The median time between diagnosis and treatment was 11.5 months (range 0.5-55 months). Complete remission occurred in six patients (86%). Reactivation of CMV during induction occurred in three patients (43%). Stem cell transplantation was undertaken in four patients: two autologous and two allogeneic. In the alemtuzumab group three patients have died: one from CMV and other complications during induction (T-PLL in PR); one from a fulminant EBV-related lymphoproliferative disorder shortly after autologous transplant (T-PLL in CR); and one from sepsis approximately one month after induction (T-PLL in CR). The median OS for the alemtuzumab cohort was not reached. Median PFS was 20.7 months. The remaining three patients received a variety of chemotherapy regimes. None achieved a complete remission. Their median overall survival was 18 months.

**Conclusion:** Alemtuzumab monotherapy induction followed by stem cell transplantation as consolidation therapy has provided relatively favourable outcomes compared to historical data in this small single institution cohort study.

## **P088. Case Report: An unusual presentation of relapsed Hodgkin lymphoma**

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### **Background**

Patients with advanced stage Hodgkin Lymphoma have a 20-30% chance of relapse. We present a case of an unusual site of relapse - the peritoneal cavity, in a 47 year old man with chronic renal failure on peritoneal dialysis.

### **Case Report**

A 47 year old man with a past history of Hodgkin Lymphoma and chronic renal failure presented with turbid peritoneal dialysate fluid.

He was originally diagnosed with stage IV Nodular Sclerosing Hodgkin lymphoma 3 years prior and treated with BEACOPP-14 x 8 chemotherapy to achieve CR1. After an 18 month remission he relapsed with limited stage disease. He commenced 2 cycles of RICE chemotherapy, had autologous stem cells collected and was planned to proceed to an autograft. Prior to autograft, his course was complicated by severe sepsis and Acute Kidney Injury. He commenced peritoneal dialysis and after confirmation of CR2 on CT and PET scanning, no further anti-lymphoma treatment was given.

Twelve months later, he was noted to have a turbid peritoneal dialysate. He also described night sweats, fatigue, and 4kg loss of weight. Microscopy of the peritoneal fluid demonstrated  $450 \times 10^6/L$  atypical lymphoid cells, demonstrating characteristic Hodgkin lymphoma morphology.



Subsequent investigations revealed omental caking with moderately enlarged lymph nodes in the peritoneum and PET demonstrated moderate 18-FDG avid diffuse soft tissue thickening in the greater omentum consistent with relapsed disease. He has since demonstrated an excellent response to salvage Vinorelbine and Gemcitabine chemotherapy and is being considered for an autograft.

### **Conclusion**

Peritoneal lymphomatosis has been described with non-Hodgkin lymphoma however not in Hodgkin lymphoma. Concurrent peritoneal dialysis allowed early detection of lymphomatosis in dialysate fluid. Response to treatment has not been hampered by site of disease, although a change to haemodialysis was required due to reduced capacity of the diseased peritoneal cavity to function as an efficient dialysis membrane.

## **P089. Expression and sub-cellular localisation of NFkappaB in lymphoproliferative diseases: optimisation of an antibody panel for imaging flow cytometry**

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**Aim:** The transcription factor NFkappaB is constitutively activated in many haematological malignancies, including multiple myeloma, acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemia and chronic lymphocytic leukaemia. Published data highlights the therapeutic and prognostic potential of NFkappaB expression in some malignancies. Studies of NFkappaB expression/activation are primarily performed by fluorescence microscopy, western blotting and enzymatic detection methods, all of which are useful but lack the quantitative and analytical power of flow cytometry.

Imaging flow cytometry is a powerful platform that couples microscopy with flow cytometry, enabling quantitative cell population analysis while at the same time tracking sub-cellular localisation of intracellular markers. This allows the translocation of activated NFkappaB from the nucleus to the cytoplasm to be visualised whilst simultaneously performing leukocyte immunophenotyping.

**Methods:** We optimised an 8-colour flow cytometry panel to observe NFkappaB expression and sub-cellular localisation in lymphocyte populations. The panel includes CD3, CD5, CD10, CD19, CD20, CD45, nuclear stain (Hoechst) and NFkappaB. Our gating strategy is modelled on the PathWest diagnostic lymphoproliferative panel which first gates lymphocytes on a CD45 vs SS scatter plot, then plots for expression of CD3, CD5, CD10, CD19 and CD20. Lymphocyte populations are then analysed for expression and localisation of NFkappaB.

**Results:** This panel allows quantitative analysis of NFkappaB expression and sub-cellular localisation in large numbers of cells and in lymphocyte sub-populations. Quantitative flow cytometry data is validated against routine analysis of samples performed by PathWest on a FACSCantoll and nuclear translocation of NFkappaB is verified using unstimulated and RANKL-stimulated RAW264.7 mouse macrophage cells.

**Conclusion:** This method enables the systematic analysis of NFkappaB expression and distribution within cells in lymphoproliferative diseases, to identify disease entities and cell populations that have aberrant activation of NFkappaB.

## **P091. Comprehensive B cell phenotyping according to normal ontogeny improves detection of peripheral blood and bone marrow involvement and demonstrates immunophenotypic and genotypic diversity.**

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**Aims:** While characterisation based on cell-of-origin (COO) into germinal centre (GC) and activated B-cell (ABC) subtypes is prognostic in diffuse large B-cell lymphoma (DLBCL), considerable heterogeneity remains unexplained. We developed comprehensive B-cell maturation flow cytometry panels for diagnosis and staging in DLBCL patients aiming to improve detection of B-cell clones and characterise their phenotypic and genotypic diversity.

**Methods and results:** Our multicolor flow cytometry panels elucidated several B-cell populations in 104 DLBCL patients including 79 peripheral blood (PB) samples, 83 bone marrow (BM) samples, and 12 lymph node samples. The mean age of the patient cohort was 64.01 years (range: 30.08 to 94.08 years). Cell of origin using the Hans algorithm was as follows: GC = 30/79 cases, ABC = 49/79 cases. Overall survival at 5 years was 56.8% (Standard error=0.08). Clonal populations were identified in 26/104 (26.5%) PB/BM samples (cf. 44/238, 18.5% historical controls). Immunophenotyping in 35 cases allowed classification based on normal B-cell ontogeny. Cases with involvement were genotyped using a customised Haloplex library preparation kit to screen for ~45 lymphoma-associated mutations. The IgM memory immunophenotypic variant was found to be less likely to have >7 lymphoma-associated somatic mutations compared to other phenotypes (p=0.03) further suggesting that the variants have differing cellular origins.

**Conclusions:** Comprehensive B cell phenotyping based on normal ontogeny can improve detection of small B cell clones in PB/BM, and demonstrate immunophenotypic diversity in DLBCL. Correlations with genotyping suggest these variants may arise from differing cellular origins and potentially improve the traditional COO classification.

## P092. Clinical characteristics, treatment patterns, and outcomes of patients with localized Gastrointestinal Follicular Lymphoma

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Peter MacCallum Cancer Centre

### Aim

To describe the clinical characteristics, patterns of treatment and outcomes of patients with localized Gastrointestinal Follicular Lymphoma (GI-FL).

### Method

Retrospective review of all patients with GI-FL at PMCC (Melbourne, Australia) April 2003-July 2013. Patients were identified through pathology database search and data were extracted from clinical, radiology and radiotherapy databases.

### Results

11 patients with GI-FL were identified. Characteristics: median age 54 (range: 43-65), male: 8 (73%), location (6 duodenum only, 2 duodenum/small intestine, 1 small intestine, 2 mesenteric lymph nodes), histology (grade 1: 5, grade 1-2: 6), Lugano stage (stage 1: 8, stage 2: 3), B symptoms (0), ECOG status (0: 9, 1: 1, 2: 1), FLIPI score (0: 8, 1: 3), *H. pylori* positive (0 of 4 tested [rapid urease test]). Investigations: PET + lesion 8 (1 upstaged), invasive (endoscopy 9, laparoscopy 2), pillcam 2 (1 identified new lesion),

	Patients	CR	Relapse/progression (timing) <sup>1</sup>	Deaths	Follow up <sup>2</sup> (months)
All Radiotherapy <sup>3</sup>	8	8	1 <sup>3</sup> (66)	1 <sup>4</sup>	18 (14-120)
Radiotherapy only <sup>5</sup>	6	6	1 (66)	0	14 (14-101)
Surgery only	1	1	0	0	21
Monitor	2	NA	0	0	52 (22-82)
Entire cohort	11	9	1 (66)	1	22 (14-120)

SD= stable disease, NA= not applicable

<sup>1</sup>months

<sup>2</sup>median months, range in brackets ()

<sup>3</sup>in & out of radiotherapy field

<sup>4</sup>death due to second malignancy (out of radiotherapy field)

<sup>5</sup>median dose: 30 Gy, 2 patients received additional boost dose (median: 4 Gy)

Radiotherapy adverse events: abdominal symptoms (grade 1-2: 6/8)

### Conclusion

Whole abdominal radiotherapy is tolerated well and achieves CR in GI-FL. Longer term follow up and larger patient population is required to accurately assess PFS/OS and long-term sequelae of radiotherapy. PET scan and full bowel visualisation (e.g. pillcam) is recommended to assess extent of disease.

## **P093. Whole genome differential microRNA profiling of Primary Central Nervous System Lymphoma (PCNSL) compared to systemic Diffuse Large B Cell Lymphoma (DLBCL)**

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### **Aim**

We investigated genome wide miRNA signatures in an attempt to identify miRNAs that are differentially expressed and are unique to PCNSL compared to systemic DLBCL.

### **Method**

RNA was extracted from FFPE samples consisting of 57 PCNSL from immunocompetent patients, 36 systemic DLBCL and normalised to control tissue (12 each of reactive lymph nodes and normal brain). We measured the expression of 752 miRNA sequences by quantitative real-time PCR using whole miRNA genome arrays. The data were analysed using the  $\Delta\Delta\text{Ct}$  method. Normalised Ct values were expressed relative to their control tissue to determine fold change and a p-value was obtained using a paired t-test. Hierarchical clustering of cases was performed using Pearson correlation.

### **Results**

96% of the miRNAs examined were expressed in 90% of the PCNSL and DLBCL cases. Of these, 18% were underexpressed and 5% were overexpressed, in PCNSL, and 17% were underexpressed and 7% overexpressed in DLBCL ( $p < 0.5$ ). 71 miRNA's were shown to be significantly overexpressed ( $p < 0.05$ ) in PCNSL compared to DLBCL, miR-1321, miR-155 and miR200c being the most overexpressed. Most differentially expressed miRNA's were downregulated in PCNSL compared to DLBCL, miR-145, miR-122, miR-497, miR-579 and miRNAs associated with the C-MYC pathway being the most underexpressed. Several novel miRNAs were overexpressed in PCNSL compared to DLBCL including miRNA-132, whose predictive target is BOB1. miRNA-212 which is enriched in neuronal cells was underexpressed in PCNSL compared to DLBCL indicating it may be acting as a tumour suppressor.

### **Conclusion**

To the best of our knowledge this study represents the first whole genome miRNA profiling of PCNSL in immunocompetent patients. The study demonstrates that PCNSL has a different miRNA expression profile from DLBCL indicating that they are two distinct biological entities. miRNA profiling of PCNSL has diagnostic and prognostic significance and can be used to further identify potential gene targets.

## **P094. Retrospective analysis of the incidence of prolonged cytopenias in patients treated with anti-metabolite drug – Fludarabine**

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*Gold Coast University Hospital*

### **Aim/Background:**

Bone marrow suppression is a known effect of the purine analog Fludarabine – an antimetabolite drug used in a variety of haematological disorders. Some patients have been noted to have prolonged, persistent cytopenias with significant and often serious sequelae.

Here we chose to review our patient population treated with Fludarabine and examine the incidence of prolonged cytopenias and characteristics of these patients.

### **Methods:**

A retrospective chart review was performed of adult patients treated with frontline Fludarabine at the Gold Coast Hospital between 1<sup>st</sup> January 2010-31<sup>st</sup> December 2012.

Data was collected on patients up to one year post the first day of their last chemotherapy cycle containing Fludarabine. Prolonged cytopenia was defined as ANC <  $2.0 \times 10^9/L$ , platelet count <  $150 \times 10^9/L$  or Hb < 120g/L at 3 months post day 1 of the last cycle of chemotherapy.

### **Results:**

52 patient charts were reviewed. Median age was 70 years (range 44 -79 years), 62% were male. 70% had received Fludarabine for chronic lymphocytic leukaemia (CLL)

The most common treatment regimen was Fludarabine in combination with Cyclophosphamide and Rituximab (FCR) in 80% of patients.

50% of patients had neutropenia at 3 months post treatment. Of these 30% had grade 3 neutropenia (ANC < 1.0) and 22% had grade 4 (ANC < 0.5). At 9 months post completion of therapy, 16% had persistent neutropenia of any grade. Thrombocytopenia was noted in 67% of patients – of these 33% had grade 3 thrombocytopenia (plts < 50) and 17% had grade 4 (plts < 25). Anaemia was seen less frequently.

### **Conclusions:**

Fludarabine is a very effective chemotherapy agent, particularly for low grade lymphomas and CLL. However it is associated with prolonged myelosuppression and ongoing monitoring of blood counts is required

Further evaluation is ongoing and will be presented

## **P095. Recurrent *Helicobacter cinaedi* bacteraemia during chemotherapy for diffuse large B cell lymphoma**

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### **Aim**

To present an unusual case of recurrent bacteraemia during chemotherapy for diffuse large B cell lymphoma (DLBCL).

### **Case history**

A 44 year old male was diagnosed with Stage IIB DLBCL in January 2014. He underwent seven cycles of standard R-CHOP chemotherapy followed by involved field radiation therapy. He was admitted with neutropenic fevers on two occasions. On the first occasion, he had several ovoid, brown, macular lesions on his torso and no other potential focus of infection. He was treated with initial IV ceftazidime and vancomycin therapy then de-escalated to oral ciprofloxacin therapy on count recovery. He was treated with IV cefepime on the second occasion. On both occasions, blood cultures became positive with gram negative bacilli after over seventy hours incubation (73 – 115 hours). On both occasions, 16S rRNA gene analysis identified the agent as *Helicobacter cinaedi*. He had no clinical signs to suggest infective endocarditis and his trans-oesophageal echocardiogram was negative. He has been treated with a prolonged course of IV ceftriaxone and oral doxycycline. Repeat surveillance cultures remain negative.

### **Discussion**

*H.cinaedi* is a gram negative bacillus believed to be an enterohepatic organism of low pathogenic potential. Infection in the form of bacteraemia or cellulitis has been described in immunocompromised patients including those with HIV/AIDS, X-linked agammaglobulinemia, SLE, end stage renal failure on haemodialysis, and malignancies undergoing chemotherapy. It has a tendency to recur. A variety of antimicrobial classes have been used to treat it.

### **Conclusion**

*H. cinaedi* bacteraemia is an unusual infection which can be recurrent in the setting of immunosuppression. Clinicians should consider this atypical organism in this group of patients. At present, the standard treatment to eradicate this organism is unclear.

## **P096. Prognostic Impact of SNP Array Karyotyping in Myelodysplastic Syndromes (MDS)**

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### **Background**

Metaphase cytogenetics (MC) is the “gold standard” for karyotypic analysis in haematological malignancies including MDS. However because of technical limitations of MC, such as low resolution and the need for dividing cells, many important genetic defects can be missed resulting in detectable genetic aberrations in only 40-50% of MDS cases. Single nucleotide polymorphism (SNP) arrays have emerged as an important tool in detecting genetic defects undetected by MC. In addition to a high level of resolution, SNP-array allows detection of acquired copy-neutral loss of heterozygosity (aCN-LOH), a common chromosomal defect in MDS (~12-20% cases), undetectable by conventional MC.

We hypothesize that a combination of conventional MC and SNP-array will improve detection of cytogenetic abnormalities in lower risk MDS cases and thus refine prognosis further.

**Aim:** To improve detection of cytogenetic abnormalities in low or intermediate risk MDS patients by combining SNP-array and conventional MC

**Method:** We selected 78 IPSS-low or intermediate risk MDS patients with known karyotype for SNP-microarray analysis. DNA was extracted from residual cytogenetics fixed cell pellets and assessed using the IlluminaCytoSNP 850K bead array. Suitable quality DNA was obtained from 75 patients.

### **Results:**

23(31%) showed karyotypic abnormality. Of the 52 (69%) karyotypically normal patients 22 (42%) were abnormal following SNP-microarray. 13/23 (57%) of the karyotypically abnormal patients showed additional cytogenetic abnormalities by SNP-microarray. The most common form of abnormality seen by array only was cnLOH and 16 chromosome arms were affected, 7q cn-LOH was the most common and was seen 6 patients, while 11qcn-LOH was seen in 3.

Median survival of patients with SNP-array ± MC abnormality was inferior than patients with normal SNP-array and MC (43 vs. 65 months)

**Conclusion:** Overall the cytogenomic abnormality rate increased from 31% by karyotyping alone to 60% by utilizing SNP-microarray, illustrating the potential improvement in detection of genetic abnormalities. Importantly in this limited patient number, median OS was inferior in patients with SNP-array abnormality compared to patients with normal SNP Array.

## **P097. Use of lenalidomide in treatment of a patient with 5q- myelodysplastic syndrome provides novel treatment prospects in management of pulmonary sarcoidosis: A case report and review of the literature**

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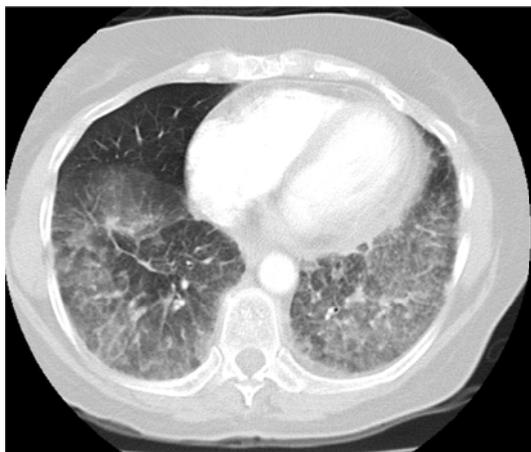
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There is established use of lenalidomide in patients with myelodysplastic syndrome (MDS) with isolated 5q- clone. There is also evidence to support the use of lenalidomide in the treatment of refractory cutaneous sarcoidosis. However, to date, there have been no reports demonstrating the potential benefits of lenalidomide as a therapeutic option in patients with pulmonary sarcoidosis.

We present the case of a 71-year-old female with history of refractory pulmonary sarcoidosis, who was investigated for persisting dyspnoea and macrocytic anaemia and found to suffer from 5q- MDS. Upon commencement of treatment with lenalidomide for her newly diagnosed 5q- MDS, her respiratory symptoms showed remarkable clinical improvement, with clear evidence of improvement in her lung function testing and of particular significance; computer tomography (CT) revealed complete clearance of bibasal alveolar infiltrates secondary to pulmonary sarcoidosis [Image 1 & 2].

It has been proposed that lenalidomide acts as an immunomodulatory agent when used in the management of 5q- MDS. Sarcoidosis is a multisystem granulomatous disorder associated with CD4+ T helper cell immune mediated response. As such, immunomodulatory and cytotoxic agents have been used in the treatment of sarcoidosis. There is evidence in the literature to establish the use of lenalidomide in cutaneous sarcoidosis as well as use of thalidomide, from which lenalidomide is an analogue, in treatment of cutaneous and pulmonary sarcoidosis. The effectiveness of use of lenalidomide in pulmonary sarcoidosis has not been reported.

This is the first case to report on efficacy and significant improvements clinically, radiologically and based on lung function testing in a patient with pulmonary sarcoidosis when using lenalidomide for management of 5q- MDS. This case demonstrates a potential role for the use of lenalidomide as a novel therapeutic agent in patients with refractory pulmonary sarcoidosis.



## **P098. Marked eosinophilia – an approach to diagnosis and why management should be a clinical individually-based approach**

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**Aim:** We present a case of marked isolated eosinophilia, review the relevant investigation pathway and highlight that marked eosinophilia is not necessarily indicative of either clonal or reactive aetiology.

**Case:** A 25yo Nepalese-born, previously well gentleman presented, on return from holiday to Nepal, with epigastric discomfort treated with esomeprazole. A FBE demonstrated isolated eosinophilia of  $30.2 \times 10^9/L$ .

We discuss subsequent investigation algorithm aimed at:

- (a) Assessment of clinical manifestations of hypereosinophilia
- (b) Exclusion of reactive causes due to allergy, infection including parasitic, pulmonary, or autoimmune disease
- (c) Exclusion of neoplastic disorders with secondary eosinophilia including T cell or Hodgkin Lymphoma, and ALL
- (d) Exclusion of neoplastic disorders with eosinophilia as part of the neoplastic clone focusing on cytogenetic and molecular markers including BCR-ABL and FIP1L1-PDGFR fusion genes, KIT and Jak2-mutation, PDGFRB or FGFR1 rearrangements, AML inv(16)(p13q22)

While the absolute level of eosinophilia is not diagnostic, male gender, splenomegaly, and elevated serum tryptase and Vitamin B12 with a normal IgE point to a clonal process. In our case, apart from gender, an elevated IgE of 1033IU/mL [ $<90IU/ml$ ] in the absence of atopic history suggested a reactive process.

In the setting of persisting eosinophilia, while awaiting results of above investigations, and despite the unpredictable nature of onset of organ involvement, rather than commencing corticosteroid or cytoreductive therapy, we opted for a watch-and-wait approach. He remained clinically well with resolution of GI symptoms and a normal eosinophil count 3 months post presentation. No clear cause was identified.

### **Conclusions:**

- (1) The absolute level of eosinophilia is not indicative of a reactive or clonal process.
- (2) Concurrent investigation of both and functional organ assessment is important.
- (3) A watch-and-wait policy may be appropriate and treatment should be an individually-based clinical decision.

## **P099. The risk factors and sequelae of primary portal vein thrombosis**

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### **Aim:**

To determine the risk factors and long-term complications of primary portal vein thrombosis (PVT) in a large tertiary centre.

### **Methods**

Radiology reports and patient discharge summaries were searched for patients with a diagnosis of primary PVT from 2000-2013. Patients with 'secondary' PVT related to cirrhosis, Budd-Chiari syndrome (BCS), recent abdominal surgery or biliary procedure, concomitant intra-abdominal sepsis or intra-abdominal malignancy were excluded.

### **Results**

Radiological findings of PVT were identified in 411 patients, of which 23 met the inclusion criteria. Of these, 13 patients had a prothrombotic risk factor including myeloproliferative neoplasms (MPNs) in 9, an inherited thrombophilia in 4 and the oral contraceptive pill as the only risk factor in one patient. 2 patients were identified as having a concurrent MPN and thrombophilia. PVT was the presenting feature of a JAK2+ MPN in 6 patients. No risk factor was identified in the remaining 10 patients. All of these patients underwent an inherited and acquired thrombophilia screen and 7 were tested for JAK2 mutation.

Acute and long-term complications of PVT were assessed in the 23 patients with primary PVT with a median follow up of 24 months (range 1-136). Thrombus extension to the mesenteric veins occurred in 12 patients, 2 of whom required a laparotomy and bowel resection due to mesenteric ischaemia. Seventeen patients (74%) developed portal hypertension, defined by radiographic or endoscopic evidence of varices, ascites or splenomegaly. Gastric or oesophageal varices were identified in 52%. Three patients suffered 4 episodes of variceal bleeding, one requiring the insertion of a decompressive porto-systemic shunt. Seven of 20 patients managed with anticoagulation therapy experienced a major non-variceal bleeding complication, which was fatal in one and required temporary or permanent cessation of therapy in the remainder. Of 21 patients with follow up imaging, 5 (24%) achieved complete recanalisation of the portal vein on anticoagulation over a median time of 4 months.

### **Conclusions**

Primary PVT is a rare but important cause of portal hypertension with frequent and dangerous acute and long-term sequelae. Given the high prevalence of MPNs in primary PVT, in our experience 35-40%, this study supports the key role of JAK2 mutation testing in PVT work-up.

## **P100. Detection of MPL mutations using high resolution melt analysis compared to allele-specific oligonucleotide PCR**

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### **Aim**

The Myeloproliferative Leukaemia virus oncogene (MPL) has gain of function mutations that have been described in Myeloproliferative Neoplasms. Acquired MPL mutations have been reported in Exon 10 at codon 515, in approximately 10% of PMF cases and 3% of ET cases. Current methodology used by this laboratory, ASO PCR, is specific only for the two most common mutations W515L and W515K. This method has other limitations; it has variable sensitivity and is time consuming. The aim of this study is to determine if a High Resolution Melt (HRM) based assay could be developed with equal or superior sensitivity to the ASO PCR.

### **Method**

HRM was performed on Life Technologies ViiA 7 analyser, using MeltDoctor master mix. Several primer sets spanning codon 515 of the MPL gene, generating PCR products of varying sizes, were tested to determine which amplicon provided optimal specificity. A primer set previously described by Boyd et al (2009) was selected and further tested using serially diluted MPL positive samples of known copy number to calculate the level of sensitivity. 30 blinded samples previously tested by NATA accredited in house ASO PCR were tested by HRM. The DNA was extracted by QIAGEN EZ1 robot.

### **Results**

Serial dilutions of both W515L and W515K positive plasmids demonstrated differentiation of normal samples from low level mutant alleles, confirming the sensitivity of the assay. HRM results for the blinded samples were concordant with results previously reported using the ASO PCR method.

### **Conclusion**

HRM provides a rapid means of screening the codon 515 'hotspot' region of the MPL gene, at a level of sensitivity comparable to the ASO PCR. Samples that had previously been identified by the ASO PCR as positive and low level positive were clearly detected using the HRM method. HRM has the added advantage of being capable of detecting other rare variants affecting codon 515, such as W515R and W515A.

## **P101. The National Myeloproliferative Neoplasm survey**

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### **Aim**

The National Myeloproliferative Neoplasms Survey is the first study to explore the experiences of people living with four myeloproliferative neoplasms (MPN) subtypes in Australia.

The aim of this survey is to explore the whole disease experience of people affected by the MPN subtypes – polycythaemia (rubra) vera (PV), essential thrombocythaemia (ET), myelofibrosis (MF) and systemic mastocytosis (SM). The survey explores the experiences of people living with a MPN, their quality of life, the Leukaemia Foundation support services they utilise and value, and the additional support services they require.

### **Methods**

The survey questions were developed based on previously validated Leukaemia Foundation surveys. The MPN-Symptom Assessment Form (MPN-SAF), a validated and reliable tool to assess symptom burden in people affected by PV, ET and MF, also was incorporated into the survey (Scherber et al, 2011).

Recruitment is through the Leukaemia Foundation database and via invitation to participate in awareness campaigns such as the Foundation's national MPN newsletter.

The survey will be open for two months and take 45-60 minutes to complete. It can be completed electronically on the Leukaemia Foundation website ([www.leukaemia.org.au](http://www.leukaemia.org.au)); as a mail out hard copy; or over the phone with a trained Leukaemia Foundation volunteer.

Data analysis will be conducted by an independent data analysis company.

### **Results**

The survey results will be presented and will focus on topics such as the circumstances around initial diagnosis, treatment – including access and clinical trials, impact of symptom burden on quality of life, geographical spread (metro, regional), complications of disease, and bone marrow transplantation, to name a few.

### **Conclusion**

This study provides the first structured insight into the whole disease experience and disease burden impact on the quality of life of people affected by these rare blood cancers in Australia. This research will give the Leukaemia Foundation a platform on which to guide the development of future support services to meet gaps identified.

\*(Scherber et al., 2011, Blood).

## **P102. Real world management of multiple myeloma: An initial snapshot from the Australia and New Zealand Myeloma and Related Diseases Registry (MRDR)**

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### **Aim**

Myeloma accounts for a high burden of disease in the community, with evidence of variation in care and disparity in patient outcomes not explained by disease characteristics alone.

### **Method**

The Myeloma and Related Diseases Registry (MRDR) aims to improve quality of care through systematic data collection on myeloma management and outcomes. Data on all incident cases at participating sites are collected via a secure web database.

### **Results**

437 patients from 9 hospitals were registered between Jan 2013-June 2014. Data were analysed on 283 patients.

Symptomatic myeloma was the diagnosis in 206 (72%) and MGUS or asymptomatic myeloma in 77 (28%). Median age was 66y; 57% were male. For symptomatic myeloma, 35% were high risk; 42% had one or more comorbidity at diagnosis. End-organ complications (CRAB) were hypercalcaemia (10%), renal impairment (10%), anaemia (27%) & bone lesions (52%).

Therapy data were available for 150 patients. Indication for first-line therapy: CRAB in 72%, plasma cell burden or rising paraprotein in 13%, other in 6% and not specified in 9%. The most common induction regimens were: bortezomib/cyclophosphamide /dexamethasone (65%); cyclophosphamide/thalidomide/dex (10%); and lenalidomide/dex (4%). 13 patients (9%) were enrolled in a clinical trial. Responses are recorded to date in 72 patients, of whom 14% had a CR, 24% VGPR, 41% PR, 10% minimal response and 11% stable disease. 9 patients went on to second line therapy, 3 for relapse and 6 for sub-optimal response. Information on ASCT was available for 81 patients, of whom 64% have or are planned to undergo ASCT, and 36% will not due to age, comorbidities or performance status.

### **Conclusion**

The MRDR will provide valuable information on the range and utility of therapies and longer-term outcomes of myeloma patients. Future intended research will include the evaluation of healthcare costs and outcomes for myeloma outside clinical trials, and a biobank will be established. More information: <https://mrdr.org.au/>

## **P103. Thrombotic microangiopathy complicating bortezomib-based therapy for multiple myeloma**

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### **Case Report**

A 70 year old lady was commenced on cyclophosphamide, bortezomib and dexamethasone (CyBorD) for progressive IgG kappa myeloma, associated with biopsy-proven cutaneous and hepatic necrobiotic xanthogranuloma. On day 20 of her fifth cycle, she was admitted for investigation of acute-onset, profuse, watery diarrhoea. Faecal analysis was negative for gastrointestinal infection, while contrast CT scans of her abdomen on day 4 of admission demonstrated diffuse proctocolitis.

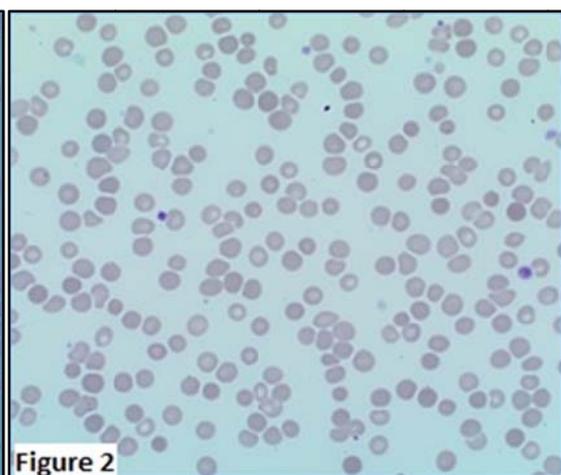
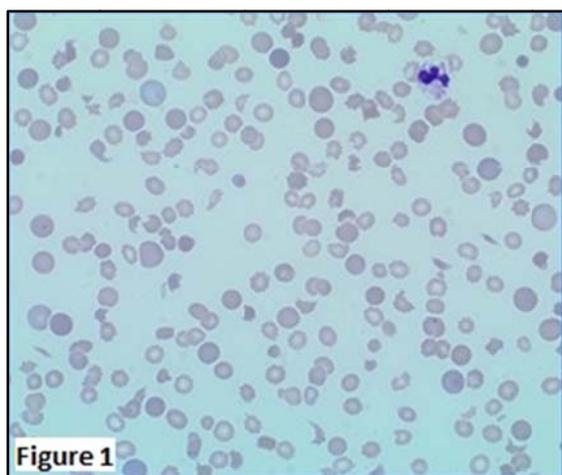
Over the next 6 days, she developed overt thrombotic microangiopathy (TMA), manifesting with marked fragmentation haemolysis [Figure 1], severe thrombocytopenia (platelets  $14 \times 10^6/L$ ), confusion and acute anuric kidney injury requiring haemodialysis. Plasma exchange, FFP and immunosuppression were not given on the basis of the patient's rapid decline and the option of best supportive care was discussed. CyBorD was ceased.

Subsequent investigations revealed a stable paraprotein level of 17 g/L, kappa FLC 1132.5 mg/L and lambda FLC 55.7 mg/L. An ADAMTS13 activity level was measured on day 20 post-onset of microangiopathy – this was mildly reduced at 25% [normal range 40-130%]. Unexpected resolution of the TMA was observed over the following 4 weeks despite no direct intervention [Figure 2]. The patient's confusion completely resolved and she became dialysis-independent. The diagnosis of a drug-associated TMA was therefore favoured, with bortezomib considered the likely precipitant.

### **Discussion**

Bortezomib-associated TMA is a highly unusual phenomenon, with an incidence confined only to sporadic reports in the literature. Mechanisms through which bortezomib may induce TMA remain speculative. Here, withdrawal of bortezomib facilitated complete resolution of microangiopathy without the need for plasmapheresis or immunosuppression, which supports its causative role.

Given the increasing use of proteasome inhibitors and the potentially catastrophic nature of drug-induced TMA, a high index of suspicion should be maintained and cessation of bortezomib considered if similar situations arise in future clinical practice.



## **P104. Epithelial-to-mesenchymal transition (EMT) is a key feature of t(4;14)-positive multiple myeloma**

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### **Aim**

Prognosis for multiple myeloma (MM) patients has improved dramatically since the introduction of novel therapies. However, prognosis in the high-risk t(4;14) subgroup, characterized by expression of MMSET and FGFR3, remains poor due to the acquisition of a highly aggressive, motile and invasive phenotype. While the term “epithelial-to-mesenchymal transition” (EMT) is not commonly used to describe MM, we hypothesise that an EMT-like process plays a critical role in t(4;14)-positive MM disease pathogenesis. In this study, we conducted a comprehensive evaluation of the association between t(4;14) and EMT in MM.

### **Method**

Expression of a core EMT-related signature, comprising 433 mesenchymal genes and 422 epithelial genes, was assessed in CD138-selected MM plasma cells from newly-diagnosed MM patients in four independent microarray datasets (E-GEOD-19784 [n=327], E-GEOD-26863 [n=304], E-MTAB-317 [n=226] and E-MTAB-363 [n=155]), accessed through Array Express (EMBL). In each dataset, gene expression was compared in t(4;14)-positive and t(4;14)-negative patients, defined by MMSET and FGFR3 expression, using linear models for microarray data (LIMMA). The regulation of EMT-related genes by MMSET was further validated in a microarray dataset (E-GEOD-50072) assessing MMSET knockdown/overexpression in KMS-11, a t(4;14)-positive human myeloma cell line.

### **Result**

85 mesenchymal genes were found to be upregulated, and 41 epithelial genes downregulated, in t(4;14)-positive patients across the 4 datasets ( $p < 0.05$ , Fisher's method). The upregulated genes included key EMT drivers (TWIST1, SOX9, HIF1A, TCF4) and genes associated with the cytoskeleton (VIM), cell-cell adhesion (CDH2, ITGB1, NCAM1) and signalling pathways involved in EMT (BMPR1A, IL6R, SOS1, TGFB2). Downregulated epithelial genes included ITGA6 and SMAD7. Regulation of the EMT drivers (TWIST1, SOX9, TCF4, HIF1A) by MMSET was confirmed in KMS11.

### **Conclusion**

This study has identified an extensive EMT-like gene expression signature driven by MMSET in t(4;14)-positive MM patients. This EMT-like phenotype may underpin the poor prognostic features of this subset of patients.

## **P105. Lack of correlation between c-Maf gene transcription and nuclear oncoprotein expression in multiple myeloma**

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### **Aim**

Translocation t(14;16), present in 5% of patients with multiple myeloma (MM), results in over-expression of the c-musculoaponeurotic-fibrosarcoma (c-Maf) oncogene with limited data suggesting that c-Maf expression may be a biomarker predictive of increased sensitivity to MEK inhibition. Moreover, some data suggest that aberrant c-MAF expression at the transcriptional but not protein level may also correlate with t(4;14). We aimed to clarify the extent of c-Maf expression in MM thus clarifying its utility as a biomarker for clinical trials of MEK inhibitors in MM.

### **Methods**

c-Maf gene transcription and protein expression in 105 primary MM patient samples were evaluated with RT-qPCR of CD138 selected MM cells and immunohistochemistry (IHC) of paired trephine biopsies, respectively. The presence of t(4;14) was confirmed with RT-qPCR on the same specimens.

### **Result**

MM patients had higher levels of c-Maf gene expression when compared to normal subjects (n=105) (p=0.0002). One patient with known t(14;16) confirmed on interphase clg FISH, had the highest level of c-Maf gene expression (>10000x normal mean). RT-qPCR identified 13 patients with t(4;14), who collectively demonstrated a higher median level of c-Maf gene expression when compared to patients without t(4;14) (p=0.0002). c-Maf nuclear-protein over-expression was seen with t(14;16) but patients with intermediate levels of c-Maf gene expression [including cases with t(4;14)] did not demonstrate detectable c-Maf nuclear-protein expression. The level of c-Maf gene expression level did not demonstrate any correlation with patient survival.

### **Conclusion**

c-Maf gene over-expression is present in both t(14;16) MM and non t(14;16) MM particularly MM with t(4;14). However, c-Maf nuclear-protein expression as evaluated by IHC is only detectable in MM with t(14;16) and therefore has limited clinical utility as a potential biomarker. Other practical assessments of c-Maf protein expression, ideally validated in clinical trial settings, are required.

## **P106. Recommendations for serum free light chains (FLC) measurement in routine laboratories**

**Mills A**<sup>1</sup>, Tate J<sup>2</sup>, Jovanovich S<sup>3</sup>, Mollee P<sup>1</sup>, Chiu W<sup>4</sup>, Wienholt L<sup>5</sup>, Gillis D<sup>6</sup>, Reibelt L<sup>7</sup>, Youdell O<sup>8</sup>, on behalf of the RCPAQAP Immunochemistry Working Party on Serum Free Light Chains (WP-SFLC)

<sup>1</sup> Haematology, Department, Princess Alexandra Hospital, <sup>2</sup> Chemical Pathology, Pathology Queensland, <sup>3</sup> RCPA Quality Assurance Programs, Flinders Medical Centre, <sup>4</sup> Chemical Pathology, Auckland City Hospital and Middlemore Hospital, <sup>5</sup> Immunology, Royal Prince Alfred Hospital, <sup>6</sup> Immunology, Pathology Queensland, <sup>7</sup> Immunology, Sullivan and Nicolaidis pathology, <sup>8</sup> Alfred Pathology, Alfred Hospital

### **Aim**

Results from the 2012 and 2013 RCPA Immunochemistry Paraprotein QAPs indicate large variability for SFLC measurement between laboratories, and for different manufacturers' assays and platforms. The RCPA convened a working party to develop guidance recommendations for SFLC measurement by routine laboratories.

### **Methods**

Information was gathered from WP-SFLC members working in routine laboratories and clinics, the manufacturers producing FLC kits (Freelite, The Binding Site and N Latex, Siemens Healthcare), who provided input through their recommendations for dilutions, and the literature. Recommendations addressed: 1) imprecision goals; 2) reference intervals; 3) sample dilutions to detect antigen excess and nonlinearity; and 4) reporting of results.

### **Results**

Manufacturers' FLC quality controls matched to specific kit lots should be within  $\pm 20\%$  CV of quoted values. Use of a serum-based sample within the normal range or close to FLC upper reference limit values is also recommended. Laboratories should validate manufacturers'  $\kappa$  and  $\lambda$  FLC reference intervals and  $\kappa/\lambda$  ratio ranges according to the CLSI document C28-A3. In end stage renal failure a different  $\kappa/\lambda$  ratio range applies when using Freelite but not when using N Latex. Laboratories should follow the manufacturers' dilution procedures for FLC measurement although for difficult samples it may be worthwhile to investigate further. Reporting of FLC concentrations should be in whole numbers from 0 to 100 mg/L and  $\kappa/\lambda$  ratio at 0 to  $< 10$  to two decimal places and  $\kappa/\lambda$  ratio  $\geq 10$  to one decimal place or as a whole number. Include assay type (Freelite or N Latex) in the report to avoid clinical misinterpretation of results.

### **Conclusion**

The WP recommends that laboratories use the same FLC assay and the same platform when monitoring disease response and to adopt a consistent approach to sample dilution procedures to reduce current variability in SFLC measurement.

## **P107. Targeted inhibition of N-cadherin as a therapeutic modality for multiple myeloma**

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### **Introduction**

Expression of the cell adhesion molecule N-cadherin (*CDH2*) is elevated in plasma cells (PCs) from approximately 50% of newly diagnosed multiple myeloma (MM) patients<sup>1,2</sup>. In addition, serum N-cadherin levels are elevated in a subset of MM patients with high-risk disease and poor prognosis<sup>1</sup>.

### **Aim**

To investigate the role of N-cadherin in the modulation of MM PC behaviour *in vitro* and MM pathogenesis *in vivo*.

### **Method**

The effects of shRNA-mediated N-cadherin knock-down and an N-cadherin inhibitor, ADH-1 (Exherin™), on MM PC adhesion and proliferation were assessed *in vitro*. The role of N-cadherin in MM tumour establishment and intramedullary growth was investigated *in vivo* using the C57BL/KalwRijHsd mouse model of MM. In this model, intravenously injected luciferase-expressing mouse MM PCs (5TGM1-SFG) home to the BM and initiate systemic MM disease.

### **Result**

N-cadherin knock-down significantly reduced 5TGM1-SFG cell adhesion to BM endothelial cells (BMECs) in a static system and under shear stress (2 dynes) in a parallel plate flow chamber assay. N-cadherin knock-down did not alter the proliferation of 5TGM1-SFG cells compared with control cells. However, treatment of 5TGM1-SFG cells with ADH-1 (0.8mg/ml) significantly reduced cell number after 3 days. Moreover, treatment of N-cadherin-expressing human MM PC lines OPM-2 and LP-1 with ADH-1 (0.6mg/ml) significantly reduced cell number after 3 days. C57BL/KalwRijHsd mice bearing 5TGM1-SFG N-cadherin knock-down cells had significantly reduced tumour burden, as assessed by bioluminescent imaging, after 4 weeks compared with mice bearing control 5TGM1-SFG cells. Furthermore, daily intraperitoneal ADH-1 administration (100mg/kg/day) to 5TGM1-SFG cell-bearing mice significantly reduced tumour burden and serum paraprotein levels compared with mice treated with vehicle alone.

### **Conclusion**

These studies demonstrate the potential role of N-cadherin in MM tumour development. Moreover, targeting of N-cadherin may represent a novel therapeutic modality to control MM disease progression in patients with aggressive disease.

<sup>1</sup>Groen *et al.* Haematologica 2011

<sup>2</sup>Vandyke *et al.* Br J Haematol 2013

**P108. Updated results from a phase 2 extension study of patients with multiple myeloma (MM) previously enrolled in carfilzomib company-sponsored phase 1 and 2 clinical trials (PX-171-010)**

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<sup>1</sup> John Theurer Cancer Center, Hackensack University Medical Center, <sup>2</sup> Sarah Cannon Research Institute/Tennessee Oncology, PLLC, <sup>3</sup> MD Anderson Cancer Center, <sup>4</sup> University of California, San Francisco, <sup>5</sup> Onyx Pharmaceuticals, Inc., an Amgen subsidiary, <sup>6</sup> Washington University School of Medicine, WA, USA

**Aim:** PX-171-010 (010; NCT00884312) is an extension study of patients who completed a phase 1/2 carfilzomib study that aims to provide insights into the long-term tolerability, safety, and clinical benefit of carfilzomib.

**Methods:** Patients who completed a carfilzomib study were eligible to enrol and continue receiving carfilzomib at the same dosing level. Addition of other approved anti-MM agent(s) at the time of progression was allowed. The primary end point was safety; efficacy was also evaluated.

**Results:** Between 2009 and 2012, 91 patients with MM were enrolled (31 from the pivotal PX-171-003-A1 study). Median carfilzomib treatment (initial study+010) was 100.3 weeks (range: 4.4–273.4 weeks); 68.1% of patients received carfilzomib for ≥18 cycles and 29.7% for ≥36 cycles. Treatment-emergent grade ≥3 adverse events (AEs) and serious AEs are presented in the Table, along with AEs that were previously reported from 4 phase 2 clinical studies (N=526). Seven patients died on the 010 study (4 due to AEs, 3 due to disease progression); none of these deaths were assessed as carfilzomib-related. The 4 AE deaths were due to myocardial infarction (MI) (n=2), pneumonia (n=1), and pneumonia with MI (n=1). The 3 patients with MI had pre-existing cardiac disease and died after 9–47 cycles on study. Most patients (79.1%) had ≥1 regimen change; 30.6% of these patients continued receiving single-agent carfilzomib at a different dose/schedule, and 69.4% received additional combination therapy. Notably, responses were observed after the first regimen change due to disease progression (overall response rates were 18.2% and 20.5% in the single-agent and combination groups, respectively).

**Conclusion:** The types and rates of AEs in 010 were similar to those previously reported with single-agent carfilzomib. Patients who remained on carfilzomib for extended periods continued receiving clinical benefit, with no new significant safety signals noted from additional cumulative exposure.

**Table.** Treatment-Emergent Grade ≥3 AEs Occurring in ≥10% of MM Patients in the PX-171-010 Extension Study or 4 Phase 2 Clinical Studies

Adverse Event Category, n (%)	PX-171-010 Extension Study (N=91)		Integrated Safety Summary from 4 Phase 2 Clinical Studies (N=526) <sup>1</sup>	
	Grade ≥3	SAEs	Grade ≥3	SAEs
Neutropenia	19 (20.9)	0	54 (10.3)	2 (0.4)
Anaemia	16 (17.6)	1 (1.1)	118 (22.4)	7 (1.3)

Adverse Event Category, n (%)	PX-171-010 Extension Study (N=91)		Integrated Safety Summary from 4 Phase 2 Clinical Studies (N=526) <sup>1</sup>	
	Grade ≥3	SAEs	Grade ≥3	SAEs
Thrombocytopenia	13 (14.3)	0	123 (23.4)	6 (1.1)
Pneumonia	11 (12.1)	10 (11.0)	55 (10.5)	52 (9.9)
Lymphopenia	5 (5.5)	0	95 (18.1)	0

<sup>1</sup>Safety data from 4 phase 2 studies (PX-171-003-A0, PX-171-003-A1, PX-171-004, and PX-171-005) which supported accelerated approval of carfilzomib for relapsed and refractory MM in the United States.

## **P109. Efficacy of Salvage Autologous Stem Cell Transplant in Relapsed Multiple Myeloma**

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*Royal Perth Hospital*

### **Aim**

Autologous stem cell transplant (ASCT) remains the standard of care with a significant mortality benefit in eligible patients diagnosed with Multiple Myeloma (MM). Although tandem ASCT has been considered evidence based practice in MM for many years, the role of delayed "salvage" ASCT is less well established. We analysed a cohort of patients from our centre who received salvage ASCT for MM to assess efficacy of this treatment modality and identify any variables that may predict outcome.

### **Method**

We retrospectively analysed all patients that received a second autologous stem cell transplant for multiple myeloma at our centre between January 1999 and June 2014. Particular outcomes of interest including progression free survival (PFS), time to next treatment (TTNT), overall survival (OS) and toxicity.

### **Result**

A total of 15 patients (9 male, 6 female) received salvage ASCT in the study period. Median age at diagnosis and at salvage ASCT was 52 and 57 respectively. Preliminary results suggest median time between first and salvage ASCT was 83 months. We will present data on PFS, lines of therapy prior to second autograft, and TTNT. Overall survival for the cohort was 111 months from diagnosis. There was 1 recorded death within 100 days post salvage ASCT.

### **Conclusion**

Our study demonstrates similar efficacy of salvage ASCT in relapsed Myeloma patients in our centre as compared to international case series. Salvage autograft remains a useful therapeutic tool in carefully selected cases.

## **P110. A Phase II single-arm safety study of Elotuzumab (E) in combination with Thalidomide and low dose dexamethasone in patients with relapsed and or refractory multiple myeloma (MM)**

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### **Aims**

Elotuzumab, humanized monoclonal-IgG1 antibody, targets SLAMF7 on MM cells. Thalidomide/dexamethasone (Td) combination used in relapse is commonly associated with grade  $\geq 3$  non-hematologic toxicities. This study evaluated safety and tolerability of E plus Td (E-Td) in MM patients with relapsed and/or refractory MM.

### **Methods**

Patients were treated with E-Td in 28 day cycles. During cycles 1 and 2, E weekly loading dose was 10 mg/kg IV on days 1, 8, 15, and 22, with dose escalation of T from 50 to 200 mg *po qhs*. From cycle 3, E was given biweekly and dexamethasone weekly at 40 mg *po*. Patients not achieving  $\geq$ partial response (PR) by cycle 5 or progressing between cycles 2 and 5 received cyclophosphamide (C) 50 mg *po qd* with E-Td (E-CTd). Treatment was continued until disease progression, unacceptable toxicity, or death. Primary endpoint was the proportion of patients who experienced  $\geq 1$  severe ( $\geq$ Grade 3) nonhematological toxicity. Additional safety parameters and efficacy endpoints were measured.

### **Results**

Forty patients consented treatment (median age; 64 years, 63% males, median time from diagnosis; 5 years, median number of prior therapies; 3). Grade  $\geq 3$  nonhematologic events were reported in 62.5% of patients, and the most common events were asthenia (35%), peripheral edema (25%), fever (25%), respiratory tract infection (23%), neuropathy (20%), back pain (20%), and constipation (20%). Six patients experienced an infusion reaction; no patient discontinued due to these events. The clinical benefit rate was 58% (7 MR, 9 PR, 4 VGPR, 2 CR, and 1 sCR). Among patients with a PR or better, 63% maintained their response at 1 year.

### **Conclusions**

Elotuzumab can be safely combined with Td or CTd. Grade  $\geq 3$  nonhematological toxicities with E-Td are consistent with Td alone. Although the trial was performed in heavily pretreated MM patients, the clinical benefit rate is encouraging.

## **P111. IL-6 induced pSTAT3- A novel prognostic biomarker for multiple myeloma identified by phospho-flow cytometry**

Suen H, Brown R, Yang S, Weatherburn C, Ho P, Gibson J, Joshua D

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### **Background**

Aberrant expression of phosphorylated signalling proteins is increasingly relevant to the diagnosis, prognosis and pharmacodynamics of haematological malignancies. Phospho-flow cytometry offers novel opportunities to investigate signalling pathways by flow cytometry and provide insights to the biology of malignancy.

### **Aim**

To investigate the diagnostic and prognostic significance of phosphorylated signalling proteins in patients with monoclonal gammopathies using a novel phospho-flow assay

### **Method**

Constitutive and IL-6 induced pSTAT3, pSTAT5, pERK and pAKT expression and IL-6 receptor levels were measured in cryopreserved malignant plasma cells (CD38++ CD138+) from patients with multiple myeloma (MM) at diagnosis (n=65), monoclonal gammopathy of undetermined significance (MGUS) (n=17), plasma cell leukemia (n=7) and aged matched normal controls (n=11).

### **Result**

Constitutive expression of pSTAT3, pSTAT5, pERK and pAKT was not related to the diagnosis or prognosis of the monoclonal gammopathies. Patients with plasma cells that were sensitive to IL-6 induced p-STAT3 had a significantly better overall survival in a univariate analysis ( $\chi^2=13.6$ ;  $p<0.0003$ ), than those with plasma cells that did not respond to IL-6 induction. In a multivariate analysis utilising the International Staging system, IL-6 induced p-STAT3 expression was a significant independent prognostic marker. High pSTAT3 expression levels correlated with existing CD45 expression and pSTAT5 expression correlated with IgG levels. Mean IL-6 receptor expression on plasma cells increased with the severity of the monoclonal gammopathy but did not correlate with overall survival.

### **Conclusion**

This is the first comprehensive study of phosphorylated signalling proteins in patients with monoclonal gammopathies. Whilst constitutive phosphorylated protein levels did not aid the differential diagnosis of monoclonal gammopathies, a novel prognostic biomarker for MM was identified. The optimised phospho-flow assay provides new opportunities for the identification of patients who may be susceptible to targeted inhibitor therapy and adds to our understanding of the biology of these disorders.

## **P112. Pleiotropic tumour effects induce hypo-responsive senescence in T cells of patients with multiple myeloma**

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### **Background**

Multiple myeloma (MM) patients exhibit widespread immune dysfunction. The T cell compartment is defective and apart from long term survivors, cytotoxic T cell clones exist in a state of proliferative hypo-responsiveness. A precise characterisation of the dysfunctional checkpoint in these cells may provide a target for restoring T cell function and offer better prospects for immune-based therapies.

### **Aim**

To characterise the nature of T cell hypo-responsiveness in MM patients and to investigate whether they demonstrate an anergic, exhausted or senescent phenotype.

### **Method**

T cell clones (CD3+CD8+TCRV $\beta$ +CD57+) from patients with MM were determined using a Betamark kit. Markers associated with anergic, senescent or exhausted T cells were analysed using flow cytometry and cell signaling pathways were analysed using a novel phospho-flow technique. Proliferation was determined by standard CFSE assays.

### **Result**

T cell clones were present in 50% of patients and failed to proliferate in response to stimulation by MACS iBeads. Clonal T cells did not express LAG-3, TIM-3, PD-1, CD28 or CTLA-4 but had high levels of CD57, CD160 and perforin suggesting the cells were senescent rather than anergic or exhausted. Low pERK levels suggested that the ERK pathway, associated with proliferation, was suppressed. In addition, elevated levels of p-SMAD suggested T cell inactivation and higher Bcl-xL levels suggested that the dysfunctional T cell clones have a survival advantage due to an inhibition of apoptosis. SHP-2 and pZAP-70 levels were suggestive of normal TCR activation.

### **Conclusion**

The T cell clones in MM patients display the phenotypic characteristics of senescent T cells. In addition, multiple cell signaling pathways appear to be dysregulated in T cell clones, most likely as a result of pleiotropic tumour effects. These data raise questions about the use of monotherapy of check point blockers in myeloma due to the myriad of defects in T cell function that are present.

## **P113. Myeloid derived suppressor cells are more potent immune inhibitors in multiple myeloma than in Waldenstrom's macroglobulinaemia**

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### **Background**

Myeloid derived suppressor cells (MDSC) are a heterogeneous population of cells that have been implicated as inhibitors of lymphopoiesis in patients with malignancies. They have a consensus phenotype of CD33+/CD11b+/HLA-DRlo/- and can be further divided into CD15+ granulocytic (G-MDSC) and CD14+ monocytic (M-MDSC) subsets.

### **Aim**

We aimed to determine the number and function of MDSC in the blood of patients with multiple myeloma (MM) and Waldenstrom's macroglobulinaemia (WM).

### **Method**

A multi-parameter flow assay was developed which determined granulocytic and monocytic MDSC. A CFSE 4 day tracking assay was used to demonstrate lymphocyte proliferation. Treg cells were defined as CD3+CD4+CD25hi CD127- cells.

### **Result**

The absolute number of G-MDSC in the blood of patients with progressive MM (n=10) was significantly higher (mean=106.0 x10<sup>7</sup>/L) than in the blood of both age-matched controls (n=12) and MM patients with stable disease (n=25) (mean=0.8 x 10<sup>7</sup>/L, U=3.00; p=0.0002 and mean=3.8 x10<sup>7</sup>/L, U=31.00; p=0.0006 respectively). Patients with WM (n=7) had no increase in G-MDSC (mean=0.5 x 10<sup>7</sup>/L). Flow-sorted MDSC from patients with MM (n=8) induced the generation of Treg cells (mean increase of 26.4%). MDSC from both MM patients (n=8) and aged-matched controls (n=5) demonstrated a dose dependent inhibition of lymphocyte proliferation (mean=29.7% vs. 21% respectively) in CFSE-tracking experiments.

### **Conclusion**

G-MDSC are significantly increased in the blood of patients with MM but not in WM. The increased MDSC are associated with inhibition of lymphocyte proliferation and the induction of Treg cells suggesting that tumour-induced immune dysfunction associated with MDSC is greater in patients with MM than WM.

## **P114. Leading the Development and Implementation of a Young Person Myeloma Group <60 Years**

**Waterman S**

*The Leukaemia Foundation*

**AIM:** To highlight the increasing number of younger people (those <60) diagnosed with myeloma and the quality of life and survivorship issues that are unique to this patient population.

**METHODS:** A literature review will be conducted using the Cochrane, Medline, CINAHL, PsycINFO databases (using the search terms: young people with myeloma, support needs, patient experiences). A needs analysis survey will also be conducted by the Leukaemia Foundation Victoria, using patients within the state database to discuss needs of this specific group.

**RESULTS:** Findings of the literature review and the focus group needs analysis will be interpreted, collated and presented in poster format.

**CONCLUSION:** Survival of young patients (defined as below 60 years of age) with myeloma has improved however it is a non-curable chronic health condition. The impact that being diagnosed with an older person's condition at a young age can be overlooked. The unique components of support, self-advocacy, empowerment and knowledge should be highlighted for this group.

## **P115. Congenital dyserythropoietic anaemia-II: A case report and review of diagnostic features**

**Adams R, Spanevello M**

*QML Pathology*

### **Case Report**

We present the case of a 24 year old primigravida, who was discovered to be moderately anaemic and mildly thrombocytopenic on routine antenatal screening. Examination of the blood film revealed a normocytic anaemia with moderate anisocytosis, ovalocytes and irregularly contracted red cells. On further testing, mild hyperbilirubinaemia, in combination with a mildly elevated lactate dehydrogenase and a markedly reduced serum haptoglobin appeared consistent with a haemolytic anaemia, however the reticulocyte response was considered inappropriately low for the degree of anaemia.

A bone marrow examination was performed, which revealed erythroid hyperplasia, and a significant proportion of binucleate erythroblasts. A Ham test was performed, which was positive.

Features were considered diagnostic of congenital dyserythropoietic anaemia-II (HEMPAS, hereditary erythroblastic multinuclearity with positive acidified serum lysis).

### **Diagnostic features of CDA-II**

The congenital dyserythropoietic anaemias are rare inherited disorders, which are characterised by ineffective erythropoiesis, and striking morphological abnormalities of erythroblasts. There are three main subtypes, designated CDA-1, -2 and -3, with a fourth category of "variant" forms. CDA-2 is the most common of the CDA's. Presentation is generally with normocytic anaemia associated with an inadequate reticulocyte count for the degree of anaemia, and an increased red cell distribution width (RDW) with poikilocytosis. Other features include hyperbilirubinaemia and variable splenomegaly.

Bone marrow examination is remarkable for the presence of hyperplastic erythropoiesis with the most striking feature being the presence of <10% binucleate and multinucleate erythroblasts. Pseudo-Gaucher cells with birefringent material can be seen in the majority of cases.

Other abnormalities which are not routinely tested include abnormal migration of membrane proteins band 3 and band 4.5 on SDS-PAGE, increased i-antigen expression with high agglutinability by anti-i sera, and membrane abnormalities on electron microscopy.

An association to a gene locus on chromosome 20 (q11.2) was described in families in Southern Italy. The molecular basis of the disorder has recently been elucidated, with mutations in the secretory coat protein complex II component, SEC23B, resulting in disordered protein transport from the ER to the Golgi.

## **P116. A single center review of requests for measuring direct oral anticoagulant (DOAC) levels**

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### **Aim:**

Routine measurement of DOACs is not recommended except in certain clinical situations. These include urgent surgery, bleeding/thrombosis or a patient with renal insufficiency whilst on DOACs.

This study aimed to retrospectively analyze the requests for DOAC levels with respect to adequacy of the information provided on the request forms and their clinical appropriateness in a tertiary referral hospital.

### **Methods:**

Requests for all DOAC levels (i.e. Dabigatran, Rivaroxaban and Apixaban) were first identified by querying the laboratory information system (LIS) for all tests performed between January 2012 and June 2014. Request forms were considered to be adequate if they stated the exact name/test of drug level requested and the indication. Requests were considered to be clinically appropriate if they fit any of the criteria mentioned in the aims. If this information was not given or inadequate, further information was sought from other scanned forms provided to the laboratory in the same period or from the local electronic medical records system. The proportion of requests that were adequate and appropriate were evaluated.

### **Results:**

A total of 76 test requests were evaluated from January 2012 to June 2014 (Inclusive). Request form information was found to be adequate in 44/76 (57%) and inadequate in 33/76 (43%). Requests for DOACa levels were found to be clinically appropriate in 57/76 (75%), inappropriate in 6/76 (8%) and unclear or unknown in 13/76 (17%).

### **Conclusion:**

Despite the relatively low frequency of tests in the 2.5 years, there was a noticeable number of inappropriate tests requested for DOACs (8%), reflecting a lack of familiarity with these tests. The proportion of inadequate/unclear request forms was high (43%), which may further reiterate the need for education regarding clinically relevant and adequate, more specific, request information. This will enable the better interpretation of the results leading to better clinical outcomes.

## **P117. Is there a role for FibroScan in patients with transfusional haemosiderosis?**

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<sup>1</sup> *Mater Health Services*, <sup>2</sup> *Princess Alexandra Hospital*

### **Background:**

FibroScan is a new diagnostic method that estimates liver stiffness and has not been extensively investigated in patients with transfusional haemosiderosis. FibroScan only takes a few minutes, is non invasive, well tolerated and performed at the bedside. FibroScan is based on the principle that the velocity of propagation of a wave through a homogeneous tissue is proportional to its elasticity, which is correlated with the amount of fibrosis.

### **Methods:**

This is a descriptive study of our local experience in the use of FibroScan to detect liver pre fibrotic changes in patients with haemosiderosis secondary to chronic transfusional support.

### **Results:**

Nine patients had a FibroScan and FerriScan. The most common cause of transfusional support was thalassaemia major (5 cases); other diagnosis included MDS (2 cases), Hb SS (1 case), and Hb S-β thal. (1 case). Elastography mean was 5.88 kPa (3.5-10.4). Expected values in patients without scarring are < 7 kPa. Just one patient showed markedly increased readings (10.4 kPa) but with normal LIC (1.4 mg/g), suggesting a different etiology from Fe overload. The mean LIC in mg/g was 6.47 (1-23.1) with a normal range between 0.17 and 1.8. Indeed, 6/9 patients (66.7%) had evidence of increased LIC by FerriScan. The two patients with sickle cell disease (Hb SS and Hb S-β thal.), had the highest values of LIC (23.1 and 16.1 mg/g, respectively), but there was no correlation with FibroScan results (6.8 and 53.9 kPa, respectively).

### **Conclusion:**

Given the small population evaluated in this cohort, it is difficult to establish a correlation between LIC estimated by FerriScan and scarring of the liver by FibroScan. There was not clear correlation between the results obtained by both tests. Follow up studies would allow us to estimate better if there is similar trend in the change of LIC and development of liver fibrosis.

## **P118. Therapeutic Plasma Exchange (TPE) and Clinical Outcomes: Experience at a Single Tertiary Institution**

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### **Background:**

In recent years in addition to standard haematological indications TPE has increasingly been employed in the treatment of various neurological, endocrinological and metabolic disorders with a lesser evidence base for efficacy. This study aims to audit the outcome of TPE in these conditions.

### **Methods:**

Analysis of patients who underwent TPE for neurological indications at Canberra Hospital between January 2007 and March 2014 was undertaken. TPE was performed using a COBE™ (and Optia) Spectra Apheresis systems. Target washed plasma volume was determined as per indication. Human albumin (4%) or cryoprecipitate poor plasma was used as plasma replacement. Data was extracted from in-house apheresis database and electronic health records.

### **Results:**

Of a total of 122 patients, 48 (39%) patients underwent 591 TPE sessions for various neurological indications. Median age was 52 years with equal M:F. Indications included Thrombotic Thrombocytopenic Purpura 13 (27%), Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) 8 (16%), Myasthenia Gravis 5 (10%), Guillian Barre Syndrome 6 (12%), Neuromyelitis Optica 5 (10%), Multiple Sclerosis 4 (8%), Voltage Gated K-channel Ab Related Disorders 5 (10%) and Catastrophic Anti-phospholipid Syndrome, Encephalomyelitis, Epilepsy in one each (6%). Median numbers of TPE sessions per patient were 12 (1-161). Overall response rate - 67%, minimal or no response - 27%. Excellent response (90%) was seen in conditions with Grade I American Society for Apheresis (ASFA) recommendations (TTP, GBS, MG, CIDP), whereas conditions as per the ASFA categories II and III recommendations (e.g. encephalomyelitis) showed only 37% response rate. Vast majority of the TPE was performed through Vascath in 38 (79%) and no major complications were noted.

### **Conclusion:**

Better responses were observed in conditions as per category I ASFA recommendations. The findings confirm TPE to be an effective treatment in certain neurological indications by causing improvement of acute conditions as well as halting disease progression.

## **P119. Establishment of telomere length testing as a diagnostic service at the Children's Hospital at Westmead**

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### **Aim**

Measurement of telomere length (TL) is important in the diagnosis of many telomere maintenance disorders such as Dyskeratosis Congenita, Aplastic anaemia and idiopathic pulmonary fibrosis. Currently, no laboratory in Australia offers TL measurement as a diagnostic service. At the Children's Hospital at Westmead we have undertaken to establish telomere length testing via two different methods, and aim to offer this as a diagnostic service.

### **Methods**

Assays were established to measure TL via Flow FISH and quantitative PCR (qPCR) using previously published methods. To validate these methods in our population, peripheral blood samples from over 200 normal subjects, aged between 0 and 90 years, were taken and analysed by the two assays. From these data, normal percentile curves were calculated. Patients with known telomere maintenance disorders were also assayed, and results compared with those obtained from existing TL results.

### **Results**

Both methods showed characteristic decline in TL with age. Reproducibility of qPCR was acceptable with CVs of 6-7%. Percentile curves were constructed so that test samples could be run and ranked. There was reasonable correlation between the 2 assays ( $R^2 = 0.46$ ). Both assays were able to identify known patients with telomere maintenance disorders.

### **Conclusion**

Telomere length testing is now established at CHW as a clinical diagnostic service. This will be supplemented with genetic testing for telomere maintenance disorders, so that a comprehensive diagnosis can be made.

## **P120. Dipyrrone induced agranulocytosis**

**Barraclough A**, Howman R, Augustson B

*Sir Charles Gairdner Hospital*

### **Introduction**

Dipyrrone (also known as metamizole), is an effective analgesic medication that has been banned for sale in Australia and the United States of America because of the potentially fatal side effect of agranulocytosis. This medication, however, is freely available for over the counter purchase in some areas in Asia, South America, Africa and Europe. We describe a case of severe, life threatening agranulocytosis in a patient returned from holiday in Bali, who purchased dipyrrone for headache relief from a local pharmacy.

### **Case study**

51 year old female, previously well, presented with fevers and severe oral and anal ulceration secondary to herpes simplex. Her full blood picture was notable for a marked leucopenia with complete absence of neutrophils. Her viral and autoimmune screen were negative. Her bone marrow biopsy showed preserved erythropoiesis, megakaryocyte and lymphocyte numbers but a complete absence of granulopoiesis. Despite a week of granulocyte colony stimulating factor, intravenous antibiotics and antiviral therapy there was no improvement.

On further questioning about her medication history, her husband recalled she had purchased an analgesic called neuralgin from a Bali pharmacy 3 weeks prior and had been taking it intermittently for headache relief. Neuralgin contains the active ingredient dipyrrone which has been known to cause agranulocytosis. As an immune mediated mechanism was suspected for drug induced agranulocytosis the patient was initially commenced on a course of intravenous immunoglobulin. With no improvement noted after 3 doses she went on to have plasma exchange. On the second day post exchange a small number of neutrophils were seen on blood film. These numbers escalated quickly with daily exchanges to within normal levels. With neutrophil recovery, her fevers and ulceration resolved.

### **Conclusion**

This case highlights the potentially fatal consequences of using unregulated medications without being aware of the possible side-effects.

## **P121. A case of an acquired Factor VIII inhibitor suspected secondary to Alemtuzumab administration for multiple sclerosis**

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### **Aim**

To describe a case of an acquired factor VIII inhibitor in the setting of previous Alemtuzumab treatment for multiple sclerosis.

### **Case report**

A 37 year old male presented with an acquired factor VIII inhibitor. Symptoms at presentation included 3 weeks of easy bruising, right ankle swelling and left thigh swelling and pain. Initial investigations demonstrated an INR of 1.1, PT 12, APTT 88 and a FibD of 4.4. Mixing studies showed complete correction. Factor levels showed a severe factor VIII deficiency of <0.01 U/mL with a factor VIII inhibitor titre of 7.0 BU/mL. Multiple investigations to identify a precipitating event returned negative results.

The patient's left thigh swelling and pain rapidly progressed with decreased peripheral pulses and a threatened compartment syndrome. Urgent activated factor VII administration occurred with resolution of symptoms.

The patient's previous medical history was significant only for multiple sclerosis which had previously received Alemtuzumab (CAMPATH – anti CD52) based therapy on trial in 2009 & 2010. A literature review was performed identifying rare cases of an association between Alemtuzumab administration and the development of factor VIII inhibitors. Of interest Alemtuzumab is currently undergoing a marketing relaunch in Australia for use in multiple sclerosis with likely increased use and possible appearance of rarer side effects (many of which involve immune dysregulation).

Prednisone at a dose of 1mg/kg and subsequently Rituxumab at a dose of 375mg/m<sup>2</sup> was administered. Serial factor VIII testing demonstrated a return to normal levels with a loss of detectable inhibitor.

### **Conclusion**

This case demonstrates the potential emergence of haematological and immunological side effects following a broadening of the use of Alemtuzumab in the greater medical community.

## **P122. Implementation of an evidence-based risk stratified model for Surgical Thrombo-Embolic Prevention in a specialist cancer centre: the STEP protocol**

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**Background:** The Agency of Healthcare Research & Quality ranks the provision of preventative thromboprophylaxis (TP) as the most important intervention to improve patient safety in hospitalized patients. Evidence from a prospective audit conducted at our institution revealed significant underutilization of perioperative TP, with less than 1.5% overall compliance with best practice guidelines in high-risk patients. A key area of failure was the poor adherence to recommended durations of TP in the post-operative/post-discharge periods.

**Aim:** To implement an institutional quality improvement (QI) strategy that ensures compliance with appropriate perioperative TP.

**Method:** The hospital wide initiative involved development of an evidence-based guideline termed the Surgical Thrombo-Embolic Prevention (STEP) protocol. The protocol provided standardized recommendations according to an assessment of patients' TE risk: low, intermediate or high, based on a pre-operative weighted risk stratification model. The aim of the protocol was to simplify risk stratification, improve adherence to appropriate TP and reduce heterogeneity in perioperative TP practice. Key stakeholders from specialty groups were engaged in protocol development and institutional education. Multidisciplinary participation by medical and non-medical staff maximized the chance of success in changing practitioner behaviour.

**Results:** Existing institutional processes were analysed and changes promoting compliance were introduced at various stages of perioperative care. This included staff and patient education, integration of STEP into the surgical time out process, modification of anaesthetic and drug charts to document risk profile and TP interventions, and development of a web-based application to guide staff in the utilization of appropriate TP according to patient's overall risk profile. A review of discharge scripts by our clinical pharmacists ensured appropriate dispensing of post-discharge TP measures. Discharge packs containing aids for self-administration of pharmacological agents were also provided to patients. A post-implementation audit was conducted to assess compliance rates with this QI initiative. Results of this audit are pending analysis.

## **P123. A phase-IV open-label study evaluating changes in bone marrow morphology in adult immune thrombocytopenia (ITP) patients receiving romiplostim: Analysis of the 1- and 2-year romiplostim cohorts**

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### **Aim**

Results of a multicentre study evaluating bone marrow biopsies for reticulin and collagen, pre- and post-treatment with romiplostim, in adult ITP patients.

### **Methods**

Eligible patients had platelet counts  $<50 \times 10^9/L$ , received  $\geq 1$  prior ITP therapy, and no collagen in baseline bone marrow biopsies (before romiplostim). Patients were scheduled for biopsies after 1 (Cohort 1), 2 (Cohort 2), or 3 years of romiplostim, dosed to maintain platelet counts at  $50\text{--}200 \times 10^9/L$ . Bone marrow biopsies were also performed if patients discontinued early or failed to achieve/maintain a response to romiplostim (platelet counts  $\leq 20 \times 10^9/L$  for 4 consecutive weeks at the maximum dose of  $10 \mu\text{g/kg}$ ). Reticulin and collagen formation were measured using the modified Bauermeister. Collagen was detected by trichrome staining and reticulin by silver staining.

### **Results**

Of the 50 patients enrolled in Cohort 1 (54% female, mean age 55.5 years), 39 patients received romiplostim and had bone marrow biopsies. Mean(SD) dose was  $3.8(2.9) \mu\text{g/kg}$ . No patients with evaluable results developed collagen. No patients had an increase  $\geq 2$  grades from baseline in reticulin. Of the 50 patients enrolled in Cohort 2 (76% female, mean age 48.6 years), 39 patients received romiplostim and had bone marrow biopsies. Mean(SD) dose was  $4.1(3.2) \mu\text{g/kg}$ . Again, of the patients with evaluable results, none developed collagen. Two patients had a 2-grade increase in reticulin (1 patient: baseline grade 0-2 at year 2; 1 patient: baseline grade 1-3 at end of treatment). The safety profile was similar to previous trials. In Cohort 1, 3 patients died, and in Cohort 2, 2 patients died (none attributed to romiplostim).

### **Conclusion**

There were no neutralising antibodies to romiplostim or thrombopoietin in the 2 Cohorts, and no evidence of collagen formation after 2 years of romiplostim treatment. The incidence of increase in reticulin was low, consistent with results of previous romiplostim studies. This study is ongoing.

## P124. Fremantle Hospital 4 years' experience on ROTEM and its impact on blood products usage

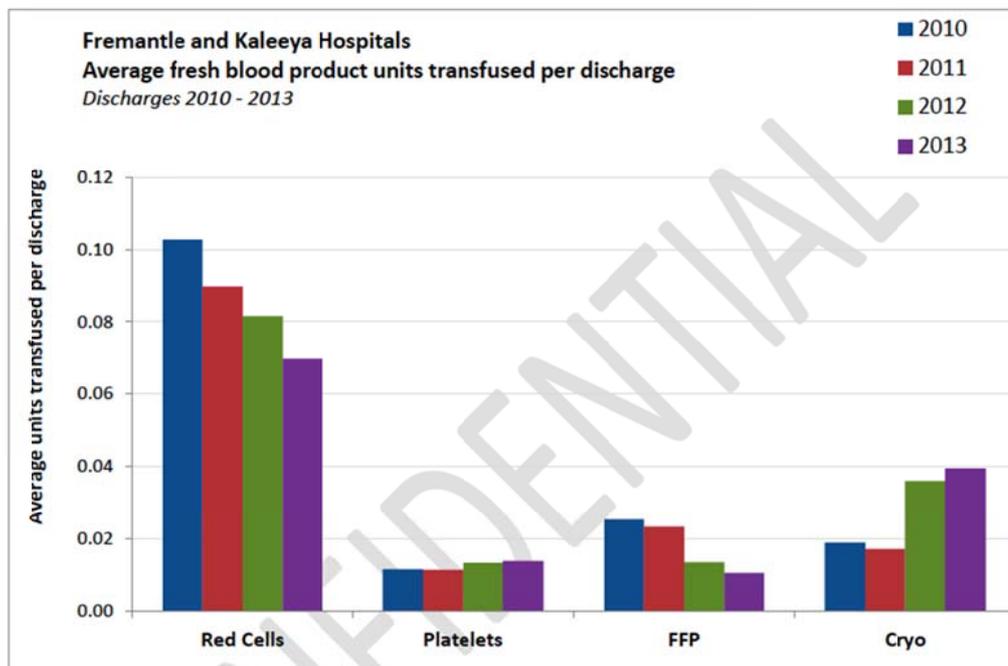
Chuah H, Kesavan M, Sciortino K, Ng F, Dixon T, Leahy M

*Fremantle Hospital*

**Aim:** Whole blood ROTEM analysis allows visual assessment of blood coagulation from clot formation, through propagation, and stabilization, until clot dissolution with rapid turn-around time. ROTEM analysis allows early discrimination between surgical bleeding and coagulopathy bleeding and facilitates targeted blood products usage. ROTEM testing was first setup at Fremantle Hospital in November 2010, predominantly used in surgical setting initially. Over the years, the use of ROTEM have increased and expanded, from surgical setting to emergency and medical fields. We aim to describe the frequency and demographic of ROTEM requests and the blood products usage over the last 4 years at Fremantle Hospital.

**Method:** All ROTEM requests from November 2010 until June 2014 are filed in hard copies and summarized on excel spreadsheets on monthly basis. These requests are analysed to determine the source of request and frequency of requests. WA Patient Blood Management Program database is used to look into the blood products usage over the same period.

### Results:



Other results are still in the process of compilation.

**Conclusion:** We show that ROTEM has been taken up widely across all surgical, acute care and medical fields for assessment of bleeding patients over the last 4 years at Fremantle Hospital. We

also show that introduction of ROTEM has changed blood products usage, with increase in cryoprecipitate and platelet and reduction in fresh frozen plasma and packed red blood cells.

## **P125. Case report: The use of intravenous bevacizumab for the treatment of recurrent iron deficiency anaemia in a patient with Hereditary Haemorrhagic Telangiectasia**

**Cirillo M, P'ng S**

*Royal Perth Hospital*

### **Aim**

Hereditary Haemorrhagic Telangiectasia (HHT) is a genetic disease characterized by vascular malformations affecting multiple organs. This 45 year old women with HHT suffered from spontaneous recurrent epistaxis, telangiectasia, and visceral lesions (pulmonary, uterine and gastrointestinal). She is confirmed on genetic testing to have HHT1 (ENG gene mutation).

The diagnosis had been known for >10 years, requiring treatment for recurrent epistaxis and gastrointestinal bleeding – including blood transfusions, iron replacement, and nasal embolization. Of note, the patient had been receiving 4 – 6 weekly nasal cautery for >6months to control symptoms. The patient was referred to our service for further management of recurrent iron deficiency; attributed to recurrent epistaxis and menorrhagia.

### **Method**

Baseline investigations including full blood picture (FBP), and iron studies were obtained. A program of intravenous iron replacement was instituted. A literature review was conducted to find a suitable anti-angiogenic therapy; Bevacizumab was selected based on published case reports (efficacy), side effect profile and availability. Intravenous therapy was selected because of multiple sites of blood loss. A fortnightly infusion of 5mg/kg, total 4 doses was given (June – August 2013). The patient was reviewed regularly, kept a symptom diary and had regular FBP and iron studies.

### **Results**

There was partial response within 4 weeks of completion, with reduction in bleeding symptoms, stabilization of haemoglobin and reduction in ED presentations due to severe bleeding. No blood transfusions were required for 10 months (August 2013 till June 2014). The effect of this treatment is not indefinite however; with return of significant epistaxis at approximately 11 months of follow-up.

### **Conclusion**

The use of Bevacizumab in this patient resulted in significant improvement in quality of life, with normalization of haemoglobin. Follow-up confirms that symptom control lasts less than 12 months. This effect is in keeping with other published case reports of IV Bevacizumab for bleeding/iron deficiency in HHT patients. The role of this therapy should be explored with larger scale clinical trials in HHT patients with recurrent haemorrhage.

## **P126. Simplifying the assessment of body iron stores in beta-thalassemia major: The comparison of liver iron concentration measured by T2\* MRI against Ferriscan®**

Clarke L, Lindeman R, Moses D

*Prince of Wales Hospital, Sydney, NSW, Australia*

### **Aim**

Regular blood transfusions in thalassaemia major result in progressive iron overload and subsequent complications. Untreated cardiac arrhythmias and cardiomyopathy are the predominant causes of death, while endocrinopathies and cirrhosis contribute to morbidity and mortality. Chelation therapy can prevent these complications and has been pivotal in improved survival. Accurate assessment of iron overload is critical in guiding the appropriate choice of chelation therapy and in managing non-adherence.

MRI Relaxometry can be used to evaluate tissue iron concentration. Liver iron concentration (LIC) has been validated as the best measure of total body iron overload however correlates poorly with cardiac iron deposition. The R2 MRI approach for determining LIC is commercially available (FerriScan®) however cardiac wall motion renders it unsuitable for simultaneous evaluation of cardiac iron loading. T2\* MRI, with a shorter acquisition time overcomes this limitation and has been validated as both a measure of intraventricular iron loading and as a predictor of associated cardiac complications. In this study we evaluated the accuracy and reproducibility of T2\* measured LIC against reference R2 (FerriScan®) based approach in patients with  $\beta$ -thalassemia major.

### **Method**

All patients (n=34) between 2008-2009 with  $\beta$ -thalassemia major undergoing routine assessment of body iron stores with FerriScan® and cardiac T2\* MRI underwent simultaneous assessment of LIC at the time of T2\* MRI. Data was analysed with the IBM SPSS statistical programme version 19.

### **Result**

Statistical analysis identified a positive linear relationship between LIC as measure by T2\* MRI and FerriScan® with a correlation coefficient (r) of 0.962, P<0.001.

### **Conclusion**

These results indicate that there is a strong correlation between T2\* MRI and FerriScan® for the assessment of LIC. This has the potential to simplify assessment of iron body stores in patients at risk of iron over load by allowing simultaneous assessment of liver and cardiac iron deposition.

## **P127. Development of an assay to Identify polymorphisms in the promoter regions of the gamma globin genes, as a possible cause of hereditary persistence of foetal haemoglobin.**

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### **Aim**

The aim of this project was to develop an assay to identify polymorphisms in the promoter regions of the gamma globin genes, as a possible cause of hereditary persistence of foetal haemoglobin.

### **Method**

Touchdown polymerase chain reaction (TD-PCR) was used to amplify the of gamma globin A and gamma globin G genes of 47 high ( $\geq 1\%$ ) foetal haemoglobin(HbF) and 22 normal HbF ( $< 1\%$ ) DNA extraction samples. The promoter regions of the two genes were then sequenced and analysed.

### **Result**

The gamma globin promoter regions of all samples were successfully sequenced. 17 known and 3 novel single nucleotide polymorphisms (SNPs) were identified in promoter regions of the cohort.

### **Conclusion**

Hereditary persistence of foetal haemoglobin (HPFH) is an inherited characteristic which results in elevated levels of foetal haemoglobin (HbF) in the circulation. Although increased levels of foetal haemoglobin are not detrimental to the health of the individual, study of this condition is of medical importance because HbF has an ameliorating effect on haemoglobinopathies, such as beta thalassaemia and sickle cell disease. Promoter regions of the gamma globin genes have been recognised as one of three quantitative trait loci which contribute to the proportion of HbF in circulation. SNPs located within the gamma globin promoters have been found to cause a resistance to the usual suppression of HbF production that occurs shortly after birth. Identifying SNPs with a strong association with HPFH has the potential to improve the management of patients with haemoglobinopathies and also improve guidance provided in prenatal genetic counselling.

## **P128. Molecular and cellular analysis of three novel alpha-2-globin gene promoter mutations (HBA2:c.-59C>T), (HBA2:c.-81C>A) and (HBA2:c.-91G>A) reveal varying patterns of transcriptional and translational activities**

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### **Aim**

While point mutations affecting the promoter region of  $\beta$ -globin are widely described, there are no well characterised reports of any point mutations currently found in the promoter of the  $\alpha_2$ -globin (HBA2) gene. We present experimental gene transcriptional activity and clinical data for three previously undescribed HBA2 gene core and proximal promoter mutations.

### **Methods**

Using an *in vitro* system designed to assess the impact of point mutations on HBA2 expression, the three novel promoter mutations (HBA2:c.-59C>T), (HBA2:c.-81C>A) and (HBA2:c.-91G>A) identified in three unrelated patients were analysed for their effects on HBA2 gene transcriptional and translational activities. Following the generation and transfection of expression vectors carrying each mutation, the HBA2 transcription activity of the promoters from each mutant was analysed with quantitative Real Time-PCR (qReTi-PCR) technique. Immunofluorochemistry (IFC) was used to analyse HBA2 protein synthesis.

### **Results**

The results showed that (HBA2:c.-59C>T) and (HBA2:c.-91G>A) reduced HBA2 transcription levels by 53.7% ( $p=0.0008$ ) and 38.3% ( $p=0.004$ ) respectively compared to the wild type. Subsequent IFC analysis confirmed that cells carrying (HBA2:c.-59C>T) and (HBA2:c.-91G>A) mutant constructs also had significantly lower HBA2 protein labelling when compared to the wild type. Conversely, the (HBA2:c.-81C>A) substitution caused 13.7% ( $p=0.089$ ) more HBA2 transcription and subsequently increased translation evidenced by increased HBA2 protein labelling when compared to the wild type.

### **Conclusions**

This study emphasises the importance of *in vitro* studies to establish the impact of base substitutions on the level of gene expression, and the value of these studies in clinicopathologic correlation so that appropriate advice can be given in genetic counselling.

## **P129. An interim analysis of a phase 2, single-arm study of platelet responses and remission rates in patients with immune thrombocytopenia (ITP) receiving romiplostim**

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<sup>1</sup> The Pathology Clinical Academic Group, The Royal London Hospital, London, United Kingdom, <sup>2</sup> Department of Internal Medicine, Centre Hospitalier Universitaire (CHU) Henri-Mondor, Creteil, France, <sup>3</sup> The Center for Cancer and Blood Disorders, Bethesda, MD, USA, <sup>4</sup> Universite Victor Segalen Bordeaux, Pessac, France, <sup>5</sup> Hospital Materno Infantil, Carretera de las Jubias, La Coruña, Spain, <sup>6</sup> Amgen Inc., South San Francisco, CA, <sup>7</sup> Amgen Australia, Sydney, NSW, Australia

### **Aim**

Platelet response and remission observed with romiplostim treatment in patients with ITP.

### **Methods**

Patients with an ITP diagnosis for <6-months who received first-line therapies only received QW romiplostim for up to 12 months in the treatment period (Fig 1). The primary objective was the number of months with a platelet response during the 12-month treatment period; secondary objectives included incidence of ITP remission and splenectomy. Interim data to March 2013 are reported.

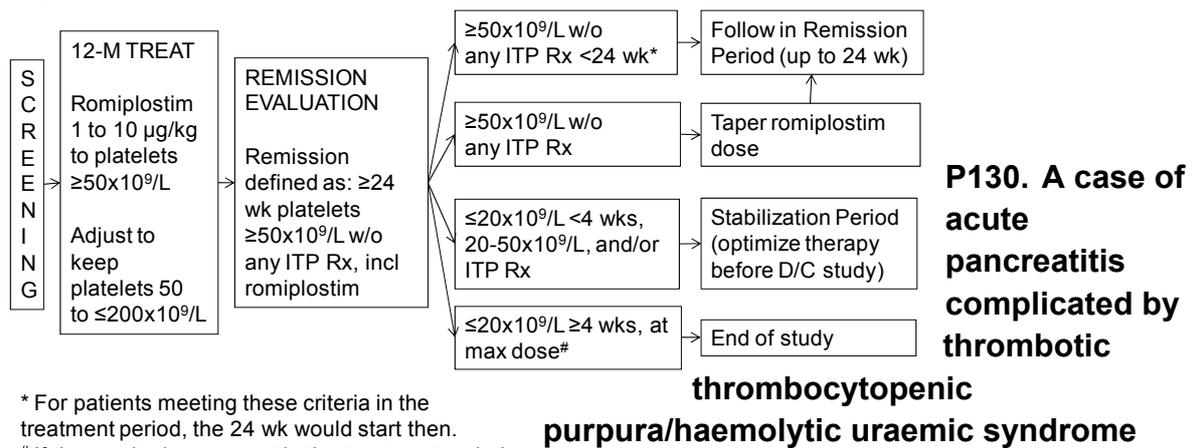
### **Results**

Of the patient population (N = 71), 59.2% were women, median age was 37 years, median time since ITP diagnosis was 2.2 months, and median platelet count at screening was  $20 \times 10^9/L$ . Thirty patients (42%) completed treatment, 31 (44%) are continuing treatment, and 10(14%) discontinued. Patients had a median of 51 weeks of treatment with an average QW dose of  $2.1 \mu g/kg$ . Sixty-six (93%) patients had a peak platelet count  $\geq 50 \times 10^9/L$ . The median time with a platelet response was 9 months; the median time to platelet response was 2.1 weeks. Of 38 evaluable patients, 11 (29%, 95%CI 15-46%) had ITP remission. One patient had a splenectomy and 6 had treatment failure (platelet count  $\leq 20 \times 10^9/L$  for 4 consecutive weeks at  $10 \mu g/kg$  QW, alternative therapy, or death). Of the 71 patients receiving romiplostim, 9 had serious adverse events. The most common AEs were headache (17%), arthralgia (13%), and nasopharyngitis (10%). The most common hemorrhage AEs were hematoma (7%), petechiae (7%), and epistaxis (7%).

### **Conclusions**

Patients with an ITP diagnosis <6 months treated with romiplostim had a response rate over 90%, with platelet responses occurring quickly (median time to response of 2 weeks) and a platelet response median of 9 months. To date, 29% of evaluable patients have shown remission (24 weeks of platelet counts  $\geq 50 \times 10^9/L$  without any ITP treatment). There were no new safety signals, and this study is ongoing.

Fig 1



\* For patients meeting these criteria in the treatment period, the 24 wk would start then.

# If these criteria were met in the treatment period, patients would be discontinued.

**Imlay-Gillespie L<sup>1</sup>, Cameron K<sup>2</sup>, Richardson L<sup>2</sup>, Dean M<sup>2</sup>**

<sup>1</sup> Haematology Department, Royal North Shore Hospital, Rozelle, NSW, Australia <sup>2</sup> Haematology Department, Gosford Hospital, Gosford, NSW, Australia

A 70 year old gentleman presented to the Emergency Department with abdominal pain and fevers on a background of previous pancreatitis. He was diagnosed with acute on chronic pancreatitis and commenced on intravenous fluids and analgesia. On day 2 of his admission he developed acute renal failure with thrombotic microangiopathy and was diagnosed with thrombotic microangiopathy/haemolytic uraemic syndrome(TTP/HUS). Therapeutic plasma exchange was commenced with an immediate response in both the clinical and laboratory parameters. Acute pancreatitis is a rare but reported trigger for TTP/HUS. A review of 42 cases reported in the international literature demonstrates a male predominance with alcohol as the leading cause of the initial pancreatitis. The majority of cases report a time to onset of TTP following the commencement of acute pancreatitis of 2 – 3 days. This is an infrequent haematological consequence of a common general medical condition which practitioners should be aware of.

## **P131. Assessment of lung function pre and post haematopoietic stem cell transplantation for severe systemic sclerosis**

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Systemic Sclerosis (SSc) is a devastating auto-immune condition resulting in collagen deposition in lungs, skin, kidneys and gut with high mortality. A subset of patients may benefit from autologous haematopoietic stem cell transplant (HSCT) and data suggests resolution of skin tightening post-transplant corresponds to functional improvement. Pulmonary involvement secondary to interstitial fibrosis may be an indication for HSCT in patients with severe disease. There is a paucity of data on the role of HSCT in improving lung function.

**Aim:** Assess and compare lung function and functional status of SSc patients prior to and following autologous HSCT and demonstrate an arrest in the deterioration of lung function.

**Methods:** Ethics approval was granted (H08/106, H10/206). 25 SSc patients who had undergone autologous HSCT between 2002 and 2013 were recruited from St Vincent's Hospital. Available pre-transplant and post-transplant pulmonary function tests including FEV1, FVC, KCO and DLCO were collated from patient records. Functional status was estimated by a validated employment questionnaire, Health Assessment Questionnaire (HAQ) scores, skin scores and visual analogue scales (VAS).

**Results:** Of 25 patients autografted, 19 were alive and eligible for analysis. 17 patients were female (90%) with a mean age 43 years at time of transplant. Average FVC pre-transplant was 68% predicted (55%-115%) and average DLCO pre-transplant was 58% predicted (40%-93%). All except two patients demonstrated stabilisation or improvement of FVC and DLCO with a mean increase of 13.3% and 15% predicted respectively.

The average FEV1 pre-transplant was 82.8% predicted (57%-114%) All patients with follow-up demonstrated an improvement with a mean increase of 15.4%. Overall, patients demonstrated statistically significant improvements in HAQ, VAS and skin scores.

**Conclusion:** Autologous transplant has the potential to arrest the deterioration of lung function in patients with SSc and this corresponds to an improvement in overall functional status.

## **P132. Hb J Oxford: A rare alpha chain variant diagnosed in the 1 and 2 gene heterozygous state**

**Lim M**, Enjeti A, Garner M

*Calvary Mater Hospital, Newcastle, NSW, Australia*

### **Aim**

High Performance Liquid chromatography (HPLC) has been shown to be reliable, reproducible, and superior to conventional hemoglobin electrophoresis for detection and identification of hemoglobin variants. Hb J Oxford is a rare alpha chain variant caused by a single amino acid substitution glycine to aspartic acid at position 15 of the alpha chain. It has been described in families of Italian and Sicilian ethnic origin with the variant Hb J Oxford reported as between 21 and 28% by Hb electrophoresis. We report the first case in Australia of heterozygous HbJ Oxford in an Afghani family diagnosed using High Performance Liquid Chromatography (HPLC) and Hb Electrophoresis as well as its effect on clinical and haematologic parameters. To our knowledge, this alpha chain variant has not previously been reported in the Afghanistan population, or its effect in the 2 gene heterozygous state.

### **Method**

The family were identified during routine screening as part of the refugee health program. All samples were analysed using the BioRad D10 HPLC system in combination with alkaline and acid Hb electrophoresis. Haematologic and biochemical studies were evaluated using standard automated analyzers while effect on clinical parameters were assessed by history and physical examination.

### **Results**

The couple had 3 children from a consanguineous marriage. Both parents were heterozygous for HbJ Oxford with a variant Hb percentage of 27%. 2 out of 3 of the couple's offspring were heterozygous for Hb J oxford with a percentage of 40%, suggesting the 2 gene heterozygous state while the third was unaffected. All 4 affected family members did not show any clinical manifestations.

### **Conclusion**

Hb J Oxford can be found in Afghanistan population and is inherited in a typical alpha gene inheritance pattern. It is clinically insignificant in the 1 or 2 gene heterozygous state.

### **P133. Reversible lead toxicity associated with self-medication using Ayurvedic proprietary medicine**

**Orchard G**, Hielscher K, Wilke A, Thomae M, Presneill J

*Mater Adult Hospital, Brisbane, QLD, Australia*

Lead is a naturally occurring heavy metal with increasing levels in the biosphere over recent centuries associated with human activity. In the past, lead toxicity was most commonly encountered in the general Australian community associated with ingestion or inhalation of particles of lead paint. From a global perspective, an important source of human exposure to lead is via industrial processes involving lead-containing compounds.

A 53 year old man presented to an Australian general hospital with a six month history of vague gastrointestinal symptoms, complicated by acute cognitive changes associated with severe hyponatraemia (nadir  $\text{Na}^+$  115 mmol/L). Accompanying biochemical abnormalities included hypokalaemia, hypochloraemia, hypophosphataemia and hypomagnesaemia suggestive of Fanconi Syndrome; and hepatic dysfunction. The full blood count was mildly leukoerythroblastic, with a normocytic anaemia, mild thrombocytosis and prominent basophilic stippling.

After consideration of this overall picture, an urgent blood lead level was requested, which was markedly elevated at 5.0 micromol/L. Further history from the patient delineated up to 9 months daily consumption of several black pills labelled Ayurvedic proprietary medicine which had been sourced from India during the patient's overseas travels.

Cessation of this medication and institution of chelation therapy with dimercaptosuccinic acid (DMSA) for a total of 3 weeks was associated with rapid symptomatic, biochemical and haematological improvement. After five weeks, the blood lead level had reduced to 2.04 micromol/L in an asymptomatic patient.

Analysis of the black pills showed them to be markedly radiodense, with a composition by weight of 11% lead and 0.8% tin. Ayurvedic medicines have been reported repeatedly overseas to be associated with lead toxicity, however such medications are less well recognised by Australian clinicians to have the potential to induce heavy metal poisoning.

## **P134. Laboratory and clinical findings in severe B12 deficiency and response to treatment**

Getta B, Crowther H, **Panicker S**

*Westmead Hospital, Westmead New South Wales Australia*

### **Aim**

To report the clinical and laboratory findings of patients with severe B12 deficiency at diagnosis and with treatment.

### **Method**

We identified four patients admitted between January 2011 and July 2013 with megaloblastic anaemia due to severe B12 deficiency. We reviewed clinical and laboratory findings at presentation and following treatment with parenteral B12.

### **Results**

Only four cases of pancytopenia due to B12 deficiency were identified. Three of the four were Indian background and the fourth Sudanese. Three presented with fever and the fourth with dyspnoea. At presentation the average haemoglobin was 52g/L, WBC  $3.1 \times 10^9/L$ , ANC  $1.4 \times 10^9/L$ , platelets  $38 \times 10^9/L$ . MCV was normal in 2 cases. Median bilirubin was 43  $\mu\text{mol/L}$  and LDH 9433 U/L. DAT was positive in 3 cases and elevated in the others. All four patients had B12 levels  $< 37\text{pmol/L}$ , folate was normal and homocystein elevated in 2 of 3 patients tested. All patients had detectable anti-parietal cell antibodies and 3 of 4 had anti-intrinsic factor antibodies, consistent with Pernicious anaemia. ANA was positive in all 4 cases. All patients had leucoerythroblastic blood films, 3 of 4 had howell-jolly bodies and one had agglutination. All responded to parenteral cobalamin. Platelets and neutrophils normalized within 9 days and reticulocytes within 5 days. All patients remained anaemic 2 weeks post treatment. There were no treatment related complications. None of the patients had typical neurological signs associated with B12 deficiency.

### **Conclusions**

In Australia severe B12 deficiency presenting with pancytopenia is very uncommon. Severe B12 deficiency in migrants of non European background is usually due to Pernicious anaemia rather than insufficient dietary intake. DAT can be positive in pernicious anaemia. With treatment, platelet and neutrophil recovery occurs within 9 days, while haemoglobin recovery takes over 2 weeks and is preceded by reticulocyte recovery at 3 days.

## **P135. Sysmex XE-5000 haematology analyser to identify dengue and distinguish them from other febrile causes of thrombocytopenia**

**San Pedro F**, Goh J, Yip C, Gan J, Yap E, Te Chih L

*National University Hospital, Singapore*

### **Introduction**

Dengue fever is a common and endemic infection in Singapore and commonly presents with moderate to severe thrombocytopenia to the emergency department. In dengue infections, lymphoplasmacytoid cells are commonly seen in the peripheral blood films. Therefore, we hypothesize that using automated cellular indices from haematology analysers may offer a preliminary and rapid distinction from acute dengue infection, sub-acute and other febrile illnesses.

### **Methods**

Samples from consecutive patients presenting to the emergency department with thrombocytopenia with suspected dengue viral infection were analysed. Infection with dengue fever is confirmed using dengue duo rapid test consisting of dengue NS1 Ag and dengue IgG/IgM. Patients with positive dengue NS1 Ag and IgM were deemed to have an acute dengue infection (within 5 days of contracting the illness). Patients with positive dengue IgG with a negative IgM and negative dengue NS1 Ag were deemed to have a subacute infection (after 5 days of contracting the illness). Patients with negative dengue duo rapid test were deemed not to have dengue fever and served as controls. All samples had a full blood count and a 6-part differential count performed on Sysmex XE-5000 haematology analysers. Statistical discriminant functions were generated, and their diagnostic performances will be assessed by ROC curve analysis.

### **Results & Conclusion**

Incorporating platelet count, absolute lymphocyte count and high-fluorescence lymphocytes count (HFLLC) could potentially identify acute dengue fever infections with high sensitivity and specificity. However, the same parameters were unable to differentiate between acute dengue infection from sub-acute infections. These parameters could potentially expedite diagnostic approaches to tropical febrile illnesses in cost-constrained settings.

## **P136. Iron inclusions in plasma cells - A clue to the diagnosis of copper deficiency**

**Sharma A, Batt T, Johnston A**

*Royal Hobart Hospital, Hobart, TAS, Australia*

Copper deficiency is a rare cause of pancytopenia. We report findings in a 26 year old female with history of Wilson's disease diagnosed 13 years previously who was found to be pancytopenic. She was admitted for replacement of a muscle relaxant (baclofen) pump. Full blood examination showed haemoglobin 73g/L, MCV 90fl, MCH 26pg, WCC  $1.5 \times 10^9/L$ , neutrophils  $0.7 \times 10^9/L$  and platelet count  $115 \times 10^9/L$ . Bone marrow examination revealed a normocellular marrow with dyserythropoiesis, sequential myeloid maturation and increased megakaryocytes. Erythroid and myeloid precursors showed vacuoles in the cytoplasm. There were prominent iron positive inclusions in the plasma cells in the iron stain.

The bone marrow findings seen in this patient have been classically described in copper deficiency. One of the treatment modalities in Wilson's disease is zinc therapy to prevent absorption of copper. The patient was on long term Zinc supplementation (220mg three times a day). Her serum zinc level was 19.2umol/L, serum copper 0.1 umol/L and ceruloplasmin 0.02 g/L. While zinc supplementation can be an effective treatment for Wilson's disease, overtreatment can induce copper deficiency leading to cytopenias with characteristic bone marrow appearance. Recognition is important as copper deficiency can be associated with neurological deterioration due to peripheral neuropathy.

## **P137. Secondary haemophagocytic syndrome in adults: A diagnostic and management dilemma**

Sidiqi H, Gurumurthi A, Kavanagh S, Cirello M, Cooney J, P'ng S

*Royal Perth Hospital, Perth, WA, Australia*

### **Aim and Background**

Haemophagocytic lymphohistiocytosis (HLH) is rare disorder characterised by inappropriate and excessive activation of lymphocytes and histiocytes leading to widespread organ damage.

HLH is subclassified into primary and secondary forms. Primary HLH is of genetic origin and is defined by inherited defects of cytotoxic cell function. Secondary HLH occurs in the setting of viral infections (most commonly EBV), malignancy (particularly lymphoproliferative diseases) and connective tissue diseases.

Although HLH has been well described in the paediatric literature, there is a paucity of evidence with respect to optimal treatment in adult populations. There has been difficulty in developing an evidence based approach to HLH treatment in adult cases due to the predominance of secondary HLH as therapy is often tailored to the underlying disease process in individual patients. Treatment in adults has largely been extrapolated from paediatric studies with novel approaches involving immunomodulatory and biologic agents having mixed success.

We report a case series of secondary HLH from Royal Perth Hospital and Sir Charles Gairdner Hospital in Western Australia. These cases highlight the heterogeneous presentations and response to treatment of this life threatening disorder.

### **Method**

Cases of HLH were identified through referral to the Haematology service at the respective hospitals. Previously published diagnostic criteria were used to establish the diagnosis. Subsequent chart review has been conducted.

### **Result**

The treatment outcomes in EBV associated HLH were highly variable ranging from rapid response to single agent corticosteroid therapy to failure of high dose steroid and immunomodulatory therapy. The case of HLH associated with underlying connective tissue disease had a favourable outcome with multiple aggressive immunomodulatory therapies including biologic agents.

### **Conclusion**

Our series illustrates the importance of early recognition of HLH. Investigation for an underlying infectious or malignant aetiology is vital. Treatment is difficult and should be determined by the severity of the syndrome and underlying cause.

## **P138. Idiopathic Plasmacytic Lymphadenopathy: A case in an Asian male successfully treated with the Interleukin-6 inhibitor Tocilizumab**

Sidiqi H, Wright M, **Simon Kavanagh**

*Royal Perth Hospital, Perth, WA, Australia*

### **Aim**

Idiopathic Plasmacytic Lymphadenopathy (IPL) with polyclonal hypergammabulinemia is a rare disorder primarily affecting Asian individuals. This disorder forms part of the spectrum of polyclonal plasma cell disorders including cutaneous and systemic plasmacytosis and plasma cell variant multicentric Castleman's disease (MPCD). Clinical features include anemia, hypergammaglobulinemia, lymphadenopathy, raised inflammatory markers and raised serum Interleukin-6 (IL-6). This highlights the importance of this cytokine in the underlying pathogenesis of the disorder. Though similar to MPCD, IPL is often associated with a better prognosis and less aggressive clinical course.

### **Method**

We report a case of a 44 year old Asian man who presented with severe anemia, cervical lymphadenopathy, rash, raised inflammatory markers and marked polyclonal hypergammaglobulinemia. Key investigational findings were marked polyclonal plasmacytosis in marrow and nodal biopsies. A diagnosis of IPL with polyclonal hypergammaglobulinemia was made based on clinical and pathological features.

### **Result**

Based on the suggestion that IPL is part of the clinical spectrum of MPCD which has previously shown good response to IL-6 inhibition, and given the poor response of IPL to standard therapies the patient was commenced on the IL-6 inhibitor tocilizumab. Therapy was fortnightly at a dose of 8mg/kg based. We document a rapid and significant improvement in clinical and laboratory features including haemoglobin, c-reactive protein, gammaglobulins and albumin levels.

### **Conclusion**

IPL should be considered in the differential of Asian patients with unexplained anaemia and polyclonal hypergammaglobulinemia. This is the first case of IPL with polyclonal hypergammaglobulinemia successfully treated with the IL-6 inhibitor Tocilizumab.

## P139. Raised Carboxyhaemoglobin levels on venous blood gas is associated with Haemolysis

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### Background

Haemolysis is a major differential diagnosis of anaemia and is traditionally defined using a pentad of raised bilirubin, elevated LDH, decreased haptoglobin, elevated reticulocytes, positive DAT and blood film features. However, diagnosis is often difficult and can be delayed. Carboxyhaemoglobin (COHb) is created when carbon monoxide (CO) binds to haemoglobin in red blood cells, this is usually seen as a result of inhalation of CO, however, also occurs as a result of endogenous production of CO, normal range 0.5-1.5%.

### Aim

Establish the relationship between carboxyhaemoglobin and haemolysis.

### Method

Retrospective review of all documented cases of all cases of haemolysis between February-June 2014, in combination with a review of patients with both haptoglobin and COHb levels performed in May 2014, including smoking history, liver disease, renal disease and ICU admission.

### Results

27 patients (11 females, 16 males) with low haptoglobin were reviewed with a median age of 70 (18-91) years. 8 patients had haemolysis, with 5 autoimmune (AIHA) and 3 non-immune causes (2 Thalassaemia, 1 sickle cell anaemia). Median COHb level was 4.0 (3.0 -8.2) % in patients with haemolysis compared to 1.4 (0.0-6.4) % in those without haemolysis (Table 1). No patient with haemolysis had a normal CoHb level. Current smokers had a higher COHb level 3.7% (1.0, 6.4) vs. ex-smokers 0.9 % ( 1.7-2.5) and non-smokers 0.45% (0-2.8%). In one patient, serial COHb levels were available and demonstrated marked reduction after treatment (Figure 1).

### Discussion

This retrospective review suggests that raised COHb levels are associated with haemolysis, including both autoimmune and non-immune causes. All patients with haemolysis had a high COHb level. This suggests that COHb via venous blood gas could facilitate rapid diagnosis in the emergency setting.

Diagnosis	Median COHb (Range)
Haemolysis	4.0% (3.0-8.2%)
Without haemolysis	1.4% (0.0-6.4%)
Smokers	3.7% (1.0, 6.4%)
Non smokers	0.45% (0-2.8%)
Ex-smokers	0.9% (1.7-2.5%)

Table 1.

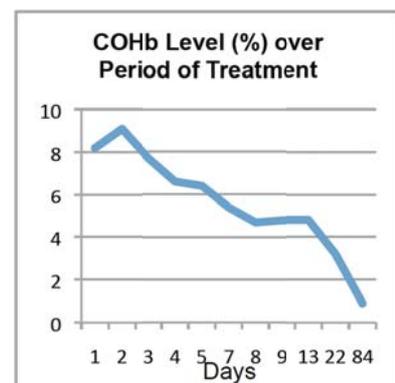


Figure 1.

## P140. Occult alpha globin gene mutations are the commonest causes of red cell microcytosis unexplained by phenotypic testing

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### Aim

Hypochromic microcytic anaemia is the hallmark phenotype of thalassaemia. Current phenotypic tests do not provide a diagnosis in a small proportion of patients with red cell microcytosis. We investigated the genetic basis of microcytosis in a cohort of such subjects.

### Method

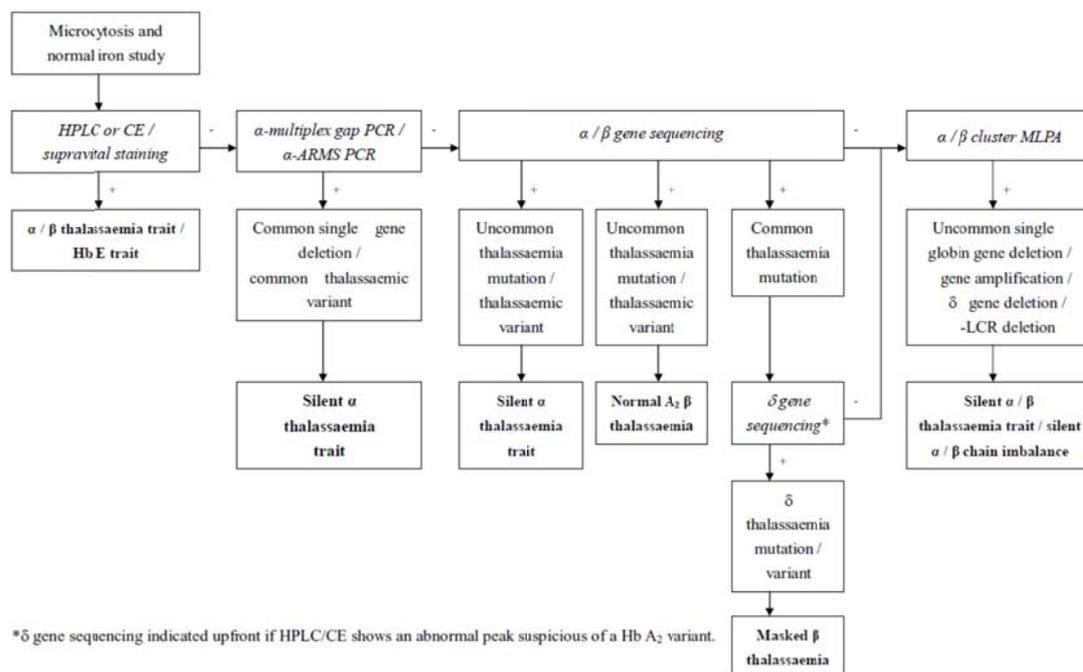
We identified from a large cohort of 1684 unselected requests for thalassaemia testing 25 Chinese subjects who had unexplained microcytosis after phenotypic haemoglobin studies. Extensive genotypic analysis of the  $\alpha$  and  $\beta$  globin gene cluster was performed in 20 of these subjects who had adequate DNA. Techniques employed included gap-polymerase chain reaction, amplification-refractory mutation system, Sanger sequencing and multiplex ligation-dependent amplification.

### Result

Occult single and double alpha globin gene (HBA1, HBA2) deletions and  $\alpha$  thalassaemic haemoglobinopathies (Haemoglobin Quong Sze, Haemoglobin Constant Spring) are the genetic basis for the microcytosis. Occult  $\beta$  globin gene (HBB) mutations, and  $\delta$  globin gene (HBD) abnormalities masking  $\beta$  thalassaemia are not seen. A cost-effective genotyping approach for the detection of these occult globin gene mutations is proposed (Figure).

### Conclusion

Occult alpha globin gene mutations are the commonest causes of red cell microcytosis unexplained by phenotypic testing. These occult mutations can produce diseases with significant morbidities if they occur together with common thalassaemia mutations. Identification of these occult mutations is important not only for making a diagnosis but also for the provision of accurate genetic counselling.



## **P141. Taliglucerase for Gaucher disease (GD) type 1: The Australian experience**

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### **Background**

Imiglucerase and velaglucerase are funded to treat GD in Australia. Taliglucerase is a plant-expressed recombinant glucocerebrosidase with which eleven patients in Australia have been treated, either as commenced within a clinical trial (3 patients) or to deliver therapy during the global shortage of imiglucerase.

### **Aim**

This study was a retrospective, descriptive review of disease-related parameters of patients treated with taliglucerase.

### **Results**

Ten patients are receiving taliglucerase as compassionate supply from Pfizer Australia with an eleventh recipient who switched back to imiglucerase due to infusion-related events after 3 years. There were 4 female and 7 male patients with a median age of 51 years (range 18-70); median age at diagnosis of GD was 15 years (range 5-50). ERT was commenced between 1997 and 2009 with imiglucerase except for two treated initially with alglucerase. Median duration of ERT at the date of switch was a 7 years (1-15) and all but one were switched directly from imiglucerase. Treatment was well tolerated by all patients, except for the patient who reverted to imiglucerase. The dose of ERT prior to commencing taliglucerase was 27.9U/kg/2 weeks (15-49) and the median duration of taliglucerase exposure was 116 months (range 64-145) with a delivered dose of 28.9U/kg/2 weeks (range 18.4-50). Platelet counts and haemoglobin concentrations remained normal or near-normal in all patients. Chitotriosidase levels were informative in 9 patients: 1265 (97-12,500) before and 330 (52-3100) most recently. There was no hepatosplenomegaly before or after therapy and seven patients had improved femoral and/or spine bone marrow burden (BMB) scores which were unchanged in the remainder.

### **Conclusion**

Taliglucerase was well-tolerated when switching from an existing ERT and resulted in stability in all measured clinical and laboratory parameters. Improvements in chitotriosidase levels and BMB scores suggested continued response of GD parameters to taliglucerase following the switch.

## **P142. Taliglucerase alfa in adult patients with Gaucher disease who were previously treated with imiglucerase: 36-month safety and efficacy results**

**Szer J**<sup>1</sup>, Pastores G<sup>2</sup>, Shankar S<sup>3</sup>, Petakov M<sup>4</sup>, Giraldo P<sup>5</sup>, Rosenbaum H<sup>6</sup>, Amato D<sup>7</sup>, Chertkoff R<sup>8</sup>, Brill-Almon E<sup>8</sup>, Zimran A<sup>9</sup>

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### **Introduction**

Taliglucerase alfa is the first approved plant cell-expressed recombinant human protein and is an enzyme replacement therapy indicated for treatment of adults with Type 1 Gaucher disease (GD). Safety and efficacy were evaluated in adult patients switched from imiglucerase to taliglucerase alfa in study PB-06-002 and extension study PB-06-003.

**Aim:** To report 36-month safety and efficacy results of taliglucerase alfa treatment of adult patients with GD who were previously treated with imiglucerase.

### **Methods**

Patients with stable disease were switched from a stable dose of imiglucerase to the same dose of taliglucerase alfa given every other week. Spleen volume, liver volume, haemoglobin concentration, platelet counts, and chitotriosidase activity were assessed through 36 months.

**Results:** Mean (SE) values at baseline and study end were as follows, respectively: spleen volume (n=11), 4.6 (1.2) and 3.7 (0.9) multiples of normal (MN); liver volume (n=12), 1.0 (0.1) and 1.0 (0.1) MN; haemoglobin concentration (n=14), 13.4 (0.4) and 13.3 (0.3) G/L; platelet counts (n=15), 171 and 172 x10<sup>9</sup>/L and chitotriosidase activity (n=10), 12,206 (4,934) and 6,551 (3,018) nmol/ml\*hr. All treatment-related adverse events (AEs) were mild/moderate and transient. The most common AEs were nasopharyngitis, arthralgia, upper respiratory tract infection, headache, and pain in extremity.

### **Discussion**

These 36-month results of taliglucerase alfa treatment in adult patients with GD who were previously treated with imiglucerase extend the clinical safety and efficacy data on taliglucerase alfa. Mean disease parameters were similar at baseline and following long-term treatment with taliglucerase alfa, suggesting ongoing disease stability.

## **P143. The prevalence of silent cerebral infarct in adults with sickle cell anaemia**

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### **Background**

Silent cerebral infarct (SCI) is the most common form of neurologic disease in children with sickle cell anaemia (SCA). However, in adult populations the prevalence of SCI is poorly studied. Presence of SCI in SCA children increases the risk of overt stroke by 14-fold and causes a progressive decline in neurocognitive function.

### **Aim**

To perform a cross-sectional study to establish the prevalence of SCI in the SCA population at a tertiary referral centre in Victoria for the management of SCA patients.

### **Method**

Surveillance MRI brain scans were offered to all adult SCA patients at our centre who had no clinical evidence of overt stroke. All MRI scans were independently reviewed by a blinded MRI neuroradiologist.

### **Results**

21 of 31 patients with SCA participated in MRI surveillance for the presence of SCI. The median age of the cohort was 39 years. 13 of the 21 (61%) patients were treated with either blood transfusion/red cell exchange or hydroxyurea for non-cerebrovascular complications. Surveillance MRI detected 6 out of 21 (28%) patients with SCI. 3 of the 6 (50%) patients with SCI were on transfusion therapy and 1 patient (17%) was on hydroxyurea therapy. 3 out of 9 (33%) patients who were on transfusion therapy had evidence of SCI, whilst 2 out of 8 patients (25%) who were not actively treated had evidence of SCI.

### **Conclusion**

This study demonstrated lower than expected rate of SCI in our cohort, where the majority of patients received intensive therapy for SCA. The higher prevalence of patients who had evidence of SCI on the transfusion/red cell exchange arm does not necessarily imply failure of treatment, as SCI may have preceded therapeutic initiation. This study highlights the high incidence of SCI in adult SCA patients and the value of surveillance MRI in detection and future management of SCI.

## **P144. Carbimazole induced pure red cell aplasia – a case report**

**Wang J, Ku M, Bazargan A**

*St Vincent's Hospital, Fitzroy, VIC, Australia*

### **Background**

Carbimazole (CMZ) treatment is associated with neutropenia and agranulocytosis in up to 1 in 1000 cases, through an idiosyncratic reaction by an as yet unknown mechanism. Pancytopenia, aplastic anaemia, and isolated thrombocytopenia have also been documented. Despite reviewing the available literature, CMZ has not been reported to be associated with pure red cell aplasia (PRCA). Here we present a case of CMZ induced PRCA, which, to our knowledge, is the first such case reported.

### **Case history**

A 45 years old woman with Grave's disease was commenced on 10mg CMZ BD. Prior to commencing treatment, her haemoglobin was 95g/L and reticulocyte count  $28 \times 10^9/L$  (Ref 20-100 $\times 10^9/L$ ). Total white cell count (WCC) was  $4.3 \times 10^9/L$ , and platelets  $363 \times 10^9/L$ .

Her past medical history included non-transfusion dependent alpha-thalassemia compound heterozygosity (HbH disease), and iron deficiency anaemia. No other medications were recorded.

Eleven days after commencing CMZ, she developed a generalised pruritic urticarial rash. CMZ was ceased 6 days later leading to complete resolution of the rash. Blood tests at the time of ceasing CMZ revealed severe reticulocytopenia ( $3 \times 10^9/L$ ; ref 20-100 $\times 10^9/L$ ).

The reticulocyte count remained low ( $3 \times 10^9/L$ ) on repeat testing. 10 days after CMZ cessation the reticulocyte count started to recover ( $17 \times 10^9/L$ ) with a stable haemoglobin of 98g/L. WCC and platelets were within normal range.

Other causes for PRCA were excluded, such as parvovirus B19, viral hepatitis, and paroxysmal nocturnal haematuria. Computerised tomography did not reveal any abnormalities.

A diagnosis of CMZ-induced PRCA was made and the patient's anti-thyroid treatment was changed to propylthiouracil (PTU).

### **Discussion**

To our knowledge, this is the first reported case of CMZ-induced PRCA. The patient's haemoglobin remained stable, as the period of reticulocytopenia was brief and much shorter than the normal life expectancy of red blood cells. PRCA should be considered as a possible side effect of CMZ.

## **P145. Microangiopathic haemolytic anaemia secondary to paracetamol overdose: A case report**

**Wight J**, Fox L, Turner P, He S, Smith C, Grigg A

*Austin Health, Heidelberg, VIC, Australia*

### **Case:**

A 20 year old female was admitted 14 hours following an intentional 21gram paracetamol overdose. There was evidence of severe liver injury at presentation with ALT and AST >6000u/L, INR 7.5 and a bilirubin of 280micromol/L. Beta-HCG was normal. An N-Acetylcysteine (NAC) infusion was commenced.

Within 3 days the liver enzymes and INR were improving, however the bilirubin continued to worsen and peaked at 850 micromol/L. There was a concomitant worsening of anaemia (lowest 62g/L) and thrombocytopenia (lowest  $16 \times 10^9/L$ ). Initial examination of the peripheral blood film was normal, however after several days demonstrated microangiopathic haemolytic anaemia (MAHA) with marked red cell fragmentation and anisopoikilocytosis. Direct antiglobulin test was negative and ADAMTS13 level was normal.

With the administration of NAC infusion and supportive care, there was clinical improvement and the MAHA slowly resolved. She did not undergo plasma exchange or liver transplantation.

### **Discussion:**

MAHA is an uncommon finding on a blood film and can be associated with drugs, pregnancy, trauma, malignancy, DIC, TTP and HUS. To our knowledge, this is the first report of MAHA in paracetamol overdose. The mechanism of microangiopathy in this case is unclear; however it is possible that the liver microvasculature was damaged in the paracetamol-induced liver injury. It is possible that co-ingestion of a second, unknown substance (although the patient denied this) may have contributed to the severity of the MAHA. A normal ADAMTS13 level was helpful in ruling out TTP although the test was not available until after the decision not to perform plasma exchange was made.

### **Conclusion:**

This case demonstrates the potential for significant paracetamol overdose and severe acute liver failure to cause MAHA. Supportive care alone was sufficient to manage the condition in this particular case and plasma exchange was avoided.

## **P146. Taliglucerase alfa in paediatric patients with Gaucher disease: Efficacy, safety, and exploratory growth and development endpoints**

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### **Background**

Taliglucerase alfa (TA) is a  $\beta$ -glucosidase enzyme replacement therapy approved in the USA and other countries for treatment of Gaucher disease (GD) in adults and the first approved plant cell-expressed biotherapeutic.

### **Objective**

To assess efficacy and safety of TA in paediatric patients with GD.

### **Methods**

Phase 3B, double-blind, multicentre, 12-month study of TA 30 and 60U/kg in treatment-naïve paediatric patients (aged 2-<18y) with GD. Eleven patients randomised: n=6, 30U/kg; n=5, 60U/kg.

### **Results**

Median (interquartile range [IQR]) % changes from baseline in haemoglobin (Hgb) concentration at mo 12 (primary endpoint): 12.2% and 14.2% for TA 30 and 60U/kg, respectively. Post hoc analysis of pts with anaemia at baseline, median (IQR) Hgb % change: 19.6% (20.2) and 17.9% (14.3) in 30 and 60U/kg groups, respectively. Platelet count increased by a mean of 45,500/mm<sup>3</sup> (30.9%) and 72,600/mm<sup>3</sup> (73.7%) for 30 and 60U/kg, respectively. Mean spleen volume decreased from 22.2 to 14.0 multiples of normal (MN) and 29.4 to 12.9 MN; mean liver volume decreased from 1.8 to 1.5 MN and 2.2 to 1.7 MN with TA 30 and 60U/kg, respectively. Chitotriosidase activity decreased by 58.5% and 66.1% for 30 and 60U/kg, respectively, at mo 12; CCL18 levels decreased by 50.6% and 52.6%, respectively. With 12 mo of 30 or 60U/kg TA, respectively, pts gained height (mean: 4.2% and 7.6%) and weight (mean: 9.6% and 14.7%), and showed advancement in mean bone age (1.9 and 1.4y). Pubertal status (Tanner stage) remained stable in majority. Child Health Questionnaire scores revealed parents/guardians felt children's health improvement after treatment more often to very good/excellent. Most AEs were mild/moderate and transient. Only 1 serious AE (gastroenteritis; pt needed hospitalization for rehydration) was reported as related. All pts completed study.

### **Conclusions**

This study provides evidence that TA has the potential to be a treatment option for children with GD.

## **P147. Primary EBV associated Pure Red Cell Aplasia**

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### **Presentation**

A previously well 58-year-old male presented with six weeks of abdominal pain, jaundice, malaise, night sweats, weight loss and lymphadenopathy. Initial lab tests showed a normal full blood count (FBC), deranged liver function tests (LFTS) with a markedly elevated LDH and a polyclonal hypergammaglobulinaemia. CT demonstrated widespread low volume lymphadenopathy and a left axillary lymph node biopsy was taken prior to discharge. He was re-admitted a week later with a suspected severe haemolytic anaemia.

### **Investigations**

*Blood results:* Haemoglobin 41g/L, reticulocytes  $2 \times 10^9/L$ , LDH 990 IU/L, bilirubin 37mmol/L, DAT C3d 2+

*Lymph node biopsy:* reactive changes morphologically. Flow cytometry demonstrated an abnormal B-cell population with normal light chain expression.

*Bone marrow biopsy:* markedly hypercellular with focal T cell aggregates and a background lymphocytosis. Absent red cell activity with a negative glycophorin stain. Immunohistochemistry was negative for B cell markers and no aberrancy was demonstrated. T cell aggregates demonstrated high Ki67 activity.

*PET scan:* diffuse skeletal uptake with no visceral or nodal changes.

*Viral serology:* Parvovirus PCR negative, Epstein-Barr virus PCR low level positivity (484 copies/ml)

*EBV-Insitu hybridisation* (performed retrospectively on lymph node): positive

### **Diagnosis and Progress**

He was initially managed with Prednisolone and transfusion support for a suspected haemolytic anaemia, but subsequent investigations confirmed the diagnosis of EBV related pure red cell aplasia (PRCA). By two months he was transfusion independent and a repeat BMAT demonstrated erythroid recovery but a persisting T cell lymphocytosis. He has now been off all treatment for over 12 months with no evidence of relapse.

### **Conclusion**

Pure red cell aplasia is associated with a number of viruses, in particular parvovirus B19. This is a rare case of EBV driven PRCA in the absence of a lymphoproliferative disorder.

## P148. ClinTrial Refer: Building networks, breaking down barriers, improving access, driving trial performance

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### Background

Significant recruitment barriers exist for haematology trials including rarity of disease, geographical challenges and clinician awareness of available studies. The Haematology Clinical Research Network (HCRN) NSW/ACT develops strategies to facilitate cross-referrals and enhance trials recruitment.

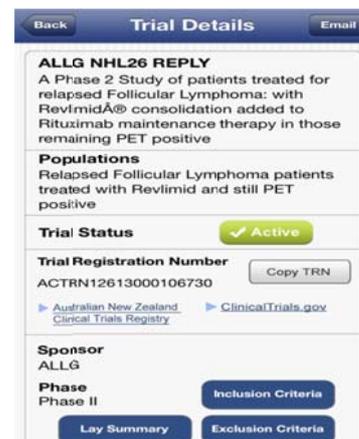


### Aim

To develop a smartphone App which is free, user-friendly and current. Primary endpoint was rate of cross-referral. Secondary endpoints were rates of trial enrolment, App usage metrics and development of other *ClinTrial Refer* Apps.

### Method

App specifications were refined following collaboration with patients, clinicians and researchers. The App has an easy to navigate list of recruiting trials and key search filters are disease, location and sponsor. Design features include: inclusion/exclusion criteria, hospital locations, hyperlinks to ClinTrials.gov and anzctr.org.au, lay summary, alert notifications, and real-time data entry into a web-based database which ensures currency of trial information. We collected referral and usage data by surveying cross-referrals clinical trials centres and mining App metrics data.



at

### Result

*ClinTrial Refer* went live on Google Play and iTunes in May 2013. Before *ClinTrial Refer*, the HCRN recorded 1-3 cross-referrals per month. After *ClinTrial Refer* was launched, the HCRN recorded an average of 11 cross-referrals per month sustained over 12 months, with notable recruitment in several international studies. Although difficult to quantify the impact of *ClinTrial Refer* within hospitals, reported usage of the App at local multidisciplinary meetings suggests increased clinical trial awareness and recruitment. Usage metrics demonstrate the App has 1293 users; with 89% returning an average of 10 times showing that *ClinTrial Refer* is widely and repetitively accessed. Adaptable to any clinical research portfolio, three other *ClinTrial Refer* Apps are live (HSANZ Victoria; TROG; AYA) with more in development.

### Conclusion

*ClinTrial Refer* is a tool for enhanced clinical trial activity in NSW. This simple innovation has driven collaboration and increased clinical research activity.

## **P149. Chronic transfusion burden of ambulatory Haematology patients at the Royal Melbourne Hospital (RMH) from 2008-2013.**

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*Royal Melbourne Hospital, Melbourne, VIC, Australia*

### **Background**

Cancer patients account for a third of all blood cells product use in Australia. Of this Haematology patients use more than half of these products.

### **Aim**

We undertook an audit of the indications for chronic packed red blood cell (PRBC) transfusion in the ambulatory setting at our institution to improve transfusion service planning and to monitor early identification of patients at risk of transfusion induced iron overload.

### **Methods**

Data was accessed and cross-referenced using written and electronic records for patients with an underlying haematological diagnosis who had received cumulatively >10 units of PRBC. Information was sourced from pathology results, transfusion laboratory, and medical and pharmacy dispensing records.

### **Results**

Between 2008- 2013, there were 108 cases of chronic PRBC transfusion identified. Myelodysplastic Syndrome (MDS) was the most common indication (53%), followed by myelofibrosis (12%), sickle cell disease (6%) and aplastic anaemia (5%). Other conditions included acute and chronic leukaemia, myeloma and lymphoproliferative disorders. Of the 108 cases, 34 (31%) had been prescribed iron chelation therapy with 32 of the 34 (94%) having a documented ferritin level above 1000 ug/L. There were 33 (97%) patients prescribed deferasirox and 2 (5%) DESFERRIOXAMINE (1 PATIENT switched from deferasirox). 5 (8%) of the MDS patients had received or were currently receiving azacitidine. Of the MDS cohort 53% had died, with a median survival from time of first transfusion, 28 months.

### **Conclusion**

Amongst patients with underlying haematological disorders, MDS patients account for more than half of chronic PRBC transfusions. However, only 31% of 108 chronically transfused patients had documented ferritin levels, although most of these had iron chelation appropriately initiated. More vigilant monitoring of ferritin levels in patients receiving chronic transfusions is indicated to ensure appropriate management of iron overload.

## **P150. Does parenteral nutrition promote microbial growth? A review of clinical and laboratory findings**

**Gavin N**<sup>1,2</sup>, **McMillan D**<sup>3</sup>, **Keogh S**<sup>1,2</sup>, **Rickard C**<sup>1,2</sup>

<sup>1</sup> *Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia*, <sup>2</sup> *Griffith University, Nathan, QLD, Australia*, <sup>3</sup> *University of the Sunshine Coast, QLD, Australia*

### **Aim**

Our aim was to synthesize clinical research findings regarding microbes colonising patients receiving parenteral nutrition (PN) versus those without PN, and to synthesize the findings of laboratory studies of microbial growth in PN versus control solutions.

### **Method**

A systematic review of journal articles reporting microbial colonisation of patients receiving PN and without PN, or laboratory papers reporting growth curves of microbes in any PN and control solutions.

### **Results**

Only one paper could be synthesized as their findings were presented as colonised PN and non-PN central venous access devices (CVADs) rather than combining the data. A total of 1140 CVADs were analysed: 23/237 (9.7%) PN and 13/903 (1.4%) non-PN CVADs were colonised. The majority of CVADs were colonised with aerobic Gram-positive cocci (12/23, 52% PN vs 8/13, 61% non-PN CVADs), followed by fungal colonisation (6/23, 26% PN vs 4/13, 31% non-PN CVADs) and aerobic Gram-negative rod colonisation (3/23, 13% PN vs 1/13, 8% non-PN CVADs).

Four papers presented microbial growth curves using a variety of PN and control solutions. A selection of microbes representing aerobic Gram-positive cocci, aerobic Gram-negative cocci, aerobic Gram-negative rods and fungi were grown in a range of PN and control solutions. Lipid solutions, broth and normal saline supported growth of the tested microbes. *Candida* grew in all test solutions (lipids, glucose, amino acids, 3-in-1, 0.9% normal saline (NaCl), 5% glucose, broth).

### **Conclusion**

There appeared to be no difference in the types of microbes colonising patients with or without PN administration, but sample sizes were small and further research is needed. A variety of microbes can grow in clinically administered solutions although microbial growth in 0.9% NaCl was slower than in lipids. Patients requiring PN are often acutely unwell with immune deficiencies which increase risk of catheter colonisation.

## **P151. "Blood School" - A new clinical educational program for haematology trainees in Victoria**

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### **Aim**

Most Haematology Advanced Trainees (HAT) participate in the Conjoint Training Program which is designed to fulfil the requirements of both The Royal College of Pathologists of Australasia (RCPA) and The Royal Australian College of Physicians (RACP). In Victoria, the RCPA Network provides a structured Laboratory Tutorial Program for Trainees in the year they are candidates for the RCPA First Part examinations. Trainees rely on work based tuition, clinical experience and self directed study to meet the requirements of the RACP Physician Readiness for Expert Practice (PREP) program. Blood School was proposed by Victorian HAT to meet their need for a structured lecture program available to all trainees their first clinical year.

### **Method**

Trainees presented the 'Blood School' proposal at a meeting in September 2013 and established support from the RCPA Network Coordinator and representatives of HSA NZ Victorian Branch. A pilot program of ten lectures was designed by Dr Jim Griffiths and Dr Kate Burbury to provide a broad introduction to Haematology. Epworth HealthCare Richmond offered a central venue. The program and monthly invitations are issued to Trainees and Speakers via the RCPA Training Network.

### **Results**

The Blood School is an excellent lecture program by experienced Haematologists for HAT in Victoria 2014. The long hours of clinical work often extend beyond rostered hours and sometimes frustrate Trainee attendance in the early evening. Senior Haematology nurses are keen to participate.

### **Conclusion**

The initial experience with Blood School is a basis for discussion and development of an education program in Clinical Haematology for HAT and senior nurses. Two hours of allocated training time would facilitate regular attendance by HAT in their first clinical year.

## **P152. A retrospective analysis of prophylactic posaconazole use in high risk haematology patients.**

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### **Aim**

To evaluate prophylactic posaconazole use and clinical utility of therapeutic drug monitoring (TDM) in high risk haematology patients.

### **Method**

Haematology inpatients prescribed posaconazole suspension 40mg/mL were identified using an electronic dispensing system. Data was collected from January 2009 to December 2011. Patients with a haematological malignancy receiving induction chemotherapy or patients undergoing allogeneic haemopoietic cell transplantation prescribed posaconazole were evaluated. Patients being treated for an existing invasive fungal infection (IFI) were excluded. Medical records were reviewed and data collected on posaconazole dose, duration of therapy, TDM, interacting drugs and immunosuppressant therapy, liver function tests (LFTs) and whether a change in anti-fungal therapy occurred. Where a change in anti-fungal therapy occurred, the reason for change was also recorded.

### **Result**

One hundred patients met the inclusion criteria. The median age was 53 (18-79 years) and 52 (52%) were male. Of the 100 patients, there were 132 episodes of posaconazole prescribing; 80 (61%) episodes were prescribed post induction chemotherapy. A serum-level was taken for 65 patients with 82 (62%) episodes of prescribing. In 50 (34%) episodes of prescribing, levels were below 500 microg/L. Changes to anti-fungal therapy occurred in 69 (52%) of 132 episodes. The main reasons for changes were due to mucositis (32, 46%), suspected IFI (17, 25%) and suspected posaconazole-induced LFT derangement (5, 7%). Posaconazole prophylaxis was changed to anti-fungal treatment in 18 (13.6%) episodes; there were a total of 21 serum-levels taken and of these, 4 were below 500 microg/L.

### **Conclusion**

The majority of patients prescribed posaconazole prophylaxis required a change in anti-fungal therapy, predominantly due to mucositis. Most episodes of posaconazole prescribing that had TDM achieved acceptable serum-levels; this was despite co-administration of proton pump inhibitors and regular anti-dopaminergics in most episodes. LFT derangement was only observed in a minority of episodes.

## **P153. Improving acceptance and utility of quality of life measures in stem cell transplant recipients**

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### **Aim**

Ongoing psychosocial assessment is strongly recommended for long-term survivors of SCT although incorporation of these recommendations into clinical practice is suboptimal. This may be influenced by the time required to complete and analyse quality of life (QoL) measures. We examined the correlation between two validated patient-reported outcome (PRO) measures in long-term survivors of SCT, one a rapidly completed distress tool and the other a standard QoL measure.

### **Method**

The National Comprehensive Cancer Network's Distress Thermometer (DT) and The Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT) were prospectively completed by patients attending a SCT late effects clinic. All survey variables, in addition to sociodemographic and clinical parameters likely to influence long-term QoL were examined for association with the 5 QoL domains (physical, social, emotional, functional, transplant specific) of the FACT-BMT. Linear regression was used for univariate and multivariable analyses.

### **Results**

Between April 2011 and June 2014, 136 patients (68 males) completed both DT and FACT-BMT concurrently. Median age at transplantation was 41 (range, 27-54) years. Median time since transplantation, either autologous (26%) or allogeneic (74%), was 4.9 (range 2-9) years. Lymphoma and acute leukaemia were the dominant SCT indications. Higher DT scores were strongly related to poorer self-reported QoL in all domains in addition to reduced overall QoL (all  $p < 0.001$ ). After controlling for age, gender, time since SCT and disease, the DT score remained strongly associated with all QoL domains (transplant specific,  $p = 0.002$ ; others  $p < 0.001$ ). Further, in allograft recipients, this strong association persisted after controlling for donor type, conditioning intensity and GVHD (all  $p < 0.001$ ).

### **Conclusion**

In long-term survivors of SCT, the DT correlates well with the FACT-BMT at both extremes of the distress scale thereby providing a quick and useful screening tool for this population. Experience with this simple tool may encourage broader use of a range of PRO measures.

## **P155. Pre-operative haemoglobin optimisation in orthopaedic surgery: Our experience**

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### **Aim/Background**

Major orthopaedic surgery is associated with high transfusion rates. An algorithm for pre-operative haemoglobin (Hb) optimisation prior to major elective orthopaedic surgery was developed and an audit performed to assess use and impact on transfusion rates.

### **Methods**

Consecutive patients undergoing major orthopaedic surgery between the 6/3/12 and 5/2/13 who presented to preadmission clinic were identified. If a patient was anaemic (Hb was <115g/L or <128g/L for females and males, respectively) a serum ferritin was measured and if less than 50mcg/L iron replacement was given. Other causes of anaemia were investigated.

### **Results**

231 patients were seen in pre-admission clinic prior to elective total knee 52% (120/231) or hip replacement 42% (98/231). 5% (13/231) underwent a revision of a joint replacement. 7% (17/231) met the anaemia criteria with 12/17 being further assessed. 30% (4/12) were iron deficient and 25% (3/12) received iron therapy. 29% (5/17) did not have a ferritin level measured or further follow up. Overall transfusion rates were 35% (80/231) and 82% (14/17) of anaemic patients were transfused. Similar results were seen in the 2009 Blood Matters report on Patient Blood Management in elective orthopaedic surgery (70% in anaemic versus 25% in non-anaemic patients) and in the National comparative audit of blood use in elective primary unilateral total hip replacement surgery in the UK (57% in anaemic versus 20% in non-anaemic patients).

### **Conclusion**

To increase use of the algorithm further education and promotion is required. Our results are consistent with previous findings that pre-operative anaemia increases the likelihood of transfusion however, despite use of this algorithm, transfusion rates were still high in this patient group. Other management options to explore, in order to reduce transfusion rates, include the use of intra-operative cell salvage and/or tranexamic acid and lowering the transfusion threshold post-operatively.

## **P156. Significance of unexpected red cell antibody screening in blood donors in a developing country**

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### **Aim**

Compatibility testing is done to ensure safe transfusion therapy. In India, unexpected antibody screening on donors is not done and there is an ongoing practice of performing a minor crossmatch before issuing platelets or plasma. The aim of this study was to evaluate the significance of unexpected antibody screening in donors and its role in pretransfusion testing.

### **Method**

This study was performed at the department of Transfusion Medicine at a tertiary care hospital from the period Dec 2013-May 2014. During this period 5087 donations were made of which 87 units were discarded due to positive (Transfusion Transmissible Infections) TTI results. These 5087 units were separated into components within 6-8 hours of collection and the respective components were brought into the inventory after TTI. Unexpected antibody screening was performed on 5000 donors that was negative for TTI using commercial three cell panel expressing the following antigens- D, C, E, c, e, M, N, S, s, P<sub>1</sub>, Le<sup>a</sup>, Le<sup>b</sup>, K, k, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup> using column agglutination technology. Minor crossmatch was done whenever plasma or platelet concentrate (random donor platelet) was requested for by the clinician. Crossmatch results and antibody screening results in the donors were compared and analysed.

### **Results**

Of the 5000 units, 2850 was O pos, 850 was B pos, 738 was A pos and 562 was AB pos. Antibody screening was performed on these 5000 donor samples and no unexpected antibody was found in these 5000 donor samples. Subsequently when crossmatch was performed on these units there was no incompatibility detected in these 5000 units implying when antibody screening in donors is negative, plasma components can be safely transfused without crossmatching.

### **Conclusion**

Performing unexpected antibody screening in donor samples can safely replace and can do away with minor crossmatch and importantly found to be timesaving.

## **P157. Safety of rapid intravenous injection of undiluted ferric carboxymaltose to patients with iron deficiency anaemia (Rapinject): An interim analysis**

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**Aim:** RAPINJECT is a phase II study examining the safety of rapid intravenous (IV) injection of undiluted ferric carboxymaltose (FCM) in patients with iron deficiency anaemia (IDA). We hypothesised that 1000mg of undiluted (5%) FCM can be safely administered over one minute, without Grade 4 or 5 treatment-related adverse events (TRAEs).

**Methods:** Non-pregnant adults with IDA were recruited. The study was conducted in 3 sequential stages. In Stage I, 36 patients received 1000mg IV FCM diluted and administered over 15 minutes, as per current licensing. In Stage II, 12 patients received FCM over 1 minute using an escalating dose of 500mg (3 patients), 800mg (3 patients) and then 1000mg (6 patients). In Stage III, currently in progress, 100 patients will receive a total replacement dose up to 1000mg FCM, given undiluted in 1 minute. All patients are followed-up for 4 weeks after drug administration for delayed TRAEs and effectiveness.

The primary outcome is the incidence of TRAEs up to 1 hour following FCM administration over 1 minute. Secondary outcomes included rate of delayed TRAEs (e.g. hypophosphataemia).

**Results:** TRAE rates at 1 hour post-FCM administration were 4/36 (11.1%) in Stage I (1 grade 4 anaphylactoid reaction and 2 grade 1 or 2 reactions with rash and lightheadedness), 1/12 (8.3%) in Stage II (Grade 1 lightheadedness), and 1/8 (12.5%) so far in Stage III (Grade 1 arm discomfort). Delayed hypophosphataemia occurred in 11/35 (31%) patients in Stage I, 2/10 (20%) patients in Stage II and 2/5 (40%) patients in Stage III.

**Conclusion:** IV administration of undiluted FCM over 1 minute has been well tolerated among the 20 patients who received doses of up to 1000mg with no Grade 4 or 5 TRAE, and shows promise as an alternative regimen for rapid replacement of iron stores.

This study is supported by a research grant from the FCM manufacturer, Vifor Pharma.

## **P158. Adverse transfusion reactions reported to the Australian Red Cross Blood Service (2006 - 2013)**

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### **Background**

Prescribers are requested to inform the Blood Service of adverse transfusion reactions (ATR) associated with the blood components it manufactures and supplies so that immediate action can be taken to recall other potentially implicated components and avoid patient harm. Of interest are ATRs with blood component safety or quality implications, most importantly transfusion-related acute lung injury (TRALI), transfusion-transmitted bacterial infection (TTBI) and transfusion-transmitted viral infections (TTI-Viral). A broad range of other ATRs are reported.

The Blood Service has implemented a number of risk mitigation strategies to minimise the likelihood of such events. The use of haemovigilance data allows us to monitor the effectiveness of these strategies or identify other trends in the occurrence of ATRs.

### **Method**

Blood Service haemovigilance data for the period 2006 to 2013 was reviewed. The primary data source was ATR reporting forms completed for each event by Blood Service staff in the relevant state. This information was compiled into a dedicated Microsoft Access<sup>®</sup> database and the relevant data extracted.

Reactions to plasma-derived products (primarily intravenous immunoglobulin) and recombinant products were not included in the analysis because they are captured separately by the manufacturers' pharmacovigilance systems.

### **Results**

The extracted data yielded 1310 ATR events including 153 cases determined to be TRALI and 11 cases of confirmed TTBI. There were an additional three cases of "highly probable" transfusion-transmitted hepatitis B identified independently through the Blood Service's "lookback" process.

### **Conclusion**

A number of initial ATR reports are subsequently not confirmed. Blood Service haemovigilance data shows that risk mitigation blood safety measures implemented by the Blood Service are successful in reducing the likelihood of TRALI, TTBI and TTI for transfusion recipients.

There are potential limitations to the data such as under-reporting of events. Where possible the Blood Service reconciles its ATR reports with those reported to state/territory haemovigilance systems to improve data integrity.

## **P159. Are red blood cells being used appropriately?**

**Bielby L**<sup>1,2</sup>, **Stevenson L**<sup>1,2</sup>, **Perillo J**<sup>1,2</sup>, **Glazebrook B**<sup>1,2</sup>, **Beard P**<sup>1,2</sup>, **Akers C**<sup>1,2</sup>, **Hogan C**<sup>3</sup>

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### **Aim**

To examine elective red blood cell (RBC) transfusion and patient blood management (PBM) practices in adult patients (≥ 16 years of age). Exploring if:

Practice aligned with National Blood Authority's PBM Guidelines Modules 2, 3 & 4

Haemoglobin (Hb) is not the only trigger for transfusion but also based on assessment of the patient's clinical status

In stable patient a single unit of RBC is followed by clinical reassessment determining the need for further transfusion

Over transfusion occurred.

### **Method**

Health Services (HS) transfusing RBC in Victoria, Tasmania, Australian Capital Territory and Northern Territory were invited to audit up to 30 patients who received an elective RBC transfusion during 2013.

Audit instructions included the PBM practice points being measured, definitions, inclusion and exclusion criteria.

Data collected by HS on a formatted EXCEL workbook and emailed to Blood Matters. Data imported to ACCESS database where a combination of programmed algorithms and medical review determined appropriateness.

### **Result**

94 HS responded to the audit, with 93 submitting data on 2179 RBC transfusions. Single unit practice was reported in some capacity at 10% of HS, cell-salvage 40%, and autologous collection 11% (predominately orthopaedics). Documentation implied the decision to transfuse was based on Hb and patient's clinical status in 86% and aligned with PBM practice points/ guidelines 88%. Non-symptomatic moderate anaemia was overly represented in the non-aligned category (n=193, 74% of all non-aligned transfusions), including 42 cases (16% of all non-aligned transfusions) with documented iron deficiency. A pre-transfusion Hb >100 was reported in 42 cases (16%), including 11 cases with no documented symptoms or recent/ongoing blood loss.

### **Conclusion**

Practice improvement is required to align with PBM guidelines specifically addressing patient assessment pre-operatively, and the management of non-symptomatic moderate anaemia and iron deficiency. Also of concern is the ongoing autologous practice outside the PBM recommendation.

## **P160. What we know about the 3 R's of acute transfusion reaction- 'recognise, react and report'.**

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### **Aim**

To determine if acute transfusion reaction (ATR) policies were available, appropriate, understood and practised.

Policies should be consistent with the national guidelines and safety standards.

### **Method**

146 health services (HS) in Victoria, Tasmania, Northern Territory and Australian Capital Territory that transfuse were invited to participate. Audit included:

Policy - audit of hospital-wide blood and blood product acute transfusion reaction policy

Procedural management – retrospective audit of ATR management (up to 10 individual randomly selected episodes)

Survey - of clinical staff awareness of ATR recognition and management (maximum 30).

HS were provided with an instruction sheet and data entered by HS designated auditor via the Blood Matters website.

### **Results**

Ninety-eight HS responded to at least one part with all reporting a written policy/procedure.

**Recognise:** 2089 staff surveyed for awareness and demonstrated good recognition of signs/symptoms of ATR (average score 3.7/4). However, procedural management (286 events) showed that patient observations varied greatly, with 3% of patients having no documented baseline and 12% having none at 15 minutes. Policy supporting patient observations varied from 85%-98%

**React:** On recognising ATR, staff were able to identify that first line of management should be stop the transfusion (97%). In practice, 9% of medical advice included continue as before or slow the rate. Policy supporting 'stop the transfusion' as the first line of management varied from 91%-100%.

**Report:** In practice, 9% of ATRs audited were not reported to any process (local, state or national). Policy supporting reporting varied from 70%-98%.

### **Conclusion**

Currently, practice is diverse in regard to initial management of ATRs, in particular mild reactions. HS should review policies to comply with national guidelines/standards, including first-line management of stopping transfusion in all ATRs. Knowledge of staff surveyed was good; however in practice stopping the transfusion and reporting requires improvement. Encourage "recognise, react and report" education.

## **P161. A national haemovigilance framework for Australia: Could this be a reality?**

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Haemovigilance (HV) is an organised set of surveillance procedures covering the whole transfusion chain. Good data and careful analysis are required to quantify risks, provide direction to develop safer systems, and conserve valuable resources.

International experience has highlighted the benefits of national HV programs, and these are now mandatory in many settings. National reports since 2001 have recommended Australia establish a national HV framework, and new national clinical standards require health services to report to local, state, or national systems. More recently, governments endorsed the “*National Blood Sector Data and Information Strategy and Scorecard 2013-16*” that includes the objective of a national HV system. However, we appear to be making slow progress toward this objective.

Contributing factors include:

Lack of consistency in data collection: a national minimum dataset and definitions were established, but these are not yet in use in all jurisdictions

Variation in completeness and quality of data available

Range of incident management software in use (AIMS, IIMS, STIR, RiskMan, PRIME) with different capabilities

Staff availability and training for case reporting and review

Voluntary nature of participation in existing arrangements (except for defined sentinel events)

Variation in use of independent case validation or review to determine imputability and severity

Without a ‘forcing function’ to ensure all jurisdictions provide information to a national HV framework, the last Australian HV report did not include data from all jurisdictions and the data provided were not validated or complete.

Despite the national HV Advisory Committee working to progress a national framework for Australia, and many efforts at local and regional level, significant barriers to engage all participants exist and must be addressed. The effectiveness of a national HV framework can be measured by the data reported, the analysis of data and its use to improve patient safety.

## **P162. Alteration of platelet ordering practices to minimise product wastage and improve clinical practice**

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### **Aim**

To report changes in platelet manufacture, distribution and ordering practices to minimise platelet wastage at a large tertiary regional hospital.

### **Background**

The Townsville Hospital is a large tertiary centre with trauma facilities, an allogeneic stem cell unit and a large emergency department. The hospital is located ~1400km from the nearest capital city. Local blood manufacturing services were ceased in June 2011 with centralization of processing to Brisbane. Subsequently, the lead time for platelet supply significantly increased, resulting in increased local inventory to meet potential demand. Additionally the platelets on inventory had a shorter expiry due to the time associated with transport. This resulted in increased wastage, especially on Monday/Tuesday given the need to order large quantities of short expiry platelets to cover potential weekend/Monday requirements.

### **Methods**

In late February 2014 the ARCBS initiated Saturday platelet processing. This enabled increased ordering frequency with platelets having a longer expiry period. The local ordering practices were adjusted to maximize the benefits of this change.

### **Results**

The results from the first 4 months (1/3/14 – 1/7/14) demonstrate an average platelet wastage of 6.5% (total number 56 units wasted) compared with the similar period in 2013 (1/3/13-1/7/13) during which time a wastage of 12.3% (total 97) occurred. This represents a 48% reduction in platelet wastage. The economic benefit of this represents saving of ~\$18000 for the 4 month period and more importantly preservation of an increasingly valuable resource.

### **Conclusion**

Simple adaptation of blood product ordering practices to match manufacturing and processing schedules can dramatically improve product utilization.

## **P164. High Titer, Low Avidity (HTLA) Antibodies (Abs): Clinical implications despite minimal risk of haemolytic consequences**

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### **Aim/Background**

To highlight aspects of HTLA Abs, including features on Ab screening that raise suspicion, offer diagnostic workup and have clinical implications.

### **Cases**

We present two cases of routine Ab screening demonstrating positivity to most red cell antigens (Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup>, S, s, M, N, C, c, and e), proving to be due to HTLA Abs and resulting in significant logistic and other challenges in the efficient and safe provision of blood.

Suspicion of HTLA Abs is first raised by the presence of Abs to high incidence antigens, with most donor cells reacting  $\leq 2+$  (low avidity). While most allo-Abs reacting  $\leq 2+$  have a titre  $< 8$ , HTLA Abs usually have a high titre of  $\geq 8$ . Abs that display HTLA characteristics include anti-Kn<sup>a</sup>, -McC<sup>a</sup>, -Cs, -Yk, -Ch, -Rg and -HH.

HTLA Abs usually cannot be identified in the routine laboratory but initial steps are undertaken to exclude underlying allo-Abs which have greater clinical implications. A negative repeat screen and panel on a 50:50 mix of patient plasma increases suspicion of anti-Ch, -Ro Abs as they are neutralised by pooled plasma. Dithiothreitol treatment destroys HTLA antigens and is used to detect underlying Abs when anti-Yk, -McC<sup>a</sup>, -HH or -Kn<sup>a</sup> are present. Patient specimens are also referred to a reference laboratory where a range of methods and rare or extended phenotyped cells can be used to identify the Abs.

### **Conclusions**

HTLA Abs are benign and don't usually cause haemolytic transfusion reactions (HTRs) but they can mask the presence of allo-Abs and contribute to delays in blood provision. Furthermore, some HTLA Abs (e.g. anti-Yk<sup>a</sup>) can cause HTRs and as a result, their presence [as seen in one of our cases], limits availability of compatible blood.

## **P165. Nine years' experience of testing for neonatal alloimmune thrombocytopenia (NAIT) at the Victorian Transplantation and Immunogenetics Service (VTIS)**

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### **Aims/Background**

The VTIS platelet reference laboratory at the Australian Red Cross Blood Service, receives samples for NAIT investigations from Victoria, South Australia and Tasmania. Key studies include human platelet antigen (HPA) genotyping, platelet serological studies, glycoprotein antigen assays and evaluation of HLA antibodies. This audit reviewed the number of NAIT investigations performed, proportion of serologically confirmed cases and specificity of HPA-antibodies detected.

### **Methods/Results**

Retrospective review of laboratory records of NAIT assessments performed between January 2005 and May 2014. Our laboratory routinely performs maternal and paternal HPA genotyping for HPA 1-6 and 15 specificities, looking for parental incompatibilities. Screening for platelet antibodies is performed by platelet immunofluorescence and was confirmed by GTI Pak12 ELISA for GPIIb/IIIa, GPIa/IIa, GPIb/IX and GPIV.

431 samples were referred for testing, of these 54 cases (12.5%) were confirmed as true NAIT based on laboratory results. Of confirmed cases, the main antibody detected was anti-HPA-1a (65%) and anti-HPA-5b (31%). Three further cases were classified as possible NAIT and in 14 cases NAIT was unable to be excluded, giving a total yield of 17%.

Unfortunately 48/431(11%) laboratory assessments were incomplete as paternal samples were not provided, or samples were insufficient or poor quality. The clinical context of the investigation was not always able to be determined, most commonly due to incomplete clinical information and the retrospective nature of this review.

### **Conclusions**

Our review reveals higher rates of anti-HPA-5b than reported in international studies. This may reflect the comprehensive nature of the current laboratory assays, or different frequencies of human platelet antigens reflecting Australia's ethnic diversity.

Investigation and interpretation of suspected NAIT is complicated and requires systematic testing using a number of complementary methodologies. Areas for future improvement include follow up of incomplete investigations and improving the quality of clinical information provided by referring clinicians.

## **P166. Evaluation of the Nanodrop ND-1000 and the HemoCue Plasma/Low Hb Photometer for assessing haemolysis in red blood cell concentrates**

**Crimmins D**

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### **Aim**

This study aimed to evaluate and validate the Nanodrop ND-1000 spectrophotometer and the HemoCue Plasma/Low Hb Photometer as new methodologies for determining free Haemoglobin (fHb) concentrations in order to assess haemolysis in leucoreduced red blood cells (RBC) after 35 days of storage.

### **Method**

Supernatant free haemoglobin (fHb) using the ND-1000 Nanodrop Spectrophotometer (Thermo Fisher Scientific) was quantified by measuring the oxyhemoglobin absorbance peak at 415 nm. The fHb levels (in grams per litre) were determined by the following calculation:  $C_{fHb} = 1.017 (167.2 A_{415} - 83.6 A_{380} - 83.6 A_{450}) / 1000$  where A absorbance at a measured wavelength. The HemoCue Plasma/Low Hb Photometer methodology oxidises haemoglobin via sodium nitrite to methemoglobin which reacts with sodium azide to form an irreversible bound azidemethemoglobin to generate free Haemoglobin readings. The absorbance is measured at two wavelengths 570 and 880 nm. The subsequent relative degree of haemolysis induced by storage at day 35 can be calculated when fHb levels are combined with Hct and total Hb values generated from the Sysmex Xs-1000i.

### **Result**

NZBS RBC units upon expiry at day 35 based on the use of both spectrophotometric technologies generated haemolysis profiles of only 0.1-0.22 %. According to current European guidelines for blood components haemolysis levels should be < 0.8% of red cell mass at the end of RBC storage to meet current quality control specifications. Statistical analysis was performed via Statistica (Statsoft) software version 12.0.

### **Conclusion**

The Nanodrop ND-1000 and HemoCue Plasma/Low Hb Photometer have been proven to be 'fit for purpose'. The HemoCue Plasma/Low Hb Photometer should be used as the primary method of determining free haemoglobin (fHb) concentration in RBC units and in turn their respective level of haemolysis.

## **P167. An audit of long term intravenous immunoglobulin in a tertiary centre**

**Crispin P**<sup>1,2</sup>, Aghanwa C<sup>2</sup>, Paxton L<sup>2</sup>, Hawkins C<sup>2,3</sup>

<sup>1</sup> Haematology Department, Canberra Hospital Garran ACT Australia, <sup>2</sup> Australian National University Medical School Acton, ACT Australia, <sup>3</sup> Immunology Department, Canberra Hospital, Garran ACT Australia

### **Aim**

To examine the decision criteria used to determine ongoing intravenous immunoglobulin (IVIg) therapy.

### **Method**

A retrospective audit of patients receiving (IVIg) for long term indications at a single tertiary centre to determine how, and how frequently the efficacy of IVIg therapy was assessed.

### **Result**

There were 97 patients identified during the study window on IVIg for long term indications covering a total of 6089 patient-months of treatment. IVIg was used for autoimmune disorders in 36 (37%) of case, secondary hypogammaglobulinaemia in 35 (36%) and primary immunodeficiency in 26 (27%) patients. The secondary hypogammaglobulinaemia patients were predominantly haematological malignancies (CLL 14, lymphoma 12, myeloma 6 and 1 post allogeneic transplant for AML).

Of the primary immunodeficiency patients, all had evidence of regular review of the frequency of infections and immunoglobulin levels on a 3 to 6 monthly basis. Only 19 of the 35 secondary hypogammaglobulinaemia patients had evidence that IVIg efficacy was actively being reviewed. There were 8 patients with haematological malignancies who had IVIg ceased, 2 as a palliative decision. Of these, 4 patients had IVIg recommenced with 3 having admission for pneumonia.

Chronic inflammatory demyelinating polyneuropathy was the predominant indication for immune modulatory therapy (19 patients) with other indications including myositis, myasthenia gravis and other neuropathies. Review was primarily clinical, with 26 having patient reported subjective improvement and 21 having objective responses (including activity assessment tools, clinical examination findings and hand muscle strength measurement).

### **Conclusion**

There is scope to improve follow-up assessments of patients on long term IVIg therapy. These data also suggest potential risk in ceasing IVIg in haematological malignancy patients and the need for more research to determine groups where a trial of IVIG withdrawal may be safely considered.

## **P168. Effective eLearning on Patient Blood Management**

**English L, Verrall T, Thomson A, Wood M, Clark T**

*BloodSafe eLearning Australia, Adelaide, SA, Australia*

Education for health professionals on patient blood management and safe clinical transfusion practice is fundamental to improved patient outcomes.

In December 2013, BloodSafe eLearning Australia released two courses, Patient Blood Management and Perioperative, aligned with the National Patient Blood Management Guidelines: Perioperative.

Uptake of the Patient Blood Management course has been significant with over 7,000 course completions in the first 6 months. In 2013 we began an initiative to evaluate clinician's feedback of the courses - this was to provide the opportunity for continuous improvement. The feedback has been valuable in refining course content, and mode of delivery.

User feedback on both courses indicates learning objectives are being met. Feedback from participants on the Patient Blood Management course includes:

- >90% improved their knowledge
- >75% will change their clinical practice
- >70% will help them to identify near misses and prevent adverse events
- >80% will improve patient outcomes/safety.

Similarly, feedback on the Perioperative course includes:

- >75% improved their knowledge
- >65% will change their clinical practice
- >75% will help them to identify near misses and prevent adverse events
- >80% will improve patient outcomes/safety.

Clinician's feedback confirms the eLearning courses are making a difference to user knowledge and practice.

A further course, based on the National Patient Blood Management Guidelines: Medical will be released later this year. The Medical course will be structured so health professionals can choose to complete modules relevant to their practice.

## **P169. Learn more – Patient Blood Management eLearning courses**

**English L, Verrall T, Thomson A, Wood M, Clark T**

*BloodSafe eLearning Australia, Adelaide, SA, Australia*

BloodSafe eLearning Australia is a national education program that provides free online courses to health professionals about patient blood management and safe, appropriate transfusion practice to improve patient outcomes.

Each year a new course is released in alignment with the National Patient Blood Management Guidelines. So far available courses include:

Patient Blood Management

Perioperative, and

Critical bleeding

A medical course is scheduled for release later this year.

This poster will outline the background of BloodSafe eLearning Australia, content of available PBM courses, and analysis of learning needs of clinicians and course evaluations.

## **P170. Reviewing the Maximal Surgical Blood Ordering Schedule (MSBOS) and the impact on current laboratory practice**

**Fleming S, Maxwell E**

*Melbourne Pathology, Melbourne, VIC, Australia*

**Aim:** To ascertain attitudes towards and the derivation of MSBOS used in laboratories across Australia and New Zealand. To compare current allocations to the 2007 ANZSBT Pre-transfusion Laboratory Practice guideline as well as available evidence on surgical blood usage in order to inform the next edition of those guidelines.

**Method:** Invitation to a confidential, online survey was extended to senior transfusion scientists via ARCBS electronic newsletter and through direct email to the membership of ANZSBT. Participants employing an MSBOS identified procedures for which they would recommend a group and screen (G&S) or the number of units allocated in either routine practice or special circumstances (antibodies).

**Results:** Attitudes to an MSBOS have been presented previously. Only 25 laboratories (36% of participants) utilize an MSBOS. Most MSBOS combined ANZSBT guideline recommendations (16) and local data (12). Branch laboratories (3/5) took guidance from their main laboratory. Variation from the 2007 guidelines included addition of a G&S for dilatation & curettage and AV shunt procedures, with reduced allocation for hip arthroplasty and hip fracture surgery, hiatus hernia surgery, cystectomy and aorto-femoral grafts. These changes are in line with published evidence on blood loss and transfusion requirements for surgical procedures.

**Conclusion:** Changes in both surgical technique and blood transfusion practice, including patient blood management principles, have led to reduction in product requirement and allocation for surgery. As a third of participating laboratories still value and use an MSBOS, retention and revision of the current MSBOS should be considered for the next edition of the Pre-transfusion Laboratory Practice guidelines.

## **P171. How do I report transfusion data and drive clinical change in a Patient Blood Management focused hospital?**

**Gallagher T, Esson A, Pyper W, Koay A**

*Department of Health Western Australia, Perth, WA, Australia*

### **Background**

Hospital Transfusion Committees have a responsibility to report ordering practices as well as the use and wastage of specific blood products. This reporting is commonly categorized into different specialty areas. Hospitals engaged in Patient Blood Management (PBM) may find it beneficial to utilise a separate type of blood product reporting for clinical leads, with the intent of applauding transfusion reduction, benchmarking practice, and to drive change in practice.

### **Aim**

We aim to demonstrate the unique elements of reporting transfusion to support a hospital-based PBM program.

### **Method**

WA Department of Health collaborated with specialist clinical and coding staff, to identify appropriate patient groups used in reporting templates for three tertiary PBM hospital programs.

### **Result**

Code groups for orthopedic and cardiac surgery were identified based on ICD-10-AM procedural codes. Two Orthopaedic and four Cardiac Surgery groups were developed: primary elective unilateral total hip replacements; primary elective unilateral total knee replacements with no grafting; primary isolated CABG, primary isolated valve repair/replacement, primary isolated percutaneous valve repair/replacement and primary combination CABG/valve repair/replacement.

Examples of exclusionary groups are bilateral and grafting procedures from the orthopedic group, non-elective admissions and re-operative procedures. These examples historically are associated with an increased risk of blood loss and frequently block the capability of preoperative optimisation of red blood cell levels or adequate iron stores. The goal in the remaining categories is to improve operative homogeneity of the reported cases.

Reports illustrating hospital and clinician red cell transfusion rates are now distributed several times annually to key clinical and hospital executive staff.

### **Conclusion**

The recipients of these reports now have the ability to benchmark their own practice against their peers, which promotes collaboration of best practice techniques in perioperative interventions.

## **P172. Raising standards in blood transfusion – Coordinated education and audit**

**Gallagher-Swann M**<sup>1,2</sup>, Ingleby B<sup>2</sup>, Pinchon D<sup>1</sup>, Arnold C<sup>2</sup>

<sup>1</sup> King Edward Memorial Hospital, Perth, WA, Australia, <sup>2</sup> PathWest Laboratory Medicine, WA, Australia

### **Aim**

International Guidelines and Australian Hospital Accreditation **NSQHS** Standards outline responsibilities regarding safe and appropriate transfusion practice. Our multidisciplinary team works cohesively to ensure key learning points are delivered effectively and consistently.

### **Method**

At King Edward Memorial Hospital we have designed a novel approach to triangulate transfusion guidelines and clinical audit within an education programme. We have a multi-disciplinary Transfusion Team leading practice, education and audit. Under the guidance of the Hospital Transfusion Committee, the team focus on safe, appropriate and evidence based use. We developed the mnemonic acronym KLAXON which outlines our strategic clinical and laboratory based education and audit objectives. We promote the use of 'KLAXON' as a mental checklist to direct principles of best transfusion practice in line with national guidelines throughout the organisation.



KEY INDICATORS : transfusion is appropriate and patient consents

LABELLING: Positive Patient Identification – specimens labelled at patient's side

ANALYSIS: clinical evaluation and timely laboratory investigations guide treatment

X CHECK: Positive Patient Identification - blood product verified at patient's side

OBSERVATIONS: patient monitored and transfusion documented

NOTE EFFICACY: outcome evaluated

### **Results**

KLAXON is being used to focus steps for education and provide a multidirectional approach to improvement. Each step is supported with evidence, information and clinical audit to cement key messages. Our reduction in red cell usage by 34% since 2007 reflects the changing attitudes at our institution and the increase in audited compliance with consent and essential documentation reflects the high level of staff awareness.

### **Conclusion**

KLAXON is a useful tool endorsed by the Transfusion Team to promote best practice in a consistent manner. This includes appropriate utilisation of blood products and key safety messages of Positive Patient Identification, appropriate investigations, clear documentation and consistent monitoring. This ensures our organisation has a cohesive approach to maintain and improve the quality of transfusion practice.

## **P173. Evaluation of the effects of implementing a blood management programme on patterns of blood product usage in a rural hospital setting**

**Gleeson T**, Longmore D, Lenton D

*Orange Base Hospital, Orange NSW Australia*

### **Aim**

To determine the effect of strict implementation of current national guidelines for transfusion on blood product usage in a rural hospital.

### **Method**

A retrospective chart review was performed at Orange Base Hospital in central-west New South Wales. The period of study encompassed transfusion before and after the institution of a policy mandating strict adherence to national transfusion guidelines which was implemented in mid 2013. The review included all transfusions of packed red blood cells, platelets, fresh frozen plasma and cryoprecipitate from August 2011 to May 2014. Data collected included indication and context of transfusion, gender, age, pre-transfusion haemoglobin level, haematinics (ferritin, B12, folate), type, quantity and date of product transfusion.

### **Result**

Institution of strict adherence to national transfusion guidelines resulted in a marked reduction in the usage of blood product, particularly red blood cells, and fresh frozen plasma. Platelet and cryoprecipitate usage was relatively stable across the study period. 5780 units of blood product were transfused during the study period: 4643 red blood cells, 258 platelets, 553 fresh frozen plasma, 326 cryoprecipitate. These products were associated with 2089 transfusions.

### **Conclusion**

The implementation of strict adherence to Patient Blood Management guidelines in a rural hospital setting has lead to a marked reduction in the usage of blood products. Future transfusion practice will continue to be guided by strict adherence to the national transfusion guidelines. Further data collection will act as a quality assurance tool in order to assess continued adherence to transfusion best practice.

## **P174. Usage of O RhD negative red cells in two SA hospitals**

**Gore C**<sup>1</sup>, Sinha R<sup>2</sup>, Roxby D<sup>1,2</sup>

<sup>1</sup> SA Pathology, SA, Australia, <sup>2</sup> Department of Haematology & Genetic Pathology, Flinders University, Bedford Park, SA, Australia

### **Background**

Balance between supply and demand for O RhD negative red cells (RC) remains a challenge for the Blood Service. The aim of this study was to review the usage of O RhD negative red cells in two South Australian hospitals.

### **Methods**

Patients who had been transfused with at least one unit of O RhD negative RCs during their first transfusion episode between January 1 2012 and 31<sup>st</sup> December 2012 were included in the study. Patient details including age, sex, blood group, admitting diagnosis, indication for transfusion and numbers of RCs transfused were reviewed.

### **Results**

404 patient transfusion episodes (691 RCs) were included for analysis during the study period. The median age of patients was 69 (52-81) years and 56% were males. The indications to transfuse were stratified into 16 categories including emergency situations where the patient's group was unknown; non O RhD negative requiring phenotype specific or CMV negative, irradiated RCs where ABO group specific RCs were not available or transfusion to non O RhD patients to prevent expiry of O RhD negative RCs. Ninety seven (14%) of O RhD negative RCs were used as emergency units with an average of 3.2 units per episode. One hundred and forty four units (21%) were transfused to non O RhD negative patients (usually O RhD positive patients) in order to prevent time expiry.

### **Conclusion**

Red cell inventories should be reviewed and adjusted regularly to limit excess holdings of O RhD negative RCs in order to minimize transfusion of O RhD negative RCs to non-O RhD negative patients except in emergency situations thus decreasing demand and avoiding wastage due to time expiry.

## **P175. Implementation of a commercial platelet alloantibody detection assay demonstrates the need for confirmatory reference techniques**

**Havelberg K**

*Australian Red Cross Blood Service, Kelvin Grove, QLD, Australia*

### **Aim:**

To verify suitability of the Immucor Lifecodes Pak LXTM kit for use in the Platelet & Neutrophil Reference Laboratories of the Blood Service (BS) compared to results from the MAIPA (Monoclonal Antibody specific Immobilisation of Platelet Antigens) assay.

### **Method:**

We tested 139 previously defined clinical or international QAP samples using the Pak LXTM kit. Testing was performed at 3 BS sites (Brisbane, Sydney & Melbourne) and by multiple operators at each site. The selected samples covered all antibody specificities detectable by Pak LXTM except anti Human Platelet Antigen (HPA)-2a - which is very rare - and some samples contained multiple antibodies. Samples which previously tested as negative for platelet specific antibodies were also included.

This exercise did not include HLA Class I antibody detection, although it is detectable by the kit, as there are other more specific methods commonly used for HLA antibody detection.

### **Result:**

The Pak LXTM detected:

96% of expected platelet specific antibodies across HPAs-1, 2, 3, 4, 5 and glycoprotein (gp) IV;

100% of expected anti HPA-1a, HPA-2b, HPA-4, HPA-5 and gpIV antibodies;

69% of anti HPA-3 antibodies, failing to identify both anti HPA-3a and anti HPA-3b. It failed to reach the expected result for the NIBSC Minimum Potency Standard anti HPA-3a serum.

91% of expected anti HPA-1b antibodies.

82% of expected broadly reactive glycoprotein specific antibodies.

Moreover, 6.2% of tests detected additional antibodies to those expected.

### **Conclusion:**

Pak LXTM is designed to detect IgG antibodies to HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, gp IV and Class I HLA in human serum. It cannot detect other clinically significant antibodies to HPAs, importantly HPA-15, nor can it be used for platelet cross matching or platelet autoantibody testing.

Pak LXTM was deemed adequate for BS application as a preliminary screening assay, to be used in conjunction with a confirmatory reference test –the MAIPA.

## **P176. Antibody(Ab)Titres using Bio-Rad Gel Station**

**Haysom H**<sup>1</sup>, **Crockett R**<sup>1</sup>, **Schischka S**<sup>1</sup>, **Cole-Sinclair M**<sup>1,2,3</sup>

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### **Aim:**

To explore moving Ab titres from manual to automated testing. Results were evaluated for consistency and equivalence between the GS and tube technique across a range of Ab specificities and titre strengths. Two concentrations of bovine serum albumin (BSA) dilutions were also evaluated.

### **Method:**

A single master titration prepared in 1) 30% BSA diluted to 5% and 2) commercial 6% BSA were run concurrently by tube and GS. The NICE titre tube method was compared against: a) NICE tube method using 6% BSA, and b) titres performed as a series of 'crossmatches' against a single 'donor unit' on the GS.

40 samples were titred in parallel between tube technique and GS. Abs selected were Anti-D, Anti-c, Anti-K, Anti-E, Anti-C, Anti-Fya.

Replicate testing with 3 Abs was performed manually by 5 scientists in parallel with the GS.

Equivalence of results was defined as an endpoint within 1 dilution.

### **Results:**

No difference was observed between titres performed in 5% and 6% BSA. With the exception of anti-Fya Abs, titre results were concordant comparing the GS and tube across all Ab specificities and titre ranges (<2 to 1024). 5 of 8 anti-Fya titres gave titre results >2 dilutions. 3 of these Ab samples were fresh, and two frozen, so sample age may have been a factor.

Carryover of high titre Abs was not observed. Replicate testing on the GS gave consistent results within 1 dilution. Of the 95 laboratories enrolled in the RCPA AA program, 22 use CAT, 5% BSA is the most commonly used diluent. No enrolled pathology laboratory is using 6% BSA.

### **Conclusion:**

Interpretation of manual titres can give different results when performed by different scientists, with potential clinical implications for the patient. GS titres enable minimisation of manual pipetting, standardised reading, scoring and determination of the Ab titre end point. SVHM now performs Ab titres on the GS using 5% BSA for all Abs except anti-Fya and Fyb.

## **P177. Platelet utilisation and triggers for haematology and oncology patients at Gold Coast Hospital and Health Service (GCHHS), a prospective analysis**

Clark F<sup>1</sup>, Dorrington A<sup>1</sup>, Bryson M<sup>1</sup>, Gutta N<sup>1</sup>, Hollis L<sup>2</sup>

<sup>1</sup> Gold Coast Hospital and Health Service ,QLD, Australia <sup>2</sup> Sunshine Coast Hospital and Health Service, QLD, Australia

### **Aim**

To review platelet transfusion procedures for all Haematology and Oncology patients against current guidelines implemented at GCHHS, which are aligned with the British Haematology Society Guidelines from 2003, and to analyse the outcomes.

### **Method**

A comprehensive prospective case study review conducted over a 2 month period (24<sup>th</sup> February 2014 to 26<sup>th</sup> April 2014) within GCHHS. All Haematology and Oncology adult patients who received a platelet transfusion were included. Data was captured on the following: patient diagnosis, platelet pre-transfusion count, clinical indication for transfusion, WHO bleeding scale (if applicable) and service area.

### **Results**

Over the 2 month period, a total of 36 patients, 93% Haematology and 7% Oncology, received a total of 125 megaunits of platelets within 114 platelet transfusion episodes. The mean pre platelet transfusion count was  $52 \times 10^9$  per L, (range of  $2-88 \times 10^9$  per L). 24% of transfusions included in the study did not comply with current local guidelines. 81% of transfusions that failed to comply with guidelines were administered to outpatients. The majority of platelet transfusions (83%) were given for bleeding prophylaxis, whilst 17% were for acute bleeding episodes. 20 platelet transfusions were given for a total of 15 bleeding episodes. Of these, there was one major bleeding episode and 14 minor episodes. There were 3 documented platelet transfusion related reactions in the examined cohort, none of which were formally reported and investigated further.

### **Conclusion**

While compliance with local platelet transfusion guidelines in inpatients is quite high, it still could be improved. Further education and awareness of platelet guidelines and the management of transfusion reactions within the service is required. The major source of non-compliance came from the outpatient setting, where there is a paucity of data guiding clinical platelet transfusion practice. Further research needs to be conducted in this area.

## **P178. Prospective view of patients understanding of informed blood consent at Gold Coast Hospital and Health Service (GCHHS)**

Clark F<sup>1</sup>, Bryson M<sup>1</sup>, Gutta N<sup>1</sup>, Hollis L<sup>2</sup>

<sup>1</sup> *Gold Coast Hospital and Health Service, QLD, Australia* <sup>2</sup> *Sunshine Coast Hospital and Health Service, QLD, Australia*

### **Aim**

The aim of this audit was to determine if patients understand the informed blood consent process within our organisation.

### **Method**

This was a randomized prospective case study review conducted over a 12 month period by the Transfusion CNC. Any adult patients who had received a red blood cell transfusion with blood consent obtained within <3 days were asked a series of questions. Exclusions included minors under the age of 18, Non-English speaking, unconscious or unwilling to participate.

### **Results**

Over the 12 month period, 100 patients were reviewed across all services within our organisation. Of this, 57% of patients did not know what type of blood product they had been transfused. It was interesting that the majority of patients, 83% did not have any concerns about receiving a transfusion. It was reassuring that 97% of patients agreed that the consent process was adequately explain to them and they all felt comfortable to ask questions and part of the decision making process. Only 58% of patients were provided with written information regarding their blood transfusion, of this only 28% were given the time to read it.

### **Conclusion**

Informed consent is a process by which the patients are provided with information, advice and warnings about the treatment, risks, benefits and alternatives. It is reassuring that 97% of patients within our organisation agreed and felt that the consent process regarding their red cell transfusion was adequate. However, only 57% of patients were able to identify the type of blood product they were transfused. Further improvements to provide patients with written information and allocated perusal time may potentially improve patient's understanding of all aspects of informed blood consent. Most importantly the majority of patients (83%) were not concerned with the risks of a blood transfusion which highlights that the decision to transfuse patients appropriately weighs heavily on the clinicians decision whether the risks outweigh the benefits to proceed with the transfusion.

## **P179. Retrospective review of platelet wastage at Gold Coast Hospital and Health Service (GCHHS)**

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<sup>1</sup> Gold Coast Hospital and Health Service, QLD, Australia, <sup>2</sup> Pathology Queensland, Herston, QLD< Australia <sup>3</sup> Sunshine Coast Hospital and Health Service, QLD, Australia

### **Aim/Background**

In 2013 the Queensland (QLD) Blood and Blood Product Wastage Reduction Strategy 2014-2017 established platelet wastage rates for our health service at 19% (national average is 18.9%). Reports identified that our compliance with platelet wastage rates was higher when compared to the state wide targets. The aim of this report was to investigate the reasons for platelet wastage within our health service and implement strategies for improvements.

### **Method**

A comprehensive retrospective review conducted over 5 months (January to May 2014) within GCHHS was undertaken and compiled from Qld pathology fate data base, Blood Net. All data related to platelet inventory, shelf life and reasons for discards was reviewed and analysed.

### **Results**

Over the 5 month period, our health service received a total of 572 platelets and discarded 158, overall average platelet wastage of 28%. Of the platelets discarded the majority, 108/158 (68%) was due to expiry. Upon further analysis of the reasons for expiry, it was identified that 117/159 (73%) platelets received by our health service had  $\leq 2$  day shelf life. The remaining 50/158 (31%) of platelets discarded was related to platelets ordered for patients and not required. During this time frame cardiac thoracic surgery commenced within our health service and requested 2 units of platelets to be allocated for each patient.

### **Conclusion**

Our platelet wastage rate (28%) is higher than the state wide target (19%); it is a high priority for our health service to rectify. Platelet usage for cardiothoracic surgical patients was monitored and modifications have already occurred by allocating 1 unit of platelets per patient. Additionally, our local blood bank has devised a platelet inventory board to closely monitor platelet requests and reallocate stock for further use if not required. After discussions the Australian Red Cross Blood Society (ARCBS) have amended dispatch processes to send platelets with  $\geq 3$  day shelf life to our health service. A shelf life of 5 days for platelets presents an inventory management challenge to any department; these strategies to improve our platelet wastage will be monitored and reviewed.

## P180. Treatment of platelet concentrates with the THERAFLEX UV-Platelets system

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**Aim:** The THERAFLEX UV-Platelets system (Macopharma, Mouvoux, France) is a pathogen reduction technology that uses short wave ultra-violet light (UVC, 254nm). In this study, buffy coat (BC) derived platelet concentrates (PCs) were treated with the THERAFLEX UV-Platelets system.

**Method:** Using a pool and split design, 2 ABO-matched buffy coat-derived PCs were pooled, supplemented with plasma to achieve a minimum of 30% plasma carryover (with 70% SSP+, Macopharma, Mouvoux, France) and split to form matched pairs (n=10 pairs). One unit was UVC-treated according to the manufacturer's instructions, and the other remained untreated as a control. All units were stored under standard blood banking conditions and sampled at days 1, 2, 5 and 7 post-collection and tested for *in vitro* quality and function. Data were analysed using a repeated measure two-way ANOVA, and post-hoc t-tests were used where statistically significant differences were identified.

**Results:** All platelet components met the manufacturer's specifications for UVC treatment (Table). During storage no significant differences were found between the control and UVC- treated PC for pH, pO<sub>2</sub>, pCO<sub>2</sub>, viability, mitochondrial membrane polarisation, the expression of platelet glycoproteins and degranulation markers (CD62P and CD63), ADP-induced aggregation, clotting time and strength (TEG) (p > 0.05). However, platelet metabolism was accelerated by day 5 following UVC treatment, as demonstrated by increased lactate production and decreased ATP levels (Table). UVC treatment also resulted in increased expression of the activated GPIIb/IIIa receptor (PAC-1 binding, p < 0.001) and reduced hypotonic shock response (p < 0.001). Externalisation of phosphatidylserine (PS) was significantly higher in the UVC-treated group by day 5 of storage and these PCs contained

	UVC treatment specifications	Control (n=10)	UVC (n=10)
Platelet count (10 <sup>9</sup> /mL)	0.8 - 1.2	0.83 ± 0.08	0.84 ± 0.09
Volume (mL)	325 - 375	369.78 ± 4.66	369.42 ± 6.27
Plasma (%)	30 - 40	31.75 ± 1.01	32.18 ± 1.29
Day 5 <i>in vitro</i> quality			
Lactate production (mmol/10 <sup>12</sup> platelets/hour)	-	0.064 ± 0.029	0.120 ± 0.044*
Total ATP (µmol/10 <sup>11</sup> platelets)	-	5.52 ± 0.73	4.53 ± 0.98*
Annexin V (%)	-	0.91 ± 0.21	1.83 ± 0.41*
CD61 <sup>+</sup> /PS <sup>+</sup> MPs (×10 <sup>6</sup> /unit)	-	433.40 ± 93.11	827.24 ± 521.90*

\*p < 0.05 using a paired two-tailed t-test

le).

significantly more PS-positive microparticles (MPs) than controls (Tab

## Conclusion

A comprehensive *in vitro* analysis demonstrates that treatment with the THERAFLEX UV-Platelets system affects some, but not all aspects of, platelet metabolism and activation. These changes are similar to those previously reported in the literature for this pathogen reduction technology.

## **P181. HLH Syndrome; from a Blood Bank perspective.**

**Jaggard J**

*New Zealand Blood Service (NZBS), Waikato, New Zealand*

### **Background**

A 39yr old male came to the Emergency Department at Waikato Hospital with anaemia, jaundice and symptoms of a viral infection. He had a previous history of abnormal liver function and hypersplenism. Blood bank testing identified the presence of a strong auto-antibody, requiring antigen matched units if transfusion required.

### **Case history**

Within 9 hours of admission, the haemoglobin dropped from 104 to 54g/L. Over the 8 days the patient was at Waikato Hospital, he was diagnosed with Auto Immune Haemolytic Anaemia, except his condition continued to deteriorate despite treatment. A Bone Marrow Aspirate was consistent with severe haemolysis, and other procedures were inconclusive. After 8 days, the patient was transferred to Auckland Hospital for further treatment.

### **Results**

The patient's ferritin was tested, with an immensely high result of 14,211ug/L. Hemophagocytic Lymphohistiocytosis, also known as HLH Syndrome, was then considered as a possible diagnosis. If left untreated, this syndrome will progress to multiple organ failure and death within a few months.

### **Conclusion**

This syndrome is rare, and difficult to diagnose due to the variability in the clinical presentation. Early diagnosis is crucial in the treatment of HLH Syndrome, to avoid permanent organ damage and death. To keep this patient supplied with appropriately matched red cells, 52 red cell units were sourced from different NZBS sites around New Zealand.

## **P182. Comprehensive review of platelet usage in the HAPS network**

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<sup>1</sup> Hunter Area Pathology Service, NSW, Australia, <sup>2</sup> Calvary Mater Newcastle, NSW, Australia

### **Aims**

(1) To review platelet usage in the Hunter Area Pathology Services (HAPS) hospital network in order to determine appropriateness of transfusion, and to analyse usage patterns to predict future requirements. (2) To develop a predictive model to inform blood bank inventory management to minimise wastage through over-ordering.

### **Methods**

Details of all platelet units issued across the HAPS network between 1 March 2011 and 28 February 2014 were extracted from the eBlood blood bank management system. All FBEs performed during that time were extracted from the laboratory information system.

### **Results**

6845 platelet units were issued to 1439 unique patients. 67% of all transfusions were used in haematology/oncology units, predominantly in adults. Overall usage is slowly reducing, but wide month-to-month variability exists. 66% of all transfusions were used at a platelet count of  $<20 \times 10^9/L$ . Usage at higher platelet counts ( $>100$ ) is increasing year-on-year.

The number of units used per day showed wide variability (median 6, mean 6.25, SD 3.15), making it difficult to predict platelet ordering requirements. However, 66% of all transfused patients had a platelet count available on the day prior to transfusion, suggesting this could be a better predictor of future transfusion. Linear regression models were developed to predict network platelet requirements on a day-to-day basis.

### **Conclusions**

While the majority of platelet transfusions were for severe thrombocytopenia, transfusion at platelet counts  $>100$  is increasing and warrants further audit. The wide variability in day-to-day platelet usage across the network makes it difficult to predict the number of units that will be required on any particular day. Linear regression models based on the number of thrombocytopenic patients show promise in predicting platelet usage and may help inform blood bank inventory management.

## **P183. National inventory management framework for red blood cells**

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### **Aim**

To:

Develop and test a calculation that defines appropriate red blood cell inventory levels

Identify best practice inventory management guidance for red blood cells

### **Method**

Red blood cell inventory bands were calculated for inventory requirements at the ABO and Rh level for one proof of concept site and seven pilot sites. The calculation was developed using safety stock methodologies that used the average and variability of the number of red blood cells that were issued compared with transfused. A stock level alert based on the minimum level of the inventory band identified when inventory levels were low. Data collection and observations were conducted to both test the calculation and to inform development of best practice inventory management guidelines.

### **Results**

Red blood cell inventory level changes during the pilot ranged from an increase of 31% to a decrease of 36%. The majority of pilot sites maintained their inventory levels following completion of the pilot, with two reducing their inventory levels further. There were differences between sites on deliveries (including urgent deliveries), orders, wastage and expiry. Product substitution (use of an ABO Rh group for a patient with a different ABO Rh group) was common.

### **Conclusion**

Pilot sites indicated that the increased focus on inventory management was a positive outcome of the pilot.

The calculation provided appropriate guidance on inventory levels for the health providers involved in the pilots. Ongoing review is required to ensure the calculation is appropriate for an individual health provider and that it reflects current clinical requirements rather than historical transfusion data. Subject to approvals, the red blood cell stock calculation will be made available to health providers to support their decisions about appropriate red blood cell inventory levels.

Observations allowed the development of best practice guidelines for red blood cell inventory management which will be published as a module to the *Managing Blood and Blood Product Inventory: Guidelines for Australian Health Providers* available on [www.blood.gov.au](http://www.blood.gov.au).

## **P184. Introduction of single unit red blood cell transfusions at St Vincent's Hospital Sydney**

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### **Aim**

Reduce unnecessary exposure to red blood cells to patients by introducing single unit red cell transfusion policy for stable non bleeding patients

### **Method**

The SVH Transfusion Committee supported the change to a single unit red blood cell practice for non-bleeding haemodynamically stable patients. Patients from operating theatre and intensive care unit were excluded from the project. Change to Single unit red blood cell transfusions was written into the blood transfusion policy and endorsed by executive . Implementation and evaluation of project occurred to further improve effectiveness.

### **Result**

Since the project implementation in March 2013 an average of 147 red blood cell units have been saved per month an almost doubling the single unit orders after the implementation of the project.

### **Conclusion**

The project objectives have been achieved including an overall reduction in red blood cell use of over 1600 units in one financial year . Continued monitoring and auditing should sustain single unit red blood cell issues.

## **P185. National trends in the distribution of Intravenous Immunoglobulin to Chronic Lymphocytic Leukaemia patients with Acquired Hypogammaglobulinaemia and severe and/or recurrent infections from 2008-2013**

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**Aim:** To investigate whether there were significant differences in the number of treatment episodes of Intravenous Immunoglobulin (IVIg) distributed to Chronic Lymphocytic Leukaemia (CLL) patients with acquired hypogammaglobulinaemia and severe and/or recurrent infections between Australian states and territories from 2008-2013.

**Method:** Retrospective data was obtained from the Blood Service's national IVIg registries pertaining to this population from 2008-2013. 48,870 treatment episodes of IVIg were distributed to 2,734 individual patients. Trend analyses were performed on the demographic data as well as the IVIg distribution data according to season, year and geographical location.

**Results:** CLL patients with acquired hypogammaglobulinaemia and severe and/or recurrent infections received 6.9% of the total national supply of IVIg from 2008-2013. Over 80% of this IVIg was distributed to CLL patients who resided in one of the three most densely populated states, namely NSW (34.3%), Queensland (28.3%) and Victoria (21.0%). However, CLL patients who resided in Queensland, Tasmania and the ACT received more IVIg treatment episodes per 1,000 capita compared to the national average for this indication. This appeared to be in part due to the demographic characteristics of these regions, but may also have been due to the prescribing preferences of their treating physicians. Moreover, there appeared to be a sustained growth of 5.5% per annum in the overall distribution of IVIg to CLL patients over this period despite the number of new CLL patients receiving IVIg remaining reasonably stable. There did not appear to be recurrent monthly peaks in IVIg distribution; however there was consistently more IVIg distributed during spring.

**Conclusion:** There were differences in the distribution of IVIg to CLL patients with hypogammaglobulinemia and severe and/or recurrent infections between Australian states and territories from 2008-2013. Even though demographic characteristics contributed to some of the differences, the impact of treating physicians prescribing preferences appeared substantial.

## **P186. Impact of a 'Pre-operative Anemia Clinic' (POAC) review in patients undergoing elective surgery**

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### **Background**

Screening of preoperative patients for anemia and correcting the anemia with haematinics or erythropoietin is associated with a reduction in the need for post-operative blood products. Optimisation of preoperative haemoglobin (Hb) may also lead to reduced length of stay (LOS), complications and readmission.

### **Aim**

To determine the effectiveness of the POAC in reducing post operative blood product requirements, LOS, complications and 30 day readmission rates and to compare these outcomes with a historical control.

### **Method**

Data was reviewed on patients referred to the POAC at Monash Health between January 2013 and May 2014. Majority of these patients were anemic (defined as male Hb<120g/L and women Hb<110g/L). Data on surgical outcome, complications, blood product requirements, LOS and readmission at 30 days was collected. This data was compared with those outcomes obtained from a historical control group but were not matched for procedures.

### **Result**

A total of 27 patients underwent 30 procedures. The mean age was 64 years and 58% were female. The mean referral Hb was 100g/L with 74% (n=23) of the patients having a ferritin less than <100µg/L. Procedures included arthroplasty (47%), major abdominal (including gynaecological) (17%), cardiothoracic (17%) and 20% other surgery.

77% of patients received either oral or parenteral iron therapy and 3 patients also received concurrent erythropoietin therapy.

26% of patients received blood products post-operatively compared to 37% in the historical control group (p>0.05 ns). The mean lowest post-op Hb following the 30 surgical procedures was 84g/L. The median acute (LOS) was 5.5 versus 2 days in the control group and 23% of patients had complications.

### **Conclusion**

This preliminary data suggests that, compared to a historical control, the POAC may lead to less post operative blood product use (26% vs 37%).

## **P187. A "why use two when one will do" campaign - Did it work?**

**King F**

*New Zealand Blood Service, Wellington, New Zealand*

A restrictive approach to red cell transfusion in the non-bleeding patient is accepted as best practice and it is considered inappropriate to prescribe two units for a top-up red cell transfusion when one unit may be sufficient to improve symptoms. Observational studies also suggest higher morbidity and mortality in patients receiving red cell transfusions.

In 2013, following discussion and initiation of a Minimal Transfusion working group, C&CDHB commenced a "Why Use Two When One Will Do" campaign across the site.

### **Aim**

To obtain a snapshot of red cell transfusion from Blood Bank following the blood conservation programme and to compare this with a 2012 audit.

### **Method**

All transfusions of red cell and whole blood units issued from Blood Bank for the month of April 2014, were assessed, excluding units issued to NICU and to Cardiothoracic theatres.

### **Results**

A total of 476 units (119 per week) were transfused to 179 patients with 273 transfusion episodes in total. The 2012 audit over one week only identified 155 units transfused to 72 patients in 85 transfusion episodes. There was no difference in the number of units transfused per week ( $p=0.68$ ). Only one unit was transfused in 39% episodes compared with 28% in 2012 ( $p=0.10$ ). However, the proportion of patients requiring multiple transfusion episodes increased (31% vs 15%,  $p=0.015$ ). The mean post-transfusion haemoglobin was 93g/L with 32% having a level over 100g/L.

### **Conclusion**

This audit has shown a trend towards increased numbers of single unit transfusion episodes. However, this is associated with a significant increase in the proportion of patients requiring multiple transfusion episodes. There did not appear to be a net reduction in the total number of units issued per week. Tackling the target haemoglobin may address this.

## **P188. Bloody data – how the National Blood Authority, Australia, can assist with evidence driven policy and practice change**

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### **Aim**

The data provided by the National Blood Authority (NBA) can substantially aid Patient Blood Management (PBM) programs, blood inventory management and discard reduction, and monitoring blood use trends at local, organisational, state and national levels.

### **Methods**

The NBA's BloodNet system holds data on all blood ordered by Australian Health Providers and issued by the Australian Red Cross Blood Service, at the individual unit level. When linked with data from Laboratory Information Systems (LIS) it provides end-to-end oversight for blood use within a facility, encompassing issues, receipts, transfusions and discards. This provides an evidence based approach to optimising prescribing practice, blood inventory management, and examining blood use trends. The data held in BloodNet can also facilitate benchmarking at health provider level in line accord with governance structures.

### **Result**

While the BloodNet data itself does not provide outcome data, it provides an insight into the health providers and states and territories that the NBA can partner with in data linkage projects and clinical audits.

Examples of data linkage and use for blood management are shown to illustrate the application of NBA data to assist with PBM programs and inventory management. These initiatives can provide support for clinical audits and evidence for quality improvement that brings the focus to patterns of transfusion and patient outcomes.

### **Conclusion**

The NBA continues to develop and implement standardised reports and blood data available to its users, and is working to automate linkage with LIS to provide greater reporting capabilities and analysis value to the clinical community. In the meantime, the NBA can support facilities implementing blood management programs with data, advice and collaboration.

## **P189. Blood group genotyping in the Red Cell Reference Laboratory: What is the rate of phenotype/genotype discordance for complex cases**

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### **Background**

The Blood Service Reference Laboratories receive patient samples from the public, private hospitals and pathology services and occasionally from overseas, for the resolution of problems relating to red cell serology. It is a challenge to phenotype patients accurately who have been recently transfused and for patients whose red cells are coated with immunoglobulin (DAT+) e.g. in autoimmune haemolytic anaemia (AIHA). Monoclonal typing reagents are specified as appropriate for use with DAT -positive patients but this has not been validated. Molecular blood group genotyping provides an alternate approach for investigating these complex cases to identify anomalies.

### **Aim**

To study the phenotype/genotype discrepancy rate for complex samples by comparing the patients' phenotype (by serology where possible) and its predicted phenotype by genotyping.

### **Method**

Patient's phenotypes for Rh, Kell, Duffy and Kidd were determined by standard serological methods. The patient cohort includes recently transfused patients and patients with positive DAT such as in AIHA (n=53) referred for investigation at the Blood Service Red Cell Reference Laboratories. Genotyping was performed using validated PCR methods or by SNP array.

### **Results -**

18/53 samples from the patient cohort tested showed discordant results between the observed phenotype and genotype based predicted phenotype. Discrepancies in patients with positive DAT were due to the strong DAT preventing serological detection of the antigen. Discrepancies were within the blood groups (RhE:n=2; Rhc:n=1; Kell:n=1; Duffy:n=11 and Kidd: n=3).

### **Conclusion**

Discrepancies between serology based phenotype and genotyped based phenotype predictions were observed for 34% of the patient cohort tested. This study is being expanded and will include genotyping for other blood group antigens. The study provides support for the application of genotyping in Red Cell Reference Laboratories to improve the accuracy of blood group phenotyping for complex cases. This is important for the provision of suitable blood for transfusion to reduce the risk of delayed transfusion reaction and to provide matched blood for patients with long term transfusions needs.

## **P190. Piperacillin induced immune mediated haemolysis in two patients with *Pseudomonas aeruginosa* infection**

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### **Background**

Piperacillin induced haemolytic anaemia (PIHA) is well described. More than 50% of published cases involve patients with cystic fibrosis (CF), most with *Pseudomonas aeruginosa* (*P. aeruginosa*) infections. The reasons for the high incidence of PIHA in this group remain poorly understood. We present two non-CF patients with *P. aeruginosa* infections with suspected PIHA.

### **Case Presentation**

Patient 1, 65 yo female, 8 months post allograft for acute myeloid leukemia, presented with neutropenic sepsis. She was commenced on Tazocin (piperacillin/tazobactam) as tissue cultures grew *P. aeruginosa*. Haemoglobin (Hb) fell to 69 g/L (baseline ~90 g/l) one week post antibiotic treatment.

Patient 2, 46 yo female with diabetes-related end stage renal failure and hyposplenism, presented with worsening infection following toe amputation. Wound culture grew *P. aeruginosa*. She had received Tazocin in the preceding two months and was re-commenced on Tazocin. Hb was 51 g/L (baseline ~90 g/L) on day 3 of antibiotic treatment. A blood film showed occasional spherocytes along with other features of hyposplenism.

Both patients had positive direct anti-globulin test with IgG and biochemical evidence of haemolysis. Elution was negative in patient 1, but demonstrated non-specific, pan-agglutinating IgG for patient 2. Both patients had positive indirect anti-globulin test (IAT) by incubating Tazocin-treated control group O red cells with the patients' plasma.

### **Discussion**

The diagnosis in both patients required a high degree of clinical suspicion due to more common causes of haemolysis post-allograft in Patient 1, and competing cause for spherocytes (i.e. hyposplenism) and the unusual antibody elution pattern in Patient 2. 'False' positives can occur in IAT testing with Tazocin-coated red cells due to naturally occurring anti-piperacillin antibodies. However, PIHA was strongly suspected in these cases due to the rapid resolution of haemolysis following cessation of Tazocin, thus highlighting the need for clinical awareness of this rare condition in non-CF patients.

## **P191. Significant decrease in red cell transfusion in haematology outpatients with single unit transfusion policy**

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### **Aim/Background**

Blood transfusion practice has developed from historical beliefs rather than evidence-based medicine. Traditionally, single-unit packed red blood cell (PRBC) transfusions were thought to be insufficient to treat anaemia, particularly in those with bone marrow suppression due to haematological malignancy and myelosuppressive chemotherapy. Recent data suggests that the number of PRBC transfusions may be safely reduced for inpatients with haematological malignancy and myelosuppressive chemotherapy. This may have implications for long term patient outcome as well as financial benefits to the community. (Reference haematological paper 2012 91(1)). Our aim was to reduce the number of multiple (2 or more) PRBC transfusions by implementing a single unit policy (SUP) into the Haematology Outpatient clinic (HOC) and assess the outcomes over a 6 month period.

### **Methods**

Under the SUP, all patients are assessed clinically in conjunction with a Haemoglobin (Hb) evaluation prior to prescribing transfusion. Transfusion data (Supplied by the Transfusion Medicine Unit's Information Technology Department) was analysed over 2 six month periods (Pre and post single unit implementation) to determine PRBC prescribing practice.

### **Results**

1519 episodes of care (EOC) were recorded in 6 months prior to the SUP with 238 (16%) of those including transfusion episodes.

In the 6 months following SUP implementation 1461 EOC were recorded, with 169 (11%) including transfusion episodes.

2013	Number of PRBC transfusion episodes	1U Prescribed	2U Prescribed	3U Prescribed	Total number of units transfused
Jan-June	238	115 (48.3%)	117 (49.2%)	6 (2.5%)	367
July-Dec	169	147 (87%)	20 (11.8%)	2 (1.2%)	193

In 2013 there were a total of 560 PRBC transfusions administered with 367 (65.5%) based on Hb alone. Over 50% of this group of patients received 2 or more units of PRBC at each attendance. Following the implementation of the SUP, the number of multiple units being transfused reduced from 51.7% to 13%.

### **Conclusion**

Our results suggest a SUP significantly reduced the amount of PRBC transfusions in the HOC. This resulted in less patient exposure to blood products, reduced time in the clinic, less short term side

effects and likely better long term outcomes. This will translate to cost saving on blood products, patient bed time, staffing and less demand for PRBC (1).

## **P192. Liberal versus restrictive transfusion strategy of platelet concentrate and cryoprecipitate in thoracic aortic surgery: A multicenter randomized trial**

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**Background:** Thoracic aortic surgeries frequently require massive transfusion due to acute coagulopathy or thrombocytopenia, or platelet dysfunction. Transfusion of platelet concentrate (PC) and cryoprecipitate is recommended, although the impact and appropriate thresholds for transfusion remain unclear.

**Aims and Methods:** We conducted a multicenter randomized trial comparing liberal versus restrictive transfusion with elective thoracic aortic open repairs to evaluate the efficacy of liberal (proactive) transfusion for reducing bleeding. With the liberal strategy, PC was transfused when platelet counts fell below  $100 \times 10^9/L$  and cryoprecipitate (approximately fibrinogen 2 g) was transfused when plasma fibrinogen concentrations fell below 1.5 g/L after weaning from cardiopulmonary bypass. With the restrictive strategy, PC was transfused when platelet counts fell below  $50 \times 10^9/L$  and fresh frozen plasma (FFP) was transfused when plasma fibrinogen concentrations fell below 1.5 g/L.

**Results: For the analyses,** 31 cases with the liberal strategy and 30 with the restrictive strategy were eligible. The total volume of allogeneic red cell concentrates transfused during surgery seemed to be lower with the liberal strategy (1463 ml vs. 2040 ml,  $p=0.151$ ), but this difference was not statistically significant. Blood drainage volume during the first 24 hours after surgery tended to be lower with the liberal versus restrictive strategy (880 mL vs. 1136 mL,  $p=0.068$ ). Increases in plasma fibrinogen during the first hour after weaning of cardiopulmonary bypass were significantly higher in patients who received cryoprecipitate compared to FFP (0.36 g/L vs. 0.02 g/L,  $p=0.005$ ). The intraoperative plasma fibrinogen nadir tended to be inversely correlated with blood drainage volume during the first 24 hours after surgery, especially in the liberal strategy group ( $r = -0.668$ ).

**Conclusion:** Liberal transfusion of PC and cryoprecipitate may reduce red cell transfusions and bleeding. Further large-scale studies are necessary, possibly with more fibrinogen (up to 8 g) and a higher plasma fibrinogen concentration to trigger fibrinogen transfusion, as recently suggested (Anesthesiology 2013;118:40-50).

## P193. Assessment of variables affecting blood product use and alloantibody formation after haematopoietic stem cell transplantation - A single centre audit.

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**Aim:** To assess variables that may affect or predict blood product usage and alloantibody formation following haematopoietic stem cell transplantation in patients with haematological disorders. The impact of providing rhesus incompatible blood (prior to engraftment) on alloantibody formation will also be examined.

**Method:** Data has been collected from 52 patients to date, who were treated with allogeneic haematopoietic stem cell transplantation from 2011-2013 at The Alfred Hospital. Variables examined include age, sex, underlying disease, conditioning regime, co-morbidities according to the HCT-CI (Sorrer *et al*, Blood 2005 106:2912), and infective complications. Transplantation was undertaken for acute myeloid and lymphoid leukaemia, myelodysplasia, lymphoma, plasma cell myeloma, aplastic anaemia and one patient with blastic plasmacytoid dendritic cell neoplasm. Blood product use (red cells, platelets, FFP, IVIg and albumin) was recorded for each patient for 12 months, or until death, following the initial infusion of haematopoietic stem cells. Patients lost to follow up before 12 months elapsed were excluded.

**Result:** Data collected to date is shown below, and data collection is ongoing with view to auditing over 200 patients in total, including a cohort of autologous transplant patients. Patients undergoing myeloablative and cord transplants used the highest amount of blood products, while those undergoing non-myeloblastic conditioning used the least, with three patients not requiring blood products at all. Only one patient developed an alloantibody.

	Patients		Rhesus & antibodies		Blood product use (average per patient)					Estimated cost
	Total pts	Alive 12 mo (%)	Rh mis (%)	Allo-ab (%)	RBC unit	Plt pool	FFP bag	IVIg gram	Alb gram	Total blood products (av. per pt)
<b>MA</b>	26	19 (73)	12 (46)	1 (3)	548 (22)	507 (20)	39 (2)	160 (6)	1560 (60)	\$397,292 (\$15,280)
<b>RIC</b>	13	10 (77)	5 (38)	0 (0)	214 (16)	99 (8)	5 (<1)	102 (8)	400 (31)	\$118,438 (\$9,111)
<b>NMA</b>	7	4 (57)	4 (57)	0 (0)	47 (7)	12 (2)	0 (0)	0 (0)	220 (31)	\$21,219 (\$3,031)
<b>DUCBT</b>	6	3 (50)	3 (50)	0 (0)	128 (21)	143 (24)	2 (<1)	1207 (201)	420 (70)	\$172,923 (\$28,820)

MA = myeloablative conditioning; RIC = reduced intensity conditioning; NMA = non-myeloablative conditioning; DUCBT = double unit cord blood transplant; Pt(s) = patient(s); mo = months; Rh mis = Rhesus mismatch; Allo-ab = new alloantibody; RBC = red blood cell; Plt = platelet; FFP = fresh frozen plasma; IVIg = intravenous immunoglobulin; Alb = albumin; av = average.

**Conclusion:** Considerable financial cost is associated with essential blood product support to patients undergoing haematopoietic stem cell transplantation. The provision of rhesus incompatible blood was not associated with an increased rate of alloantibody formation in this population. These data are potentially relevant from a health economics perspective.

## **P195. A flow cytometric method to determine red cell levels in platelet components prepared for transfusion.**

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### **Background**

Platelet transfusions are required for patients to arrest or prevent bleeding. Ideally, a unit of platelets should contain less than  $3 \times 10^9$  red cells as high numbers can elicit an immune response to blood group antigens that the recipient lacks. Improved blood component preparation has resulted in platelet products where the number of residual cells is below the linear range of haematology analysers. The purpose of this study was to develop a flow cytometric method to count the low levels of red cells present in platelet concentrates manufactured at the New Zealand Blood Service Waikato processing facility.

### **Methods**

EDTA samples obtained from donors were diluted in group AB plasma. Diluted RBCs were stained with CD235a in Trucount tubes using a no lyse/no wash method and were analysed by flow cytometry (BD FACS-Calibur). Sample aliquots from 239 units of platelets prepared at the Waikato NZBS Blood Processing site were tested for levels of RBC contamination using the same method.

### **Results**

The flow cytometric method showed close correlation between the results obtained from the haematology Sysmex XE 2000 and a comparative flow cytometric method established by Canterbury Health Laboratories. Both the apheresis and the pooled platelet units showed sufficiently high levels of red cell contamination to elicit a possible immune response. Apheresis platelets showed a mean volume of 0.05mLs of RBCs/unit and pooled platelets showed a mean volume of 0.13mLs of RBCs/unit.

### **Conclusion**

The flow cytometric method showed good sensitivity to low levels of RBCs in platelet preparations. Further work needs to be done to validate the method and evaluate its performance against commercial controls before its implementation as a routine test.

## **P196. The trials and tribulations of acquired anti-JMH antibodies**

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### **Background**

Patients with antibodies to high frequency antigens are technically difficult to resolve in the laboratory and provide challenges in sourcing suitable blood.

### **Case report**

An 83-year-old man presented for work up prior to endoluminal aortic aneurysm repair. A pan-reactive antibody with a weakly positive direct antiglobulin test (DAT) was found. Initial investigations included an extended antigen phenotype to detect a null phenotype and additional panel cells negative for high frequency antigens. All panel cells tested were positive. The procedure was cancelled as compatible blood could not be provided. Further testing performed by the Australian Red Cross Blood Service defined the antibody as anti-JMH. Laboratory testing proved difficult as no suitable cells were available to exclude the presence of additional underlying alloantibodies. Papain and dithiothreitol panels were set up as both cleave JM H antigen and cover all significant antigens. No additional underlying alloantibodies were detected.

The JM H antigen (JM H1) is an 80kd glycoprotein bound by a glycosylphosphatidylinositol (GPI) linkage. The glycoprotein is semaphorin 7A, a signalling protein with a role in T-cell regulation and neural functions. Many cases of JM H:-1 are acquired and found in the elderly. Often, as in our case, JM H is not totally lost and the red cells give a weakly positive DAT.

Anti-JM H antibodies are not usually considered clinically significant, but the literature is limited. No suitable donors were available from the Blood Service. A patient blood management plan was instituted. Theatre logistical problems caused surgery to be delayed twice, further complicating provision of suitable blood. Rh,K and Fy phenotype-matched cells were sourced to minimise the chance of alloimmunisation. If transfusion is needed careful monitoring for signs of haemolysis will be required.

### **Conclusion**

Comprehensive blood management plans and good communication between clinical and transfusion medicine teams are needed to manage patients with antibodies to high frequency antigens such as anti-JM H.

## P197. Where blood goes: Fresh product use in WA public metropolitan hospitals

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**Aim:** Appreciating patterns of blood product use is important in understanding transfusion practice and assists in supply planning to ensure product is available to meet clinical need. We aimed to examine fresh blood component utilisation in WA metropolitan public hospitals (MPHs) over a four year period, from 2010 to 2013.

**Method:** WA fresh blood product issuance data and fresh blood product transfusion data covering admissions discharged from WA MPHs were examined by hospital type, admission type, major diagnostic category (MDC) and diagnostic related group (DRG).

### Results:

	Issuance		Transfusion									
	WA units*	WA MPH units*	All MPH unit s*	WA TI**	Non- Elective Surgical unit s*	TI**	Non- Elective Medical unit s*	TI**	Elective Surgical unit s*	TI**	Elective Medical unit s*	TI**
<b>Red</b>												
Total	5878	311	282	0.0	500	0.0	311	0.0	852	0.0	895	0.3
change	-12%	-	-	-	-	-	-	-	-	-	-	-
<b>Platelet</b>												
Total	9731	752	645	0.0	189	0.0	874	0.0	216	0.0	123	0.0
change	7%	4%	-2%	-	2%	-	1%	-	-	-	-	-
<b>FFP<sup>†</sup></b>												
Total	8638	662	858	0.0	799	0.0	675	0.0	236	0.0	412	0.1
change	-28%	-	-	-	-	-	-	-	-	-	-	-
<b>Cryo<sup>‡</sup></b>												
Total	1196	103	770	0.0	606	0.0	135	0.0	172	0.0	342	0.1
change	68%	76%	79%	65	256	236	58	39	71	57	65	50

\* Paediatric units counted as: 0.25 units in issuance data, 1 unit in transfusion data; \*\* TI = Transfusion index (mean number of units transfused per hospital discharge); <sup>†</sup> FFP = Fresh frozen plasma; <sup>‡</sup> Cryo = Cryoprecipitate

WA MPHs received the majority of all fresh product issued in WA in 2013. Transfusion data for all five tertiary sites and five of the six general hospitals was included. In 2013, discharges for hematological disorders accounted for the largest group of red cell use in MPHs (16%) at a rate of 0.542 units per discharge (17% decrease from 2010). Other large users of red cells were discharges related to the digestive system (14%) and neoplastic disorders (13%), which also showed decreases in TI of 10% and 14% respectively. Neoplastic discharges accounted for 32% of platelets transfused in 2013 and haematological discharges received 17%. The TI remained constant for neoplastic discharges but showed a steady decrease in haematological cases (30% from 2010-13). Elective medical discharges accounted for 48% of FFP and 45% of cryoprecipitate used. Non-elective major procedures

associated with any diagnostic group received 19% of platelets, 34% of FFP and 26% of cryoprecipitate.

**Conclusion:** From 2010 to 2013, overall both total units and average units transfused per discharge for red cells, platelets and FFP in WA MPHs decreased, while use of cryoprecipitate increased. Changes in patterns of blood component use may be attributable to changing transfusion practice in both medical and surgical areas, potentially including uptake of technologies such as ROTEM.

## **P198. Prevalence study of iron deficiency in a tertiary hospital advanced heart failure clinic**

**P'ng S**, Pavey W, Shah A, Chamberlain J, Tonkin C

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### **Aim**

There is increasing awareness of the functional improvement associated with iron deficiency even in the absence of anaemia in patients with heart failure. We undertook a study to evaluate the prevalence of iron deficiency particularly functional iron deficiency in an advanced heart failure population.

### **Methods**

Outpatient attendees at a tertiary hospital advanced heart failure clinic within a 6 month period between July 2013-Dec 2013\*\* were reviewed. Iron deficiency was defined as ferritin <30ug/L and functional iron deficiency was defined as ferritin <300ug/L with Transferrin saturations <20%. Anaemia was defined as Hb<120g/L in females and Hb <130g/L in males.

### **Results**

61 new patients were identified of which 14 (23%) had anaemia at first presentation and 3 developed anaemia with follow up. 2 (3%) had iron deficiency (1 also anaemic) and 27 (44%) functional iron deficiency. Data is pending for the NYHA classification and BNP for these patients. 100 follow up patients were identified; 2 patients had no results available at time of analysis. Of those available 45 had anaemia, 15 (15%) iron deficiency (4 also anaemic) and 73 (73%) functional iron deficiency. 25 patients did not have iron studies performed. Analysis is still pending for the number of patients who had iron administered and the number of patients who were on oral medication known to interact with absorption of oral iron was.

### **Conclusion**

There is a significant population of outpatient attendees who have functional iron deficiency and who may have benefit to their overall functional status with IV iron administration.

## **P199. Blood management in an elective orthopaedic population: Further improvement in low state transfusion rates**

**P'ng S**, Pavey W, Kuster M, Chamberlain J, Reay P, Trentino K

*Royal Perth Hospital, Perth, WA, Australia*

### **Aim**

Patient blood management programs (PBM) in surgical populations aims to optimise preoperative haemoglobin, minimize intraoperative blood loss and increase tolerance of postoperative anaemia. We would like to report on our PBM program in orthopaedic patients during an 8 month period focusing on oral iron management.

### **Method**

Patient data sourced from hospital and laboratory databases was collected on all patients scheduled for joint replacement (primary, redo and staged) surgery at a tertiary facility between July 2013-February 2014. Oral iron therapy was used if scheduled surgery was > 30 days and IV iron therapy if surgery was within 30 days of assessment. Iron deficiency was defined as ferritin <30ug/L (<20ug/L premenopausal female) with functional iron deficiency defined as ferritin <100ug/L with transferrin saturations <20%.

### **Result**

Of the 404 patients assessed, 31 (13.5 %) females and 37 (21 %) males were anaemic and 13(5.7 %) females and 6 (3.4%) males were iron deficient. 332 (82%) patients had their surgical procedure completed of which 19 (10%) females and 29 (20.3%) males were anaemic and 18(7.9%) females and 10(5.7%) males were iron deficient. 80 patients had functional iron deficiency. IV iron was administered in 29 patients compared with oral iron in 17 patients. During the period of intervention, the transfusion rate in the primary elective joint replacement population was 8.9% compared with 12.75% in the 12 month preceding period. Total length of stay was significantly reduced in patients not transfused in all patient groups compared with those transfused.

### **Conclusion**

An effective PBM program can help decrease the transfusion rates and length of stay in the elective joint replacement population. Oral iron therapy is effective in optimizing patients if timely preoperative administration is achieved.

## **P200. Infections are an uncommon cause of admission in an Australian haemoglobinopathy cohort**

**Premawardena C**<sup>2</sup>, Bowden D<sup>1,2</sup>, Kaplan Z<sup>1,2</sup>, McQuilten Z<sup>1,2</sup>, Dendle C<sup>1,2</sup>, Woolley I<sup>1,2</sup>

<sup>1</sup> *Monash Health, VIC, Australia* <sup>2</sup> *Monash University, VIC, Australia*

### **Aim**

Infection is a commonly reported cause of morbidity and mortality in haemoglobinopathy populations in international studies. Less is known about the impact of infection in these patients in Australia. We examined the causes of hospital admission in a large Victorian cohort of haemoglobinopathy patients at Monash Health using an administrative dataset.

### **Method**

A retrospective analysis of an administrative dataset listing hospital admissions was conducted. The dataset contained information on all hospital admissions at Monash Health affiliated sites between June 1998 and June 2014 for any patient recorded to have a haemoglobinopathy. The information was analysed using the Australian refined diagnosis-related groups (AR-DRGs) and International Classification of Diseases, 10<sup>th</sup> revision (ICD-10) coding recorded for each admission.

### **Result**

There were 42309 admissions recorded for 807 patients, 278 (34.4%) of whom were male. Of these admissions, 40910 were for blood transfusions which were recorded in 282 patients (mean = 145, range 1 to 605). Out of the remaining 1399 admissions, 48 (3.4%) had an infection-related primary (AR-DRG) code. An additional 189 (13.5%) admissions had at least one infection-related secondary (ICD-10) code. For the 1351 admissions not related primarily to transfusion or infection, there were 294(21.8%) presentations to emergency. The other common causes of admission related to obstetrics (255, 18.9%), general medicine (195, 14.4%), haematology (178, 13.1%) and surgery (118, 8.7%).

### **Conclusion**

Haemoglobinopathy patients are known to be predisposed to infection. However, it is an uncommon reason for hospital admission in this population in Victoria. Most patients with an infection acquire it secondarily during other admissions.

## **P202. To evaluate the incidence of inhibitor development and their management in relation to type of blood products / components used to treat patients born after 2000 in paediatric hemophilia clinic in Lady Ridgeway Children's Hospital**

**Ramanayake R**

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### **Introduction**

Hemophilia is one of the oldest diseases known to mankind. According to factor activity levels, hemophilia is classified as: severe (<1%), moderate (1-5%) or mild (6-25%). The treatment of hemophilia is based on the replacement of the missing clotting factors when bleeding occurs (on-demand treatment) or is made on a regular and continuous way regularly (prophylactic treatment). One of the treatment related complications is the development of inhibitors. Immune tolerance induction (ITI) regimens are the one of available methods to reduce the levels of alloantibodies against factors VIII and IX.

### **General Objective**

The purpose of this study is to evaluate the incidence of inhibitor development and their management in relation to type of blood products / components used to treat patients born after 2000 in paediatric hemophilia clinic in Lady Ridgeway Children's Hospital.

### **Study Design and Duration of Study**

A cross sectional hospital based study and Five months from 01.08.2011 to -31.12.2011. Sample consist of haemophilia patients who take prophylactic treatment Patients' records are reviewed to collect data to evaluate the type of product ,the number of exposure days and number of units of transfusion(IU/kg/year). To assess the inhibitor status, patient's records will be checked.

### **Results**

A total of 42 male paediatric patients were studied with 35 HA,06 HB and 01 FXIII deficiency. Only one patient had taken locally prepared cryoprecipitate and 15 patients had taken purified FVIII/FIX concentration alone and 26 had taken both locally prepared products and Purified or recombinant products. The mean prophylactic Factor VIII usage with cryoprecipitate in each haemophilia A varied from 267.84IU/kg/year(SD 33.57+\_2SD, CV-14.4% ) to 1784.6 IU /kg/ year (SD 707.17+\_2SD,CV-45.7).The mean Factor IX concentrate usage with FFP in haemophilia B varied from 234.15IU/kg/year(SD-200.14+\_2SD,CV-32.7%) to 4688.12IU/kg/year(SD-1483.09+\_2SD,CV-.44.9%). Nine of them had developed inhibitors to factor VIII and five of them were undergone ITI successfully.

## **P203. First case of Haemolytic disease of new born baby due to Anti- Mur in Sri Lanka - A case report**

**Ramanayake R<sup>1</sup>, Alawala A<sup>2</sup>, Rupasinghe R<sup>3</sup>**

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**Introduction :** Anti – Mi a is one of the most common alloantibody of potential clinical significance in some Asian populations . This case describes the first case of haemolytic disease of the newborn due to anti-Mur in Sri Lanka .

**Case Report:** A 23 year old lady who had a history of one previous abortion , came to maternity unit with a POA of 34 weeks . Patient's history did not include any significant medical disease or transfusions with no any other pregnancy related problems.. At 37 weeks , she was undergone emergency cesarean section due to fetal distress and she delivered a baby girl with body weight of 2.84 kg . On the first day, the infant had a haemoglobin of 4.1 g dl and total bilirubin of 3.3 ng/dl. Peripheral blood morphology revealed spherocytosis, polychromasia nucleated red blood cells and fragmented red blood cells.

**Materials and methods :** Standard techniques as defined by in the national guidelines ( BCSH ) were employed.

**Results :** Both the mother and the patient were Group 0, RhD+, and hence HDN due to ABO and Rhesus D incompatibility was excluded. The antigen typings in the main blood group systems of the father showed that the father was of the GP. Mur phenotype. The direct antiglobulin test of the patient was moderately strongly positive (3+) with polyspecific AHG and anti-IgG. Maternal serum contained anti-Mur only. Two RBC transfusion were done and on day 07, baby was discharged.

**Discussion:** The severity of the HDN was moderate and development of severe neonatal anaemia. The results of the immunological investigation clearly indicated the haemolytic potential of the anti-Mi in maternal serum .Previous pregnancy was probably responsible for the primary immunization, while the patient in this case was probably responsible for the secondary stimulus which caused the HDN.

## **P204. Rapid uptake of patient blood management guidelines**

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*National Blood Authority, Canberra, ACT, Australia*

### **Aim**

The PBM Guidelines Implementation strategy has been developed by the National Blood Authority to support Jurisdictions and health providers implement the PBM Guidelines.

### **Method**

The national strategy uses a multifaceted, collaborative approach by drawing on high quality materials and expertise across Australia to identify, promote and support rapid uptake of the guidelines. The mandatory accreditation requirements for Blood and Blood products in the National Safety and Quality Health Service (NSQHS) Standard 7 have supported PBM guideline uptake and increased the demand for tools, education, training, data and information resources.

### **Activities under the national strategy are grouped into four main elements:**

**PBM Tools.** The identification, development and promotion of a national reference set of best practice tools to support PBM Guideline implementation.

**Education and Training.** The establishment of a supporting education and training framework.

**Promotion/Communication.** A series of targeted promotional campaigns and materials to raise awareness and motivate health provider, clinician and patient interest.

**Data.** The development of key data sets to support the implementation of PBM.

### **Results**

Jurisdictional and local health service implementation of the PBM guidelines across Australia, supported by the requirements of NSQHS Standard 7, has been pervasive and rapid. This uptake has led to a substantial reduction in red blood cell use.

### **Conclusion**

The operational and cultural change required to implement best practice clinical measures at a health provider level are significant and sometimes require complex changes in business process and clinical practice. A government facilitated multifaceted strategy has been shown to support rapid uptake of Australian evidence-based PBM Guidelines.

## **P205. False positive DATs in a red cell reagent panel**

**Ryan C**

*New Zealand Blood Service, Wellington, New Zealand*

Recently, the in-house red cell panel at Wellington Blood Bank unexpectedly returned a series of strongly positive DATs. Initially only one cell in the panel repeatedly returned a positive DAT, but within one week, three cells reacted in the same way and the panel was therefore unable to be used. However, after a replacement panel was received, the same cells again returned positive DATs. An elution on the positive cells revealed that they were contaminated with anti-D. Unlike commercial panels, our in-house panel uses 4mL tubes that are longer than the length of the pipette tip. As this panel is regularly used for quality control, the cells in question often have lower volumes, therefore the pipette must reach deeply into the tube. As this can also be the case with sample tubes, there is consequently a risk that the barrel of the pipette may come into contact with the side of either of these tubes, thereby transferring material and causing contamination. An examination of the dates that the positive DATs were discovered revealed that they coincided with the dates that samples arrived from a regular patient, Mrs W. Mrs W has an extremely high anti-D titre that has caused several incidences of contamination of other samples on the AutoVue analyser. She has an anti-D titre of over 65,000, around 4,000 times stronger than a 'significant' titre. Considering the high titre in Mrs W's sample, it is clear that a minute amount of her potent plasma contaminated the panel cells via contact with the barrel of the pipette. As a result of this we now routinely wipe the pipette barrel with an alcohol wipe before use.

## **P206. What happens after a Transfusion Reaction?**

**Sadani D**

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### **Aim:**

Transfusion of blood components to patients in hospital results in a small number of patients experiencing Transfusion Reaction (TR). These are in most cases managed appropriately with the blood services being informed who report these to the Haemovigilance system. However, there is paucity of data on the use of blood components in this group of patients.

A retrospective study was conducted in a Tertiary Referral Hospital to ask the following questions:

Do patients get transfused another component or because they experienced a TR, no further products are requested.

Even if alternative products are subsequently reordered, how much of a delay does this lead to?

How many of these patients experience repeat TRs?

Which patients particularly are at risk of experiencing a TR?

Which products do patients commonly react to?

### **Method:**

A retrospective study of over 225 patients was conducted who had a reported TR over a two year period from 2011 – 2013. Data from Haemovigilance reports was analysed to identify these patients. Transfusion records of these patients were accessed to record if more blood components were issued and the time delay in issuing these components was also analysed.

### **Result:**

A significant number of patients who had TR's experienced significant delays in getting secondary component transfused. A more detailed analysis will be presented as a poster.

### **Conclusion:**

TRs lead to significant delays in procuring much needed components to meet the primary transfusion requirements of patients in hospitals. In a proportion of cases they end up with no further components being either requested or transfused. This raises the question if the primary component was ordered appropriately and was clinically indicated in the first place.

## **P207. Transient anti-LW in malignancy : Laboratory nightmare**

**Sharma A, Davis R, Gunson H, Tegg L, Murphy N**

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### **Introduction**

The LW blood group system is phenotypically related to the Rh system. In 1982 the LW blood group system was resolved into a three antigen system –LW<sup>a</sup>, LW<sup>b</sup> and LW<sup>ab</sup>. LW antigen has strong expression on both Rh D+ and Rh D- cord cells in contrast, adult Rh D+ cells react strongly than adult Rh D- cells. An anti-LW is easily mistaken for anti D unless red blood cells are treated with dithiothreitol (it denatures LW antigens but not Rh) and/or tested with red cells from umbilical cord blood. LW<sup>a</sup> is a high frequency antigen and LW<sup>a</sup>- phenotypes are rare. A transient form of LW<sup>a</sup>- with associated anti-LW, known as acquired LW- has been described in patients with malignancies.

### **Case description**

A 72 year old woman presented at midnight with recent diagnosis of multiple myeloma for urgent transfusion due to associated symptomatic anaemia.

### **Results**

Blood group – B Rh D+

Rh phenotype – CCDee

Kell – negative

Antibody screen – positive

Red cell panel – Suggestive of pattern in keeping with an anti-D

DAT – negative

Autocontrol - negative

Reaction of patient plasma against ORh D+ cord cells - positive

Reaction of patient plasma against ORh D- cord cells - positive

Reaction of patient plasma against ORh D+ adult cells - positive

Reaction of patient plasma against ORh D- adult cells - negative

### **Interpretation**

Consistent with an anti LW.

Manual crossmatch with two O Rh D- units was compatible and were transfused without incident.

Follow up 2 months later showed a negative antibody screen.

### **Conclusion**

This case demonstrates identification of a transient anti-LW likely associated with multiple myeloma and the potential laboratory difficulties in distinguishing between anti-D and anti-LW using standard antibody identification methods.

## P208. Optimisation of protocols to fluorescently label ovine red blood cells for characterisation of post-transfusion survival

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Critical Care Research Group, The Prince Charles Hospital, Chermside, QLD, Australia

### Background and Aim:

The sheep has been established as an important model animal in which the effects of blood transfusion can be studied. To characterise the post-transfusion survival of sheep red blood cells (RBCs), an *ex vivo* method of labelling RBCs to provide rapid and sensitive quantification of these cells post-transfusion is required. The aim of this study was to compare two methods of labelling sheep RBCs: biotin-streptavidin (Thermo Scientific; Mock *et al.* Transfusion 2012; 52:963-973) and KODE Function-Spacer-Lipid (FSL) constructs (KODE Biotech Materials; Oliver *et al.* Transfusion 2011; 51:1723-1730).

### Methods:

PRBC units (n=8) were prepared from sheep whole blood collected into standard Fresenius blood packs. Leucodepleted PRBC units were labelled with either sulfosuccinimidobiotin (sulfo-NHS-biotin; n=4) or KODE FSL-fluorescein (FLRO4; n=4). As an additional step, dichlorotriazinylaminofluorescein (DTAF)-streptavidin (5µg/mL) was required to detect sulfo-NHS-biotin labelled RBC. The fluorescence intensity of labelled RBCs was compared to matched unlabelled RBCs by flow cytometry. FSL-FITC was titrated to determine the lowest level required to provide discrimination for *in vivo* studies.

### Results:

Labelling ovine RBCs with 12µg/mL of sulfo-NHS-biotin and detection with DTAF-streptavidin, did not result in a clear separation of the labelled RBC population from the unlabelled RBC population (Table 1). Increasing the concentration of sulfo-NHS-biotin to 50µg/mL resulted in a clearly defined labelled RBC population (Table 1). As an alternative, labelling with FSL-FITC gave a superior separation between labelled and unlabelled ovine RBCs (Table 1). Titration of the FSL-FITC demonstrated that 10µg/mL was the minimal concentration required for clear discrimination between labelled and unlabelled ovine RBCs (Table 1).

Table 1: Median fluorescence intensity of labelled RBCs

	Biotin-streptavidin-DTAF labelled RBCs (biotin conc. µg/mL)					
	0	12	50			
Mean (SEM)	21 (2)	236 (26)	1,159 (111)			
	KODE FSL-FITC labelled RBCs (FSL-FITC conc. µg/mL)					
	0	1	5	10	25	50
Mean (SEM)	35 (12)	103 (12)	488 (209)	1,433 (385)	4,687 (961)	11,902 (1499)

### Conclusions:

Labelling sheep RBCs with FSL-FITC was the superior method based upon efficacy (~6-10 fold higher MFI vs. similar concentrations of sulfo-NHS-biotin) and ease of use (no secondary labelling required). These data demonstrate that labelling with 10µg/mL FSL-FITC provides a basis for quantification of the *in vivo* post-transfusion survival of RBC in sheep transfusion models.

## **P209. Understanding the “tour of duty” of red cells issued to South Australian Transfusion laboratories**

**Sinha R**, Ireland S, Tocchetti R

*Blood Organ and Tissue Programs, Department for Health and Ageing, SA, Australia*

### **Aim**

An investigative exercise was undertaken using public sector Laboratory Information Systems and the National Blood Authority's BloodNet databases to understand the fate of red cells (RBC) units issued from the Blood Service to South Australian (SA) transfusion laboratories.

### **Method**

All RBC units issued to SA laboratories between 1 July 2012 and 30 June 2013 were linked to various databases to facilitate tracking of units for transfusion/discard fate and the transfer activity between laboratories.

### **Result**

During the study period, 66759 RBC (31% CMV Neg and 19% irradiated) units were issued to SA laboratories with 74.8% or 49969 of the total RBC units issued to 17 public and 16790 units issued to 5 private laboratories. Of the 66759, a total of 49353 RBC units were issued from public laboratories for transfusion, 2377 RBC units (3.6%) were discarded and 9 RBC units were utilised for research. The remaining 15020 non-linkable RBC units were presumed to be issued from the private laboratories for transfusion. 5213 (7.8%) units were transferred between laboratories (private to public, country to metro etc.) and 355 (6.8%) were discarded. To avoid expiry, the majority (4914) of these units were transferred to larger metropolitan public laboratories from regional, private or specialty services (obstetric and paediatric). Of these transferred RBC units, 94% (4600) units were able to be transfused prior to expiry.

### **Conclusion**

Units issued by public laboratories, units transferred between laboratories and units discarded at laboratories were all exclusively traced. Transfers of near expiry units between laboratories can be monitored to better understand the contribution made to overall discard rates.

## **P210. Don't forget about G**

**Stern D**<sup>1</sup>, MacCallum S<sup>1,2</sup>, Shand A<sup>3</sup>

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### **Case**

LT, 33 year old G2.P0, was found to have anti-C+D on routine antenatal screening. She had never been transfused, but had a termination at 15 weeks gestation in 2001 without anti-D prophylaxis. Her phenotype was cde, probably genotype rr. Her partner was phenotyped as C-, c+, D+, E+, e+, probable genotype R2r. A sample sent to Australian Red Cross Blood Service (ARCBS) Sydney in Dec 2013 confirmed the presence of anti-G. The anti-D quantitation was 12.8IU/ml. A second sample tested by two stage absorption/elution procedure with Ror and r'r cells confirmed the presence of anti-C+D+G. Foetal DNA isolated from a maternal sample at ARCBS Brisbane genotyped as rr. The anti-D quantitation remained stable as expected and a baby born at 40 weeks was group A Rh(D) negative, cde (rr).

### **Discussion**

The G antigen was first reported in 1958 and has ISBT symbol RH12. Anti-G reacts with red cells expressing the D and/or C antigen. There must be a serine residue at position 103 of *RHD* or *RHCE* for reactivity to occur. Sera showing anti-C+D reactivity may in fact have anti-C+G, anti-D+G or anti-C+D+G specificity. Anti-G is known to cause Haemolytic Disease of the Foetus and Newborn (HDFN) but this is rare and is seldom severe. It has also been reported to cause haemolytic transfusion reactions, generally delayed. When considering anti-G in pregnancy, it is also important to prove the presence/absence of anti-D in such sera as, in the absence of anti-D, Rh(D) prophylaxis should occur.

### **Conclusion**

In the presence of anti-C+D in pregnancy anti-G should also be considered in order to assess the need for Rh(D) prophylaxis. If possible the foetal genotype should be used to establish the need for prophylaxis and to alleviate parental worry.

## **P211. Comparative observational audit of bedside checking procedures at Princess Margaret and King Edward Memorial Hospitals in Western Australia**

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### **Aim**

To determine compliance with ANZSBT Guidelines for the Administration of Blood Products and Hospital Transfusion Medicine Protocols, regarding procedures for bedside checking and administration of blood products at two hospitals specialising in Women's and Children's healthcare.

### **Methods**

An observational audit was performed on clinical staff undertaking the bedside checking procedure and administration of blood products at the two sites. The audit included a sample of patients who received a blood product between May 2013 and February 2014. Practices observed included: checking validity of patient consent, prescription, visual check of blood product; verification of positive patient identification with patient and blood product label, time to start of infusion; and correct administration procedures. The presence of appropriate documentation including date and time, checking signatures and observations was also included. The observed practices were assessed against the ANZSBT Administration Guidelines and the Hospital Transfusion Medicine Protocols.

### **Results**

Sixty two transfusion episodes were audited. The results showed excellent compliance with validity of prescription (PMH 100%, KEMH 100%), documentation (PMH 98%, KEMH 100%) and administration procedures (PMH 98%, KEMH 100%). However the audit highlighted some key areas where expected practice was not observed. These included checking of consent (PMH 88%, KEMH 78%) and performance of verbal positive patient identification (PMH 55%, KEMH 69%). Although all audited staff cross checked the blood product and identification band with the prescription, they did not always verbally confirm the name and date of birth with the patient, as mandated by National Guidelines and organisational protocols.

### **Conclusion**

This observational audit demonstrates that transfusion practices at both sites were consistent with blood product administration standards. However key areas of improvement included the essential verbal positive patient identification step. Interventions to promote improved practice, including feedback and education on positive patient identification have commenced and we will reaudit in 2015.

## **P212. Development and implementation of a state-wide platelet wastage minimisation strategy based upon transfusion service laboratory redistribution of near expiry product**

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Platelet wastage is almost exclusively due to product expiry and is an increasing challenge to healthcare cost containment. Currently, platelet wastage rates in SA are 17% (approximate cost \$0.9 million). Minimisation of this wastage is difficult primarily because of the short product expiry times. The South Australian Platelet Wastage Minimisation project (BloodMove Platelet) plans to adopt similar measures as those implemented with the SA BloodMove country red cell program, in particular, product rotation to minimise platelet wastage due to expiry.

The key factors identified in the action plan of the project are

use of existing datasets (BloodNet & Laboratory Information System) and audits to understand non-urgent (haematology/oncology) and urgent usage of platelets at each hospital in SA and the number of discards at each hospital

development and implementation of a platelet inventory database (SA Platelet Net) to act as a real time dashboard at each site displaying the current available stocks of platelets throughout all transfusion laboratories.

validation of an alternate shipper that is simple and easy to pack and able to transport platelets across sites

examining alternate courier systems for faster, effective and secure transfer of platelets between sites

platelet ordering practices –collaboration with clinical areas and haematology testing laboratories to improve prompt daily elective platelet ordering practices, this being critical to improved platelet inventory management and prompt transfers between sites of Day 5 expiring platelets.

The above mentioned plan will form the basis of a multifaceted state-wide platelet wastage minimisation strategy involving transfer and utilisation of near expiry platelets across sites. This strategy is being implemented initially across the public transfusion services. Private pathology providers will be invited to participate.

## **P213. Audit of availability and use of Day 5 platelets in South Australian metropolitan transfusion laboratories**

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### **Background**

Platelet wastage is almost exclusively due to product expiry and is an increasing challenge to healthcare cost containment. The study aim was to evaluate the number of platelets expiring daily in South Australian (SA) metropolitan transfusion laboratories and the number of Day 5 platelets used and discarded.

### **Methods**

An audit on the availability and use of Day 5 platelets at each SA Pathology metropolitan hospital transfusion service laboratory was performed for a 3 week period. Six laboratories were asked to collect details of platelets expiring on each day in the morning (0700hr) and at night (2300hr). BloodNet data and Laboratory Information System data was utilised for platelet stock issues from the Blood Service, product discarded and patient transfusion details respectively.

Results: Six (2 larger, 2 medium and 2 smaller) laboratories participated in the audit. During the 3 week period, total number of Day 5 platelets available at all sites was 282 with 167 apheresis and 115 pooled platelets, with 37% CMV negative units. Of those, 198 (70%) platelet units were transfused and 84 (30%) platelets were discarded. The overall median number of Day 5 expiring platelets was 10 (Interquartile range [IQR] 2-15) including 7 (IQR 4-9) and 3 (IQR 2-4) for the two larger laboratories. The two smallest laboratories had a practice of platelet transfer to a larger site which included 23 (8%) near expiry units transferred during the audit period. Of those, 21 were transfused at the site transferred to i.e. 96% success in likelihood of transfusion

### **Conclusion**

With the current arrangement, 70% of Day 5 expiring platelets were transfused. This audit facilitated an understanding of laboratory practices around the usage and transfers of near expiring platelets - information useful in future wastage minimisation strategies.

## **P214. Use of IVIG (Intragam) to treat a severe case of fetal Haemolytic Disease of the Newborn**

**Traino T, Williams V**

*SA Pathology at the Womens and Childrens Hospital, SA, Australia*

### **Aim**

The aim of this study is to assess the effect of IVIG administered to a mother in the case of severe fetal HDN.

### **Method**

This study contains test details and investigations throughout three pregnancies in a mother (A.P) DOB 1/2/85. The main focus was during the third pregnancy where a severe HDN was identified by use of serial antibody titres (Anti-D), Anti-D quantification levels and use of MCA (Middle cerebral arterial) blood flow measurements.

The study includes fetal transfusion episodes and fetal test results which confirmed a severe HDN in the third pregnancy.

The Maternal-Fetal consultant (Womens and Childrens Hospital, North Adelaide, South Australia) decided after the first fetal transfusion that Intragam should be administered to the mother for the remainder of the pregnancy. The desired outcomes were:

1. To minimize the effect of the maternal anti-D against the fetal red cells.
2. To reduce the total numbers of fetal transfusion required for this pregnancy.

### **Result**

At the 27 week gestation stage of this pregnancy, it was observed that the last two fetal transfusions were required monthly. This was viewed as a desirable outcome as previous cases of severe HDNs have required fortnightly fetal transfusions. This study will continue to monitor the effect of maternal Intragam administration through to the end of this pregnancy.

### **Conclusion**

Maternal administration of IVIG should be considered in cases of severe fetal HDN.

This decision rests with the Maternal – Fetal consultant involved in the case of severe HDN.

The “Criteria for the clinical use of IVIG in Australia” booklet (Second edition, July 2012) lists the use of IVIG for cases of HDN as “Conditions for which IVIG use is in exceptional circumstances only”.

## **P215. Transfusion practice in gastrointestinal haemorrhage (GIH) patients requiring massive transfusion: Initial results from the Australian and New Zealand Massive Transfusion Registry (ANZ-MTR)**

Aoki N, Venardos K, Andrianopoulos N, McQuilten Z, Wood E

*Monash University, Melbourne, VIC, Australia*

### **Background/Aim:**

GIH is a common and important cause of morbidity & mortality. Recommendations on GIH management have been updated but questions regarding optimal transfusion practice remain.

The ANZ-MTR generates observational data on current management and outcomes in critical bleeding patients receiving massive transfusion (MT) across all clinical settings. Here we describe preliminary findings on the transfusion management of MT patients with severe upper and lower GIH.

### **Method**

All patients presenting with a GIH receiving a MT ( $\geq 5$  units of red blood cells [RBC] in 4h) were identified at 16 Australian & NZ hospitals. Patient data were extracted including transfusion history, laboratory results & hospital administration data.

### **Results**

A total of 317 GIH patients were identified, representing 13.9% of total MT events in the ANZ-MTR. Table 1 presents patient characteristics & outcomes. Within 24h from MT onset, patients received a median [IQR] of 7 [6-10] units of RBCs but 16%, 46% and 65% patients did not receive fresh frozen plasma (FFP), platelets or cryoprecipitate, respectively. Of those patients who received FFP and/or platelets, 40% received a FFP:RBC ratio  $\leq 1:2$  and 42% received a Platelets:RBC ratio  $\leq 1:4$ . Prothrombinex-HT was administered to 42 (13%) patients. Of the 212 (67%) patients with an aPTT or INR result available both prior to MT onset and 24h post-MT, 70 (33%) were coagulopathic (INR $>1.5$  or aPTT $>60$ s) before the MT and 78 (37%) were coagulopathic within 24h post-MT onset. Mean [SD] haemoglobin level 6-12h post-MT onset was 95.3g/L [18.4]. In-hospital mortality was 27% (versus total ANZ-MTR 21%).

### **Conclusion**

Patients with GIH accounted for a substantial proportion of cases in the MTR. They had high comorbidity rates and worse outcomes than other patients. With an ageing population and greater use of a range of anticoagulant drugs, better understanding of the management and outcomes of patients with GIH is needed.

Total GIH cohort; n	317
Male; n (%)	220 (69.4)
Median age [IQR]	66 [53-81]
Comorbidity present; n (%)	257 (81.1)
Median hospital LOS, days [IQR]	9 [5-19]
ICU admission; n (%)	218 (68.8)
Median ventilation time, hours; [IQR]	41 [19-97]
In-hospital mortality; n (%)	86 (27.1)

## **P216. Postpartum haemorrhage: Can we improve our transfusion support?**

Maclachlan K, Hardikar R, **Vilcassim S**, Rushford K, Wallace E, Wood E

*Monash Health, VIC, Australia*

### **Background**

Postpartum haemorrhage (PPH) is a major cause of obstetric morbidity & mortality. Upcoming national patient blood management (PBM) guidelines will address obstetric issues.

### **Aim**

Review institutional PPH transfusion practice, as a basis for practice improvement and guideline implementation.

### **Method**

Retrospective audit of patients coded with both PPH and transfusion on discharge during 2013, using clinical & laboratory records from a large metropolitan teaching hospital. Results were referenced to published guidelines<sup>(1,2)</sup> and to our own massive transfusion protocol. Review included risk factors<sup>(2,3)</sup> (RF), estimated blood loss (EBL), haemoglobin (Hb) fall and nadir, coagulation testing and products transfused.

### **Result**

In 192 episodes of PPH median EBL was 1500mL (range 300- >9000mL). Correlation between EBL and Hb fall was very poor ( $R=0.14$ ). Where data were available, 182/187 patients (97%) had  $\geq 1$  RF, and 62/187 (35%) had  $\geq 4$  RF. Of 125 patients with EBL >1000mL, 52% had no coagulation testing. Of 56 patients receiving  $\geq 4$  units RBC, 30% had no coagulation testing. All patients transfused received RBC, with 48% receiving  $\leq 2$  units. Only 13% received FFP, 12% platelets and 4% cryoprecipitate; 31 patients received  $\geq 4$  units RBC without associated non-RBC products.

### **Conclusion**

Most patients had PPH RFs. Published guidelines and hospital protocols were not followed in half of major PPHs. Clinical EBL is difficult – Hb & coagulation testing is indicated to guide transfusion decisions. Many patients transfused had only minor/moderate PPH. Few patients, even with large volume EBL, received recommended coagulation testing or non-RBC products. Our institutional transfusion practice around PPH could be improved. Baseline data will help monitor implementation of national obstetric PBM guidelines.

### **References**

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Prevention and management of PPH. 2011, RCOG Greentop Guideline No 52.

3. McLintock C. Obstetric haemorrhage. *J Thromb Haem*, 2011, 9:1441-51.

## **P217. After-hours transfusions from a satellite hospital refrigerator: Opportunities to improve clinical appropriateness and efficiency**

**Vilcassim S**, Rushford K, Michael C, Maclachlan K, Dunstan T, Wood E

*Monash Health, VIC, Australia*

### **Background and Aims**

Haemovigilance data show that storing blood in refrigerators outside the laboratory is associated with increased risks of storage and handling errors, and that after-hours transfusions are more likely to be associated with errors and transfusion reactions.

We conducted an audit at one campus of our large, multi-site, metropolitan hospital network to assess the effectiveness of:

Compliance with maintaining “cold chain” for blood components stored in the laboratory blood refrigerator, with non-laboratory staff access out of hours

Clinical appropriateness of ‘out of hours’ transfusion

### **Method**

Transfusions occurring between 1<sup>st</sup> August and 31<sup>st</sup> October 2013 were selected at random from the blood product register (n=30). Time of component departure from the central laboratory to receipt at the branch laboratory, time leaving the satellite refrigerator and commencement of transfusion, were retrieved. Medical records review was performed to assess clinical urgency according to national guidelines.

### **Results**

In 28 of 30 episodes, blood was transported to the peripheral laboratory within the validated transport time. For two episodes departure time was not recorded. In all cases, transfusion was commenced within 30 minutes of product leaving the blood fridge.

11 of 30 transfusion episodes were ‘out of hours’ (after 20:00h and before 08:00h). 3 of these required special couriers for delivery. Clinical contexts for after-hours transfusions were oncology (n=5) or elective (3) or emergency surgery (3). Only two transfusions (one oncology, one following elective surgery) were considered clinically urgent justifying after-hours transfusion, with the remainder due to delays in organising the transfusion.

### **Conclusion**

37% of blood components issued from this satellite refrigerator were transfused after-hours. Most of these transfusions could have been arranged earlier or deferred until the next day. Minimisation of out of hours transfusions will reduce clinical risks and courier costs. Cold chain documentation was not 100% compliant and could be improved.

## **P220. The New Zealand Blood Service expiry project**

**Williamson J**

*New Zealand Blood Service, Auckland, New Zealand*

### **Background**

The New Zealand Blood Service (NZBS) manages the collection, processing and distribution of blood and blood products throughout New Zealand. Six of the blood banks within New Zealand are run by the NZBS, the rest are under the control of their local District Health Board. Recent trends in blood transfusion have meant that the number of red cells transfused within New Zealand hospitals has declined; this in turn has led to a drop in revenue for the NZBS.

### **Aim**

The expiry project team led by Dr Peter Flanagan was formed in February 2013 to reduce the number of red cells being expired throughout New Zealand. A decrease in the number of red cells expired would result in financial savings for the organisation, and also decrease the wastage of a precious resource provided by volunteer donors. The aim was to decrease the expiry to below 3%

### **Method**

The NZBS has a vein to vein blood management system called eProgesa. A data warehouse has been developed by NZBS so that data from eProgesa and other systems can be extracted and analysed. The project team were able to use this information to set levels of red cells to be held at each of the NZBS blood banks. These blood banks were then given the task of deciding how to make these new levels work. The blood banks run by the District Health Boards had their red cell return policies modified to ensure that they no longer sent back short expiry blood that couldn't be used elsewhere.

### **Results**

In order to work within the red cell levels set by the project team, the culture within the blood banks had to change. At Auckland blood bank change was inevitable, the cool room is no longer filled up "just in case". Each morning, before blood orders are placed, stock reports are printed out and checked against the set stock levels. Reduced numbers of the minor blood groups are held; extra can be requested if needed for specific patients. O negative emergency units held at the blood bank and at satellite hospitals are changed regularly and monitored carefully so that there is an extended time period to use them once they are returned to stock. Twenty four "Group confirmed" O positive, O negative and A positive red cells are held at the Blood Processing department ensuring that in an emergency during out of hours shifts, there is a safety net for the blood bankers working.

Red cell expiry around the country is carefully monitored and those units with a short expiry can be moved to blood banks where they will be used.

### **Conclusion**

This has been an ongoing project which has been monitored using information from the data warehouse. The stock levels that were set have been modified when it has been shown that they have not been appropriate. When target levels are exceeded or abnormal numbers of units are expired, questions are asked ensuring that there is ongoing compliance. The target of 3% red cell expiry was achieved quickly and has been passed with the level dropping to 1.8% recently. The NZBS project team has achieved its goal and in the process of doing this has forced a change in the culture within the organisation which has led to a decrease in wastage of donated blood and financial savings for the organisation.

## **P221. Case study on a patient requiring a transfusion reaction investigation with a possible drug reaction to Ceftriaxone**

**Woolacott D**

*New Zealand Blood Service, Auckland, New Zealand*

### **Aim**

To ascertain whether a transfusion reaction had occurred or whether the patient had an immune drug related haemolytic anaemia.

The patient was reported as developing a fever with tachycardia during the transfusion. The drug being investigated was a third generation cephalosporin, ceftriaxone, administered at the same time or shortly before, the transfusion of a red cell unit.

### **Method**

A transfusion reaction investigation revealed a positive Direct Antiglobulin Test (DAT), IgG specificity, on pre and post transfusion samples. The pre transfusion samples resulted with a negative antibody screen, while the post transfusion samples displayed panagglutination with all cells tested. Negative results were obtained with elution studies. The following three investigations were performed; drug-dependent antibodies reacting in the presence of drug, drug-dependent antibodies reacting with drug-treated red blood cells, and cephalothin-dependent antibodies as per Judd (2008).

### **Result**

A drug dependent antibody to ceftriaxone was demonstrated in the patient's plasma.

Ceftriaxone was discontinued at the time of the transfusion related event.

### **Conclusion**

Second and third generation cephalosporins contribute to the majority of DIIHA, in particular cefotetan and ceftriaxone. Cefotetan as the cause of DIIHA, occurs more frequently than ceftriaxone, but, ceftriaxone has a higher association with fatalities.

Ceftriaxone can stimulate the production of antibodies. These antibodies bind with circulating ceftriaxone and form immune complexes. These immune complexes then bind with no specificity to other red cells and complement is activated. In DIIHA removal of the drug will stop haemolysis occurring. Previous exposure to ceftriaxone has usually occurred prior to DIIHA occurring, with ceftriaxone antibodies normally only detectable by testing sera in the presence of the drug. DIIHA can occur within a day or two of a patient being on medication.

## **P222. Novel mutation in exon 28 of VWF gene causing type 2M VWD**

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<sup>1</sup> *Mater Pathology, Mater Health Services, Brisbane, QLD, Australia* <sup>2</sup> *Institute of Clinical Pathology and Medical Research (ICPMR), Pathology West, Westmead Hospital, Sydney, NSW, Australia*

### **Case presentation:**

A 55 year old male presenting with recurrent episodes of haematuria and epistaxis and no structural causes had normal PT, APTT and platelet count. Von Willebrand screening showed supranormal concentration of FVIII:C, VWF:Ag, VWF:CB and severely reduced VWF:RCo (<0.05 U/mL). PFA-100 showed prolonged closure times for both cartridges Col/Epi and Col/ADP. Repeated testing some weeks apart showed similar results. Additional functional tests including VWF activity, based on a gain in function mutation of GP Ib/IX and not ristocetin (Innovance, Siemens) showed no activity. VWF:Multimers were normal. Platelet aggregation studies were normal for all agonists except for ristocetin at high concentration. Platelet glycoprotein analysis showed normal expression of glycoproteins Ib/IX and IIb/IIIa. Suspecting a type 2M vWD, exon 28 sequencing was requested and confirmed a homozygous missense variant in the VWF gene not previously described: transversion C→T in the nucleotide 3974 causing the amino acid substitution Ser 1325 Phe.

### **Discussion:**

We report a novel mutation causing VWD type 2M. Exon 28 encodes for the A1 loop of VWF that is responsible for the interaction with platelet GPIb and explains most type 2B and 2M VWD. There are 31 mutations reported in the database of the ISTH/University of Sheffield for type 2M VWD; 24 of them involving exon 28. None of these mutations have been reported in the residue 1325. There are reports of mutations involving the residue 1324 and gene expression studies that confirm mutations in this position cause type 2M vWD. Available free access online software that help to predict the functional effect of a single nucleotide substitution in the biological properties of peptides predict that this particular mutation we describe here is most likely deleterious (PROVEAN and Align GVGD).

## P223. Evaluation of sensitivity of various PT, APTT reagents for DOACS

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<sup>1</sup> Calvary Mater Hospital, Waratah, NSW, Australia<sup>2</sup> Pathology North, Sydney, NSW, Australia

### AIM:

Direct oral anticoagulants (DOACS) are an alternative to vitamin K antagonists for prevention as well as management of venous thromboembolism. Each anticoagulant varies in its own effect on the routine and special coagulation tests. Although it is acknowledged routine testing is unnecessary there are a few situations when testing is important such as in urgent surgery or bleeding. We aimed to test commonly used reagents and evaluate their sensitivity to DOACS .

### METHOD:

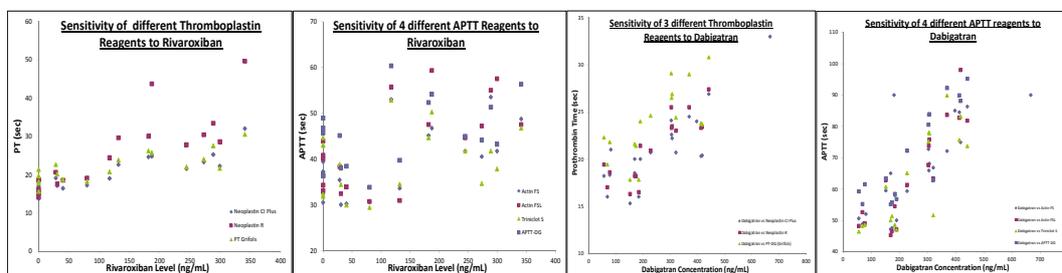
Ssamples received at HAPS on whom DOAC testing was requested were analysed. There were 19 samples for Dabigatran and 18 for Rivaroxaban. APTT reagents used include Actin FS, Actin FSL, Triniclot S, Grifols S and PT reagents include Neoplastin R, Neoplastin CL Plus and Grifols. The testing was done by mechanical clot detection on Stago Star. Dabigatran levels were tested by dilute thrombin time and rivaroxaban level by anti X a level read off rivaroxaban calibrator in our lab.

### RESULT:

All APTT reagents were sensitive to dabigatran even at lower drug concentrations. Grifols S was noted to be more sensitive than others. PT reagents were noted to have variable sensitivity and Grifols S was noted to be relatively more sensitive. In case of rivaroxaban, all PT reagents are sensitive with Neoplastine R being more sensitive at all drug concentrations. APTT reagents have shown variable sensitivity for rivaroxaban and Grifols S was again noted to be more sensitive

### CONCLUSION:

Routine coagulation assays are reagent and method dependent. With the increasing use of DOACS laboratories should be aware of their drug specific sensitivities to atleast routinely requested coagulation tests. Currently used reagents are noted to be sensitive , with Grifol S being the most sensitive APTT reagent. The PT reagents have variable sensitivity with Grifol S for Dabigatran and Neoplastine R for Rivaroxaban.



## **P224. Cross-sectional survey of management of isolated lower limb superficial vein thrombosis**

**Bennett A, Tran H, Chunilal S**

*Monash Health, Vic, Australia*

### **Aim**

Lower limb superficial vein thrombosis (SVT) is a common and important condition with no established 'gold standard' for management. This survey aimed to capture the range of therapeutic strategies used by Australasian clinicians in a non-clinical trial setting.

### **Methods**

Members of the Haematology Society of Australia and New Zealand (HSANZ) and the Australasian Society for Thrombosis and Haemostasis (ASTH) were invited to complete a 10 minute online survey on isolated lower limb SVT. Questions included information about the respondent (e.g. occupation, location), and information about their general management of SVT (e.g. use of ultrasound to confirm the diagnosis, concordance with the American College of Chest Physicians (ACCP) guidelines, and whether management was influenced by proximity to the saphenofemoral junction (SFJ)). Respondents were also asked how they would manage 3 case scenarios, each designed to highlight a specific issue around the management of lower limb SVT.

### **Results**

62 of the 64 valid responses received were from haematologists or haematology trainees. 59/64 (92%) of respondents confirm the diagnosis of SVT with ultrasound. 46/50 (92%) consider the distance from the SFJ, with most choosing a threshold of  $\leq 3$ cm before being concerned about risk of deep venous extension. A gradient of management was observed across the 3 case scenarios, with SVT close to the SFJ being treated and monitored more aggressively than distant SVT. That in turn was treated more aggressively than an isolated thrombosed varix. 8/64 (12.5%) of participants believed their practice reflected the ACCP guidelines, with the remainder disagreeing on account of duration or choice of anticoagulant.

### **Conclusion**

The results highlighted the heterogeneity of practice in Australasia with regards to management of lower limb SVT, and demonstrated that clinicians do not apply evidence based guidelines indiscriminately in this condition, rather modifying their treatment strategy after considering various clinical factors.

## **P225. A review and suggested approach for performing cardiac surgery in patients with hereditary bleeding disorders**

**Bhave P**, McGiffin D, Shaw J, Walsh M, McCarthy P, Tran H, Davis A

*Alfred Hospital, Melbourne, VIC, Australia*

### **Aim**

Significant advances have been made in the healthcare of patients with hereditary bleeding disorders (HBDs). This has led to an increase in age related comorbidities including an increased requirement for cardiac surgery. This study presents the experience of a large Australian hospital in performing cardiac surgery on HBD patients and provides a suggested approach for their perioperative management.

### **Method**

Medical records of patients with HBDs who underwent cardiac surgical procedures from January 1997 to December 2013 were reviewed.

### **Results**

Seventeen patients were included in this study, thirteen with Haemophilia A, one symptomatic Haemophilia A carrier, one with Haemophilia B and two with von Willebrand Disease. Cardiac surgical procedures performed include ten coronary artery bypass graft (CABG) operations, two aortic valve replacements, two mitral valve repairs, two aortic root replacements and one combined aortic valve replacement and CABG. Perioperative management centred on factor substitution via continuous infusion to maintain normal factor levels. Perioperative outcomes including length of hospital stay (LOS), mortality and return to theatre for bleeding were recorded (see Table 1). Two patients returned to theatre for bleeding, one patient on the first postoperative day and one patient at day twenty postoperatively with pericardial tamponade. Limitations of this study include the small number of patients with HBDs undergoing cardiac surgery and the lack of a control group.

Outcomes	The Alfred HBD patients 1997-2013 (n = 17)	Victorian Public Hospital patients post CABG 2010-2011 (n = 1510)
LOS (median)	11 days	9 days
Mortality	0%	2%
Return to theatre for bleeding	12%	1.9%

Table 1: Outcomes post cardiac surgery in HBD patients.

### **Conclusions**

Through meticulous planning, a multi-disciplinary team approach and stringent postoperative monitoring, cardiac surgery employing cardiopulmonary bypass may be safely performed in HBD patients. However, further research is required to determine robust perioperative management guidelines.

## **P226. Lessons learnt from a case of severe protein C deficiency diagnosed in adulthood**

**Boey J, Sobieraj-Teague M, Ross D, Gallus A**

*SA Pathology Flinders Medical Centre, SA, Australia*

Compound heterozygous protein C deficiency is exceedingly rare. Our patient presented at 18 years old in January 2011 with unprovoked proximal left leg thrombosis. Thrombus extension occurred during initial treatment with enoxaparin and warfarin eventually leading to the development of compartment syndrome and below-knee amputation. Compartment syndrome is a rare complication of venous thrombosis and was not recognised early. (Lesson 1) Thrombophilia testing was performed at presentation but results indicating severe deficiency were not recognised and replacement was commenced late. (Lesson 2) Genetic testing revealed a novel frameshift mutation not previously associated with protein C deficiency.

Medical history included mild right leg spastic monoparesis. MRI in 2003 showed possible neonatal cerebral infarction. Thrombophilia screening was not done at the time. (Lesson 3)

After discharge from hospital in May 2011, our patient was treated with warfarin (INR target 2.5-3.5) and weekly intravenous protein C at a dose of 100 units/kg/week giving trough protein C levels of 3% (functional chromogenic assay). She remained well except for a breakthrough event of a small splenic infarct after omitting one dose of protein C.

Rivaroxaban 20mg daily replaced warfarin in April 2013 with an increase of trough protein C levels to 12 – 18%. However, she suffered a breakthrough right arm DVT in March 2014 after missing 2 doses of rivaroxaban. (Lesson 4)

Twice-weekly subcutaneous protein C infusions, in the same total weekly dose, were trialled from April 2014 to enable self-administration at home. Results of pharmacokinetic studies comparing intravenous and subcutaneous dosing (table) mirror the few reports in the literature which show lower protein C levels with subcutaneous administration. Our patient has tolerated subcutaneous infusions well.

	0h	1h	2h	4h	8h	24h	72h
Intravenous	16	99	96	90	80	40	27
Subcutaneous	15	18	18	20	21	25	21

Table: Protein C activity (%) after 3000 units of protein C given intravenously and subcutaneously

Subcutaneous protein C infusion may be a viable option for replacement therapy in severe deficiency. (Lesson 5) A safe minimum protein C level remains to be defined.

## **P227. Implementation of specialist haemophilia physiotherapy is associated with a reduction in cost of FVIII concentrate use**

**Buyck H, Ramsay B, Dixon H, Phillips J**

*Wellington Blood and Cancer Centre, Wellington, New Zealand*

**Aim.** To evaluate changes in the use of factor VIII concentrates associated with the introduction of specialist haemophilia physiotherapy.

**Method.** In 2011, for the first time a specialist haemophilia physiotherapist joined the comprehensive haemophilia treatment centre in Wellington, NZ with responsibilities for assessment and rehabilitation of acute and chronic musculoskeletal presentations, surgical pre-habilitation and rehabilitation, exercise and weight control. We interrogated the New Zealand national bleeding disorder database to determine the costs of coagulation factor VIII concentrate usage for people with bleeding disorders treated by the centre for 2010 (prior to the appointment of a specialist haemophilia physiotherapist) and for 2013 (after two years of a specialist haemophilia physiotherapy service). People with inhibitors, symptomatic female carriers and people who moved into or out of the region between 2010 and 2013 were excluded from the analysis. No adjustments were made for changes in weight during this period.

**Result.** 38 patients with haemophilia A received treatment in 2010 but 5 were excluded (3 inhibitors and 2 moved out of the region). 33 subjects with haemophilia A (severe in 23), one with von Willebrand disorder, and one with both conditions were studied. Age on 31st December 2010 ranged from seven months to 76yrs (median 35yrs). All were male. A total of 5,078,350 IU FVIII was used in 2010. The majority of this was recombinant product at 4,467,000 IU (88 %). Seven patients converted from plasma derived to recombinant factor VIII during the study period. Between 2010 and 2013 total factor VIII product usage fell by 342,712 IU, representing a 6.8% reduction. This equates to a cost reduction of NZ\$273,753 (6.6% of 2010 cost).

**Conclusion.** The implementation of a specialist haemophilia physiotherapy service has been temporally associated with a large (approximately 7%) reduction in the cost of FVIII product use in our centre.

## **P228. Clot based direct oral anticoagulant assays**

Chapman K, **Enjeti A**

*Pathology North Hunter, Sydney, NSW, Australia*

### **Aim**

To create a clot based assay for the quantitation of direct oral anticoagulants (DOAC).

### **Method**

A standard protocol was developed to quantitate Rivaroxaban (Rivacloct), Apixaban (Apixaclot), Dabigatran (Dabicloct) and Edoxaban (Edoxaclot). A calibration curve was prepared for each DOAC using commercially available calibration plasmas. Where available, patient samples were then used to compare results from the new assays with the current methodology (Anti-Xa assay) used to quantitate these anticoagulants.

### **Results**

Calibration curves displayed good sensitivity to all levels of Rivaroxiban, Apixaban, Dabigatran and Edoxaban. There was excellent correlation between the currently available Anti-Xa assay and the clot based assays.

### **Conclusion**

Clot based assays for direct oral anticoagulants provide an economical and comparable alternative to the current Anti-Xa based assays.

## **P229. Congenital, late onset thrombotic thrombocytopenic purpura: A case report**

Chapman K<sup>1</sup>, Enjeti A<sup>2</sup>, Meldrum C<sup>3</sup>, Taylor P<sup>3</sup>

<sup>1</sup> Pathology North Hunter, Haematology Department, NSW, Australia<sup>2</sup> Calvary Mater Hospital, Haematology Department, NSW, Australia<sup>3</sup> Pathology North Hunter, Division of Molecular Medicine, NSW, Australia

### **Aim**

We present a 54 year old lady who has been recorded to have numerous episodes of Thrombotic thrombocytopenic purpura (TTP) since her early thirties. This is on the background of seronegative arthritis and varying levels of immunosuppression since her initial diagnosis which occurred in pregnancy. She has been plasma dependent at least since 2008 and has not had any flare ups of TTP since then. We therefore aimed to determine if this was a case of congenital TTP.

### **Method**

ADAMTS-13 activity and antibody assays were performed using commercially available ELISA based assays from Technoclone. PCR amplicons representing the entire coding region of the ADAMTS13 gene were amplified on a Fluidigm Access Array (Fluidigm) to create a Next Generation (NGS) DNA sequencing library. The library was sequenced using an Illumina MiSeq instrument and the data analysed by SoftGenetics NEXTGENe software (Softgenetics).

### **Results**

ADAMTS-13 activity at diagnosis and was <1% and there were no antibodies to ADAMTS-13 detected. NGS revealed two ADAMTS13 missense mutations; c. 1378C>T:p.Arg1060Trp, which encodes an arginine to tryptophan substitution and c. 1058TC>T:p.Pro353Leu, which encodes a proline to leucine substitution.

### **Conclusion**

The two mutations identified by NGS were able to explain the underlying cause of this patient's congenital TTP. The c. 1378C>T mutation has been shown to result in severe intracellular retention of ADAMTS-13 thereby leading to a deficiency in plasma. This mutation is also associated with late onset TTP which is consistent with the patient's presentation. The c. 1058TC>T mutation has been shown to result in a moderate reduction in secretion of ADAMTS-13 as well as almost complete ablation of ADAMTS-13 activity. Recognition of mutations that are implicated in late onset TTP affect long term management strategies for patients with TTP.

## **P230. Pre-analytical variables in calibration and plasma analyte measurements for microvesicles using 'Nanosight'**

**Enjeti A**<sup>1,2</sup>, **Warwick E**<sup>1</sup>, **Lincz L**<sup>1,2</sup>

<sup>1</sup> *Calvary Mater Newcastle, NSW, Australia* <sup>2</sup> *University of Newcastle, NSW, Australia*

**Aim:** Microvesicles are derived from various endovascular cells and play an important role in pathophysiology of diseases. Measurement of these cell-derived microvesicles can be of prognostic/diagnostic value. This study aimed at evaluating pre-analytical variables in calibration and the measurement of microvesicles in normal plasma using Nanosight, a novel technique utilizing Brownian motion and tracking to count particles in the nanometer range.

**Method:** Commercial beads (200nm and 400nm) and human plasma from a volunteer donor were used to test the calibration and pre-analytical variables. A total of 36 bead and 175 plasma measurements were undertaken with triplicate runs. The pre-analytical variables included measuring neat, supernatant and pelleted samples using two different centrifugation conditions (21000g for 10 minutes or 1 hour). The beads were measured in various dilutions as well as a mix of the two sizes. All samples were run on Nanosight NS500 and statistical analysis performed on Graphpad.

**Results:** The beads showed a CV of <10% for all dilutions for both 200nm and 400nm at all dilutions as well mix of beads. The CVs for triplicate results of plasma samples varied between 3-43%, with samples frozen for 1 week showing the least CV spread (5-15%). The results for neat as well as supernatant were not significantly different. Consistently lower results were obtained for pellet compared to supernatant or fresh (significantly different on ANOVA analysis). There was no difference in the pellet measurements after 10mins or 1 hour of centrifugation.

**Conclusions:** Commercial beads can be used to simulate run conditions for microvesicle estimation. Microvesicle measurement on Nanosight can be performed on fresh and/or frozen samples provided similar storage and centrifugation conditions are used. The CVs for plasma samples are high indicating the need to run them under standardized conditions. Freeze-thaw may interfere with microvesicle integrity. Additional centrifugation may result in production of microvesicles and may need to be avoided.

## **P231. Retrospective single center audit of initial treatment of immune thrombocytopenia (ITP) in the thrombopoietin mimetic (TPO) era**

**Gilbertson M**<sup>1</sup>, Chen M<sup>1</sup>, Grigoriadis G<sup>1,2</sup>, Shortt J<sup>1</sup>, Curtin N<sup>1</sup>, Opat S<sup>1</sup>, Wood E<sup>1</sup>, Chunilal S<sup>1</sup>

<sup>1</sup> *Department of Haematology, Monash Health, Clayton, VIC, Australia,* <sup>2</sup> *Department of Medicine, Monash University, Clayton, VIC, Australia*

### **Aim:**

*Primary objectives:* to describe the current treatment strategies for patients with primary ITP, including the frequency of non-corticosteroid based therapies, the overall response rates, time to platelet response and, best platelet response.

*Secondary objectives:* incidence of high dose steroid dependence beyond 28 and 56 days.

### **Methods:**

Retrospective single center cohort series including all adult patients with a platelet count of  $\leq 30 \times 10^9/L$  over a two-year period were identified. Only patients satisfying diagnostic criteria for newly diagnosed ITP were eligible for inclusion.

### **Results:**

A total of 34 adult patients with primary ITP were identified. Most (68%) were female and most (65%) had primary ITP. Overall response rate at 56 days, 26 and 52 weeks was 91%, 89%, 90% respectively, but complete response was only present in 50%, 60% and 70%. There was a high degree of steroid dependence  $>25\text{mg/day}$  beyond 8 weeks of therapy (48%). A total of 70% had steroid sparing additional immunosuppression, including Azathioprine, Hydroxychloroquine, Cyclophosphamide, Cyclosporin and Rituximab. A total of 3 patients required splenectomy for acute refractory ITP and one for chronic ITP from this cohort. TPO mimetics were used infrequently reflecting current prescribing restrictions (3 patients (9%) with newly diagnosed ITP and prior splenectomy).

### **Conclusion:**

Although 70% of our patients achieved a CR by 52 weeks a significant number (48%) remain on high dose steroid based immunosuppression beyond 56 days despite widespread use of steroid sparing agents. These data reflect poor access to non-steroid based therapies (TPO mimetics).

Conflict of interest: This audit was supported by an unrestricted educational grant from Amgen Australia.

## **P232. Outcomes of early anticoagulation in high risk patients with Acute Ischaemic Stroke**

**Gurumurthi A**, Sidiqi H, P'ng S, Ghia D

*Royal Perth Hospital, Perth, WA, Australia*

### **Aim**

Early therapeutic anticoagulation in ischaemic stroke is warranted for secondary prevention of recurrent cardioembolic stroke and treatment of co-morbid venous thromboembolism. However given RCT evidence has demonstrated excess haemorrhagic complications from early anticoagulation, the prevailing practice is to ideally delay commencement of therapeutic anticoagulation to 14 days. This is individualised based on the risk of thrombosis, with high risk patients including those with hypercoagulability disorders, mechanical valves, intracardiac thrombus and acute venous thromboembolism being considered for earlier intervention.

We aimed to identify the subset of patients with the highest risk for recurrent ischaemic stroke from a cardioembolic source or systemic hypercoagulability, as well as those with acute venous thromboembolism and to establish the safety of early anticoagulation in the setting of acute ischemic stroke.

### **Methods**

We retrospectively analysed all patients that presented to Royal Perth Hospital during a 4 year period from 1<sup>st</sup> January 2010 to 31<sup>st</sup> December 2013 with acute ischaemic stroke and received early therapeutic anticoagulation prior to 14 days of infarct diagnosis. Chart review was conducted including review of pathology and imaging results.

### **Results**

We identified 47 cases who received early anticoagulation in the setting of acute ischaemic stroke. The indications for anticoagulation included treatment of venous thromboembolism in 12 cases, treatment of right atrial thrombus in 1 case, prevention of recurrent stroke in hypercoagulability disorders in 8 cases and prevention of recurrent cardioembolic stroke in 18 cases with mechanical heart valves and 8 cases with left ventricle thrombus .

The primary outcome of interest was mortality from haemorrhagic complications of early anticoagulant therapy which was 6.4% (3/47) respectively.

### **Conclusion**

Our series provides some data to help guide early anticoagulation and its associated complications in high risk patients.

## **P233. An audit of indications for thrombophilia screening at PathWest Laboratory Medicine, Nedlands WA shows significant potential cost saving with a rationalised testing approach**

Hogan F, Cull G, Grove C, Michalopoulos N, Finlayson J

*Sir Charles Gairdner Hospital, Perth, WA, Australia*

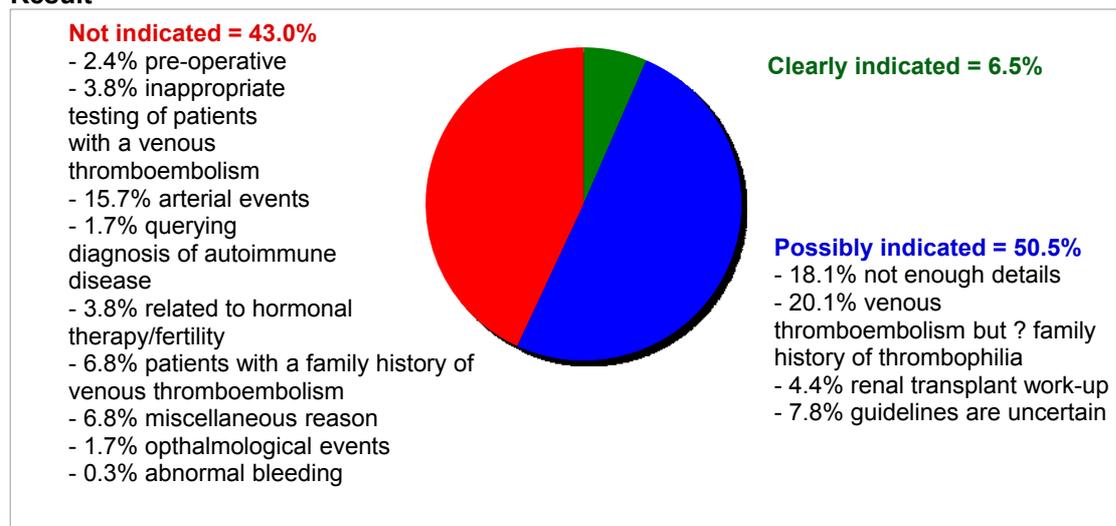
### **Aim**

The 2010 British Committee for Standards in Haematology guidelines recommend that testing for inherited thrombophilias in unselected patients with venous thrombosis is not indicated. Furthermore, testing should only be performed when the result will influence treatment. In the absence of a local guideline we propose that a significant number of thrombophilia screens are being performed inappropriately in Australia.

### **Method**

We audited the indications for full thrombophilia screens completed during March and April 2014 at PathWest Laboratory Medicine, Nedlands WA. 293 laboratory requests were categorized as clearly indicated, possibly indicated or not indicated based on the British Guidelines and using the clinical information provided. Using the number of inappropriate requests and the Medicare rebate for thrombophilia screening (\$100.75) we estimate the annual cost to the health system from inappropriate referrals.

### **Result**



Only 12 (4.1%) of the thrombophilia screens were requested by Haematologists.

An estimate of the annual cost of the thrombophilia screens which are not indicated is \$76,167. As a further 42.6% of the tests are of uncertain indication due to insufficient data, it is likely this is an underestimate.

### **Conclusion**

A significant number of thrombophilia screens are being performed when they are not indicated. This equates to a significant unnecessary annual cost to the health care system. The results of this audit provide support for the need for an Australian guideline and further education of requesting doctors.

## **P234. Unexpectedly significant coagulopathy in setting of Apixaban use requires consideration of concomitant factors**

Hogan F<sup>1</sup>, Grove C<sup>1,2</sup>, Crawford J<sup>1,2</sup>

<sup>1</sup> Sir Charles Gairdner Hospital, Nedlands, WA, Australia, <sup>2</sup> PathWest, Nedlands, WA, Australia,

### **Introduction:**

Apixaban, a renally cleared new oral anticoagulant (NOAC), can variably affect PT/INR and aPTT, depending on assay sensitivity, but has no discernable effect on TCT. If PT/INR and aPTT testing is done within 1-3 hours of intake it may lead to elevated PT/INR (up to 1.7-2.5) and aPTT (35-40sec). After this, the apixaban effect on PT/INR and aPTT diminishes. Normal PT/INR and aPTT may be found at trough, despite presence of therapeutic apixaban drug levels.

### **Results:**

We report a 78 year old man who experienced unexpectedly prolonged coagulation testing whilst taking Apixaban. He was admitted for right upper lobectomy for management of malignancy after coronary angiography and placement of drug eluting stent the previous month. He experienced atrial fibrillation 1 day post operatively and chest pain 2 days later. He commenced apixaban 5mg PO BD and amiodarone 200mg PO TDS and discharged from hospital day8 post surgery, day4 Apixaban plus dual anti-platelet therapy (Ticagrelor and aspirin).

He represented within 24 hours with acute dyspnoea and collapse. Imaging studies showed right pleural effusion and chest wall haematoma. Apixaban was last taken >17hours previously. Re-admission tests showed Hb 123, platelets 579, INR 2.7, APTT 41.7s, apixaban 139.7. Anticoagulation and anti-platelet therapy was suspended and 25U/kg prothrombinex and 1U SD platelets given. Apixaban measured 55.9 36 hours post dosing. The coagulation profile became increasingly deranged (INR 2.8, APTT 52.9s). Factor levels on day6 demonstrated multi-factor deficiencies (INR-1.7, APTT-40.8s VII-25%, X-54%, XI-38%) without liver dysfunction. Vitamin K 10mg IV was administered for presumed nutritional deficiency. Coagulation profile and factor deficiencies normalised within 48 hours.

### **Conclusion:**

Although NOAC lead to coagulation profile perturbations it's important not to attribute these solely to NOAC use, especially when concomitant factors are present. The markedly altered coagulation profile on re-admission should have prompted examination for contributors to coagulopathy beyond Apixaban.

## **P235. Evaluation of a rapid automated assay as an alternative test for investigation of Heparin-Induced Thrombocytopenia**

Htun K<sup>1</sup>, Vilcassim S<sup>2</sup>, James V<sup>1</sup>, Dauer R<sup>1</sup>, Butler J<sup>3</sup>, Malan E<sup>3</sup>, Chunilal S<sup>2</sup>, Morgan S<sup>1</sup>

<sup>1</sup> *Department of Laboratory Haematology, Alfred Health/Monash University, Central Clinical School Melbourne, VIC, Australia,* <sup>2</sup> *Department of Haematology, Monash Health, Melbourne, VIC, Australia,* <sup>3</sup> *Monash Pathology, Monash Health, Melbourne, VIC, Australia*

### **Aim**

Heparin-induced thrombocytopenia (HIT) is a potentially serious thrombotic condition caused by development of IgG antibodies against heparin-PF4 complexes. As thrombocytopenia is common in hospitalized patients receiving heparin, rapid diagnostic assays would be useful to diagnose/exclude HIT. We aimed to evaluate the accuracy of Acustar™ HIT automated immunoassay compared to ELISA assay, in conjunction with clinical 4T scoring.

### **Methods**

Citrated plasma from 28 patients with clinical suspicion of HIT was analyzed with chemiluminescent Acustar™ and ELISA Heparin/PF4 IgG assays at 2 different institutions in blinded manner. The test results and clinical 4T score (Warkentin, BJH, 2003) were analyzed by an independent investigator.

### **Results**

Out of 28 patients, 23 (82%) had concordant results in both assays (15 negative and 8 positive). Discordant results (positive ELISA but negative Acustar™) were detected in 5 of 28 patients (18%). Of these, 2 had high 4T score (6-8) while the other 3 had intermediate 4T score (4-6). 11 of 11 intermediate/high risk patients had positive ELISA; only 6 of 11 tested positive with Acustar™. All 15 patients with negative antibody assays had low 4T score (<4). In antibody positive group, 6 of 8 patients had intermediate/high 4T score while 2 had low score. Overall, Acustar™ assay showed moderate agreement (Cohen's Kappa score of 0.58) (0.4-0.6) with ELISA assay.

### **Conclusion**

In low risk patients, Acustar™ HIT IgG assay correlated well with ELISA assay and could be used to exclude HIT in this group. However, in intermediate/high risk patients there was a poor agreement with only 55% of ELISA-positive patients detected by Acustar™. The new test may be more accurate, excluding false positive ELISA tests, or less sensitive. A prospective evaluation with a reference test for HIT (ie serotonin release assay) should be performed to further determine the accuracy of the new test.

## **P236. "Cross talk" between the receptors of dermcidin isoform-2 and estriol in resistance of the effect of estriol in platelet aggregation in myocardial infarction**

**Jana P, Sinha A**

*Sinha Institute of Medical Science and Technology, Kolkata, India*

**Aim:** Estriol, one of the estrogens inhibits platelet aggregation through nitric oxide synthase (NOS) activation. Furthermore, estriol could not inhibit platelet aggregation and synthesize NO in PRP from the subjects with acute myocardial infarction (AMI). Aim of this investigation was carried out to determine the mechanism of non-genomic expression of NO synthesis in platelet and to find the reason of the failure of the estrogen to inhibit platelet aggregation and to stimulate NO synthesis in AMI platelets.

**Methods:** Dermcidin isoform-2 (DMC-2) was prepared by electrophoresis. NO was determined by methemoglobin methods. The platelet membrane NOS was isolated by gel-electrophoresis. The binding characteristics of estriol to platelets were determined by enzyme linked immunosorbent assay.

**Results:** The increased synthesis of NO by 8fold through allosteric activation was inhibited by tamoxifen, an estrogen receptor antagonist. The treatment of AMI platelets with 0.6nM estriol failed to increase both NO synthesis and to inhibit platelet aggregation by 2.0 $\mu$ M ADP due to down-regulation of estriol binding by 1.6fold to its own receptor for the preexisting binding of DMC-2.

**Conclusion:** The failure of estriol to inhibit platelet aggregation via NO synthesis was due to "cross-talk" between DMC-2 and estriol in the platelet surface in AMI.

## **P237. Evaluation of Multiplate® whole blood impedance aggregometry in routine investigation of bleeding disorders**

**Jarvis S**, Low J, Joseph J

*SydPath, St Vincents Hospital, Sydney, NSW, Australia*

### **Aim**

The Multiplate® platelet analyser (MEA) performs impedance aggregometry in whole blood and provides rapid screening of platelet inhibition by aspirin and clopidogrel. The aim of this study was to evaluate its potential role in routine diagnosis of bleeding disorders by correlating with traditional platelet function testing.

### **Method**

All 86 patients studied presented with a history of bleeding and/or bruising; including von Willebrand disease (vWD) or suspected vWD (24), thrombocytopenia (10) or a myeloproliferative disorder (6). Patients known to be on antiplatelet therapy at the time of testing were excluded. MEA was performed with the agonists ADP, ADP with prostaglandin E, arachidonic acid, TRAP, collagen and high and low dose ristocetin. Other tests (performed on the majority of the patients) were light transmission platelet aggregation (LTA) on an AggRam aggregometer (assessed by maximal amplitude only), Innovance® PFA-200 and VWD Screen (performed by Pathology West).

### **Result**

On MEA, 36 patients had an abnormally low response to one or more agonists. A total of 20 patients were found to have thrombocytopenia (mostly mild) and half of these had at least one defect (mostly adp). 7 patients' results were consistent with aspirin ingestion. vWD screening identified 24 patients with vwd or borderline vWD. On mea, only 1/4 with type 1 vwd (3 moderate and 1 mild), 3/7 with type 2 and 3/13 with borderline vWD had a reduced response to ristocetin whereas 17 patients had an elevated PFA-200. Interestingly, only one patient had an abnormal response to Ristocetin by lta. Two patients with vwd had mea performed serially after infusion of desmopressin and biostate®.

### **Conclusion**

The diagnosis of bleeding disorders, including vWD does not appear to be enhanced by the addition of Multiplate® testing to existing assays.

## **P238. Evaluation of the Cepheid GeneXpert® Factor II and Factor V genotyping assay**

**Jarvis S**, Low J, Joseph J

*SydPath, St Vincents Hospital, Sydney, NSW, Australia*

### **Aim**

We aimed to perform a preliminary evaluation of the Cepheid Gene Xpert® Factor II and Factor V Assay, a qualitative diagnostic test for the detection of Factor II (G20210A)(FII) and Factor V Leiden (G1691A)(FVL), in individuals with suspected thrombophilia.

### **Method**

The GeneXpert® system (Cepheid Holdings) consists of an instrument and a PC with preloaded software. The assay is performed in single-use disposable cartridges using 50µl whole blood. Sample purification, nucleic acid amplification and detection of the target sequence using real-time PCR assays are automated. The results are interpreted by the instrument from measured fluorescent signals to identify genotypes. The test was performed on either fresh or frozen EDTA blood from 36 individuals who had had thrombophilia testing, including genotyping (performed externally by Pathology West) requested. A control panel was obtained from Maine Molecular Quality Controls (MMQCI).

### **Results**

among the 36 patients, there were 7 fvl heterozygotes, 1 fvl homozygote, 11 fii heterozygotes, 1 compound fvl and fii heterozygote and 17 normals. genotyping performed by the Gene Xpert® was in complete concordance with the external laboratory's results. The MMQCI controls - a normal, a compound heterozygote and a double homozygote, gave the expected results. Two of the five 2013 RCPA Quality Assurance Program specimens (which were extracted DNA and therefore not optimised for this instrument), gave "invalid" results. However, an examination of the fluorescence traces indicated that the genotypes had been identified correctly.

### **Conclusion**

The results from the GeneXpert® obtained in our small number of patients were in complete concordance with the external laboratory's results. The instrument was very easy to use, provided individual results on a small amount of whole blood within 30 minutes and has the potential to markedly improve the assay turnaround time for our laboratory.

## **P239. Assessment of the AcuStar Chemiluminescent analyser for VWD screening assays - vWF Antigen (vWF:Ag) and Ristocetin Cofactor (vWF:RCoF)**

**Just S**, Prawitha D, Brighton T

*SEALS North - NSW Health Pathology, NSW, Australia*

### **Aim**

The AcuStar Chemiluminescent analyser is new to the market in Australia. We assessed its ability to provide on-demand, automated vWF screening assays - vWF Antigen (vWF:Ag) and vWF:Ristocetin Cofactor (vWF:RCoF), comparing results to the current assays used in our laboratory.

### **Method**

Testing was performed on known von Willebrand (vWD) patients (n = 24), normal donors (n=20), normal patients (n=10) and vWD patients receiving Biostate or DDAVP therapy (n=10). The known vWD patients were divided into seven Type I, eight Type II, one Type III, two acquired vWD and four type IIM and two borderline low patients. The results from the AcuStar were compared to results obtained using similar automated assays on the ACL TOP analyser, vWF:Ag on the Stago STAR analyser and vWF:RCoF by platelet aggregometry.

### **Results**

The results from the AcuStar for vWF:Ag compared well to both the ACL TOP assay and the Stago STAR vWF Liatest assay. Passing Bablok regression showed good agreement between methods and across all levels of results. The difference plot showed a mean difference of 3.21 between AcuStar and Stago Liatest vWF:Ag.

The results from the AcuStar for vWF:RCoF compared well to both the ACL TOP assay and our manual platelet aggregation method. Passing Bablok regression showed good agreement between methods, particularly at the lower range of results, which is important for subtyping of vWD. The difference plot of results showed a mean difference overall of -5.19 between AcuStar and platelet aggregometry RCoF.

### **Conclusion**

This evaluation showed that the AcuStar is able to provide on-demand testing for vWF:Ag and vWF:RCoF, with results available in 30 minutes. There was good agreement between our current screening assays, the ACL TOP assays and the AcuStar across all levels of vWF tested.

## **P240. National supply of products for bleeding disorders in Australia**

Stone M, Kemp I

*National Blood Authority, Canberra, ACT, Australia*

### **Aim**

Since 2003 Australia has maintained national arrangements for providing a safe, secure, adequate and affordable supply of blood products, including key products to meet the clinical needs of patient with bleeding disorders. These arrangements include national policy setting, planning, funding and tendering for products on a national product list, and consideration of proposed additions to the national product list. Factors relevant to decision making within the national arrangements include clinical need, macroeconomic cost, security of supply, clinical efficacy and patient safety.

### **Methods**

The National Blood Authority (NBA) is proud of its achievements over the last 10 years to support the haemophilia community – by providing reliable supply of both recombinant and plasma derived products, including to support the introduction of full funding for recombinant products; achieving improved value for money, and improved service levels, through each successive tender round, while also increasing the range of products supplied; and providing and supporting the redeveloped Australian Bleeding Disorder Registry for clinical and supply data recording.

### **Results**

The issues affecting national supply of products for bleeding disorders continue to evolve, including: the limited but growing number of suppliers in the market place; the rapid advance of new product technologies and generations with a short-term shortage of post-market data availability; supplier claims of differential product safety or efficacy profiles; changing demographics of a long-term dependent patient base.

### **Conclusion**

The approach adopted by the NBA involves a progressive decision making process involving consultation with all key stakeholders. This approach has resulted in active involvement of patient advocates, specialist healthcare providers and product suppliers. In order to continue to achieve best value for money, there is a need to balance the benefit of patient choice against maintaining competitive pressure on price and service levels – particularly given the potential opportunities and challenges which may lie ahead.

## **P241. The Australian framework for management of bleeding disorders**

**Stone M**<sup>1</sup>, Barnes C<sup>2</sup>, **Kemp I**<sup>1</sup>

<sup>1</sup> *National Blood Authority, Canberra, ACT, Australia*<sup>2</sup> *Australian Bleeding Disorders Registry Steering Committee, Canberra, ACT, Australia*

### **Aim**

The framework for the management of treatment for persons with bleeding disorders in Australia is a collaborative national partnership involving a number of groups that represent patients and carers, specialist health care professionals, haemophilia treatment centres, industry partnerships and all Australian governments.

### **Methods**

It is founded on a comprehensive care model which encourages the management of bleeding disorders in a manner which obtains the greatest patient benefit from multidisciplinary care.

### **Results**

The contribution of elements within this framework has evolved since the establishment of national arrangements for blood products in 2003. Patient care has been greatly enhanced by the safe and adequate supply of publicly funded clotting factor products provided under the national blood arrangements. It has enabled the availability and ongoing development of the Australian Bleeding Disorders Registry and its associated availability of data for improved patient care. The framework has enabled the commissioning of work to develop contemporary Australian haemophilia care guidelines, and will further assist the sector to address future arrangements for imported plasma and recombinant products, including the introduction of new and next generation products.

### **Conclusion**

The National Blood Authority is able to facilitate the articulation of an Australian framework, the structures for collaboration between stakeholders, the terms of reference for collaborative processes and a range of tools to achieve the best outcomes for persons with bleeding disorders and the sector as a whole. It is intended that this approach will further the appropriate management of patients with bleeding disorders in Australia by providing national priority setting and enhanced collaboration into the future.

## **P242. Trajectory of platelets in pregnancy - Do low-risk women need an intrapartum full blood count (FBC) prior to epidural?**

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<sup>1</sup> SEALS, Prince of Wales Hospital, Sydney, NSW, Australia <sup>2</sup> University of New South Wales, Sydney, NSW, Australia <sup>3</sup> St George Hospital, Kogarah, NSW, Australia

### **Aim**

To ascertain whether, in uncomplicated pregnancies, there is a threshold level of platelets at 28 weeks such that the intrapartum platelets will remain above  $100 \times 10^9/L$  (conservative lower limit for an epidural block).

### **Method**

Medical records of women who delivered at St George public hospital (SGH) during 2010-2012 were identified using obstetric databases and correlated with hospital pathology database to obtain 28 week (26-30 weeks) and labour (within 2 days of birth) FBC. Demographic, maternal and pregnancy details, anaesthetic and birth/neonatal outcomes were recorded. Preterm birth, multiple pregnancy, women with known hypertensive disorders, haematological conditions, or where 28 week or labour FBC result was not available (or platelets were  $<150 \times 10^9/L$  at 28 weeks) were excluded.

### **Results**

7879 women gave birth at SGH during the study period, of whom 1844 had a term, low risk, singleton pregnancy and with sufficient haematological data for inclusion. These 1844 low-risk women with a platelet count  $>150 \times 10^9/L$  at 28 weeks all had a platelet count  $>100 \times 10^9/L$  during labour. Mean platelet count at 28 weeks was  $230 \times 10^9/L$  and mean platelet count during labour was  $216 \times 10^9/L$  (mean drop  $13.6 \pm 36.2 \times 10^9/L$ ).

Mean maternal age was 30, mean gestation at birth 39 weeks, and 34.4% were caesarean births. 1089 women (59%) had epidural and/or spinal analgesia, of which 2 had a dural tap, none had epidural haematoma. 47 (2.6%) women had  $>1000\text{mL}$  blood loss postpartum, most commonly secondary to atonic uterus. 2.5% of women required a blood transfusion post partum.

### **Conclusions**

In low risk women who have singleton pregnancies and had a platelet count  $>150 \times 10^9/L$  at 28 weeks, 100% also had pre-birth platelets at term of  $>100 \times 10^9/L$ . This suggests that low risk women with normal platelet counts at 28 weeks do not require an intrapartum FBC for the purpose of checking their platelets prior to epidural.

## P243. The use of NOAC in the elderly - What is the evidence?

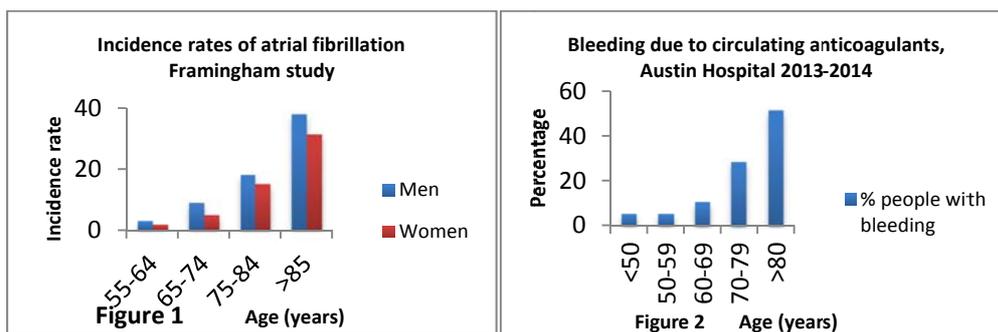
La Brooy B<sup>1</sup>, Lim K<sup>2</sup>, Ho P<sup>1</sup>

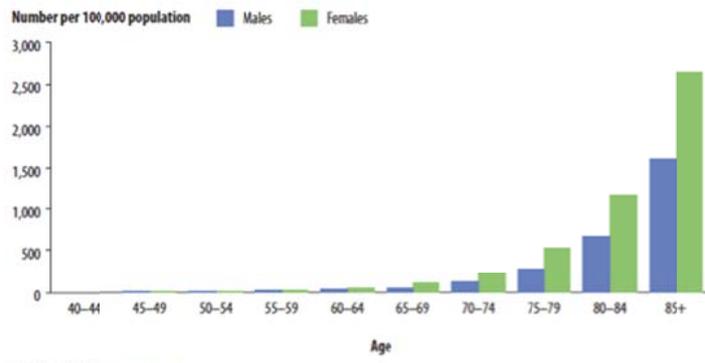
<sup>1</sup> Austin and Northern Hospitals, Melbourne, VIC, Australia, <sup>2</sup> Northern Hospital, Melbourne, VIC, Australia

**Aim + Methods:** New oral anticoagulants (NOAC) are being increasingly used due to better drug stability and patient convenience, and have been shown to be non-inferior to warfarin. However, there remain specific concerns regarding lack of reversibility and laboratory monitoring, which are particularly important in at risk populations such as those with renal failure, extremes of weight and the elderly. Recent ASTH NOAC guidelines have identified some at risk populations, but the elderly are neglected in this discussion. We aim to evaluate the specific concerns regarding anticoagulation in the elderly through literature review and retrospective analysis of bleeding complications at the Austin Hospital over a 14-month period.

**Results:** The Elderly are at greatest risk of non-valvular atrial fibrillation thus requiring anticoagulation (Figure1) and are also more likely to bleed. Local analysis of >500 patients demonstrated 79% of bleeding complications occurred in patients over 70 years and 51% in >80 years (Figure2). Australian population studies clearly show that the elderly are also more likely to fall and sustain fractures requiring surgery (Figure3). The landmark NOAC phase III clinical trials (RE-LY, ROCKET AF and ARISTOTLE) demonstrated that at best, <25% of study participants were aged >80years, had a creatinine clearance <50ml/min or were <60kg. Recent ROCKET AF sub-analysis of patients aged >75 years showed higher rates of major extracranial bleeding with rivaroxaban compared to warfarin.

**Conclusion:** The elderly population have a disproportionately high risk of bleeding, falls and fractures requiring operative intervention and unfortunately, most clinical trials are not representative of 'real world elderly'. These factors are important considerations in the use of anticoagulation, specifically the use of NOAC, particularly due to the lack of monitoring and reversal. Dedicated guidelines to address the use of NOAC in the elderly are necessary.





Note: Persons aged 10 years or over.  
Source: AIHW National Hospital Morbidity Database.

Figure3

## **P244. Clinical experience with apixaban monitoring in Australia**

**Lebret S, Whiteman I, Thomas G**

*Pfizer Australia, West Ryde, NSW, Australia*

### **Aim**

Since September 2013, apixaban has been reimbursed on the Australian Pharmaceutical Benefits Scheme for stroke prevention in patients with non-valvular atrial fibrillation (AF) and one or more risk factors for stroke. Some concern has been noted among healthcare professionals regarding the inability to accurately monitor apixaban levels. In October 2013, research-use only (RUO) apixaban-specific calibrators and controls became available for use with STA®-Liquid anti-Xa reagent (Diagnostica Stago, Inc.). To date, 51 sets of controls and 38 sets of calibrators have been purchased in Australia.

### **Method**

We reviewed the use of the STA®-liquid anti-Xa reagent and apixaban-specific calibrators and controls across a number of large public hospital laboratories to determine the frequency of assay use, urgency, reasons for monitoring apixaban patients, average turnaround times and expected apixaban levels.

### **Result (data collection ongoing)**

Of the centres that have provided feedback to date, all had the RUO apixaban-specific assay set up and running well with reproducible, reliable results. The majority of the centres report a rapid turnaround (30mins-1hr), with many able to run the assay 24 hours/7 days. Despite this, there has been little clinical need to assess patient samples in most centres, with those who have, reporting analyses of no more than 5 patient samples to date; none of which were stated as 'urgent requests'. Few laboratories also report expected peak and trough levels.

### **Conclusion**

This early feedback anecdotally confirms relatively widespread availability of apixaban-specific assays in major hospitals, with rapid turnaround times and 24-hour availability. To date, the necessity of apixaban monitoring appears infrequent.

Reporting of expected peak and trough levels may assist in the management of patients receiving apixaban in the interim, as we await the results of ongoing local and global research aimed at establishing a therapeutic reference range for apixaban in AF patients.

## **P245. Assessment of correlation and agreement between the derived fibrinogen and Clauss methods in a tertiary hospital**

Lee N, Chunilal S, Wheeler M, Malan E, Wallace J

*Department of Clinical Haematology, Monash Health, Clayton VIC, Australia<sup>8</sup>*

### **Aim**

The Clauss fibrinogen (CF) method is the most widely-used assay for assessing plasma fibrinogen. The derived fibrinogen (DF) method estimates fibrinogen levels based on a clot curve from the prothrombin time test, which is more convenient and economical than the Clauss method.

This study compares the two methods in a tertiary hospital laboratory.

### **Method**

Plasma fibrinogen was measured on fresh plasma samples referred for coagulation testing. Samples included both normal and abnormal INRs with a range of fibrinogen levels. All testing was conducted on the ACL Top (IL Werfen) using Siemens bovine thrombin for the CF and Recombiplastin 2G for the DF.

### **Results**

Table 1	All results n = 212	Normal INR n = 82	Elevated INR (Warfarin) n=25	Elevated INR (non warfarin) n=59
Mean DF (g/L)	3.64 +/- SD 2.82	4.33 +/- SD 2.92	4.15 +/- SD 1.30	2.82 +/- SD 3.41
Mean CF (g/L)	3.58 +/-SD 2.66	4.19 +/- SD 2.71	4.17 +/- SD 1.53	2.68 +/- SD 3.11
Difference in mean (g/L)	0.061	0.142	-0.012	0.143
Pearson Correlation (r)	0.985	0.989	0.965	0.984

Of 212 samples tested, clinical information was available for 130 patients. The strong overall correlation between the DF and CF was relatively maintained in the specified groups with no statistically significant differences between the mean DF and CF results. Bland-Altman plots demonstrated good agreement overall, in normal patients and for patients with non-warfarinised abnormal INRs. Warfarinised patients showed proportional error – DF results trended lower than CF as fibrinogen levels increased.

### **Conclusion**

This limited study suggests strong correlation and agreement between CF and DF methods across all subgroups suggesting that the derived fibrinogen method is a robust method for routine fibrinogen testing in our laboratory setting. However, further assessment of correlation and agreement is required for warfarinised patient samples.

## **P246. Venous thromboembolism and cancer in Northeast Melbourne: The evaluation of epidemiology, risk factors, associations and outcomes**

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<sup>1</sup> *Department of Clinical Haematology, Austin Health, Heidelberg, VIC, Australia,* <sup>2</sup> *Department of Clinical Haematology, Northern Health, Epping, VIC, Australia*

### **Aim**

Cancer is a well-recognised risk factor for venous thromboembolism (VTE) and conversely VTE is a major cause of morbidity and mortality in cancer patients. We aim to provide an overview of the relationship between VTE and cancer in our local population.

### **Method**

Retrospective evaluation of VTE from July 2011 to December 2012 at Austin and Northern Health, Melbourne, comparing cancer and non-cancer patients, including demographics, provoking factors, associations and outcomes.

### **Result**

233 (23%) of the 1003 patients had active malignancy at time of VTE with 14 (1.4%) subsequently diagnosed. When compared with non-cancer patients, cancer patients were older (67 vs 61 years,  $p<0.001$ ) with male predominance (59% vs 49%,  $p=0.005$ ). They had higher clot burden with more pulmonary embolism (PE) (64% vs 53%,  $p=0.004$ ), proximal deep venous thrombosis (DVT) (63% vs 46%,  $p=0.0008$ ) and bilateral DVT (16% vs 5%,  $p<0.001$ ) reported. Patients with metastatic cancer were more likely to have unprovoked events ( $p=0.015$ ). Incidental VTE was more common (17% vs 4%,  $p<0.001$ ) and most received enoxaparin. Cancer patients were more likely to require IVC filters (9% vs 3.6%,  $p<0.001$ ) and lifelong anticoagulation (35% vs 18%,  $p<0.001$ ). Interestingly, bleeding rates in cancer patients treated with long-term enoxaparin compared to warfarin were similar. Overall, cancer patients had more recurrent thrombosis (16% vs 8%,  $p<0.001$ ) and Grade III/IV bleeding (9% vs 5%,  $p=0.025$ ). There was a trend towards more recurrence in cancer patients with unprovoked VTE compared to provoked (19.5% vs 12.5%,  $p=0.087$ ). Mortality rate in the cancer and non-cancer patients was 63% and 11% respectively, with higher incidence of complications-related deaths ( $p<0.001$ ) in the former.

### **Conclusion**

Cancer patients have higher clot burden, thrombosis recurrence, bleeding complications and all-cause mortality compared to non-malignant patients. Given these substantial complications, further evaluation of new treatment strategies as well as clinical and laboratory risk assessments are required to improve the management for cancer-related VTE.

## **P247. Circulating CD36+ microparticles are not altered by eicosapentaenoic or docosahexaenoic acid supplementation**

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### **Aim**

Circulating microparticles (MP) have been shown to be the source of a plasma derived form of the scavenger receptor CD36, termed soluble (s)CD36, the levels of which correlate with markers of atherosclerosis and other risk factors for cardiovascular disease. The long chain n-3 polyunsaturated fatty acids (n-3 PUFAs) Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) have cardioprotective effects that we have shown to be gender specific. The aim of this study was to determine if EPA and/or DHA affect circulating CD36+MP levels, and if this occurred differentially in healthy men and women.

### **Method**

Ninety four participants (43M, 51F) aged 39.6±1.7 years received 4 weeks of daily supplementation with EPA rich (1000mg EPA;200mg DHA), DHA rich (200mg EPA;1000mg DHA) or placebo (sunola) oil in a double-blinded, randomized, placebo controlled trial. Compliance was ensured by determining plasma fatty acid composition. Plasma CD36+MP were detected with a CD36 specific antibody and enumerated by flow cytometry at baseline and again post supplementation. Differences between genders and treatments were evaluated by Student's or paired t-test and one way ANOVA.

### **Results**

Males and females had similar levels of CD36+MP at baseline (mean=1018±325 vs. 980±318; p=0.577) and these were not significantly changed after EPA (M, p=0.361; F, p=0.901) or DHA (M, p=0.571; F, p=0.444) supplementation. Likewise, the overall percentage of change in these levels were not different between supplement cohorts when all participants were combined (% change in CD36+MP: EPA= -3.4 ±35.4, DHA =5.7±37.5, placebo=-11.5±32.9, p= 0.158) or stratified by gender (M, EPA= -15.1±20.1, DHA =-2.6±30.6, placebo=-21.4±28.7, p= 0.187; F, EPA=6.8 ±42.9, DHA= 11.7±41.5, placebo= -2.8±34.7, p= 0.552).

### **Conclusion**

EPA and DHA supplementation did not reduce CD36+MP levels in healthy males or females, suggesting that the cardioprotective effects of these n-3 PUFAs do not act through a CD36+MP mechanism.

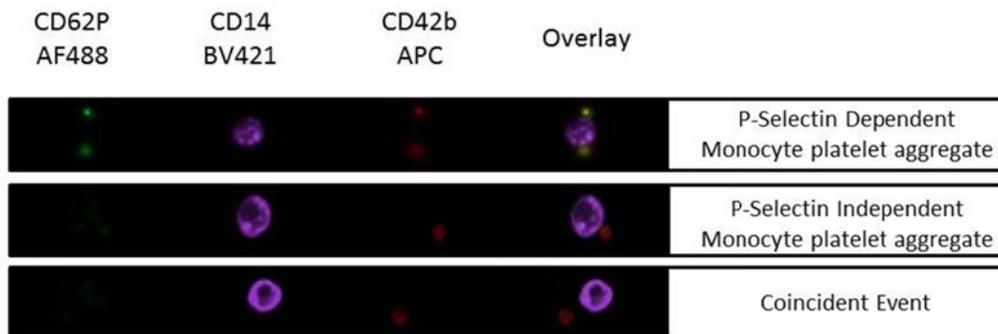
## P248. Measurement of circulating monocyte-platelet aggregates by imaging flow cytometry

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<sup>2</sup> School of Pathology and Laboratory Medicine, University of Western Australia, WA, Australia

Platelets are subcellular blood elements with a well-established role in haemostasis. Upon activation platelets express P-selectin on the cell membrane and bind to PSGL-1 expressing monocytes, influencing them toward a pro-adhesive and pro-inflammatory phenotype. It is well established that elevated circulating monocyte-platelet aggregates are linked to atherothrombosis in high risk patients. However, whole blood flow cytometry has recently shown that circulating monocyte-platelet aggregates may also occur in the absence of platelet activation, particularly in healthy children. A potential limitation of conventional flow cytometry is the potential for coincident events to resemble monocyte platelet aggregates. Here we report a novel imaging cytometry approach to further characterise monocyte-platelet aggregate formation by P-selectin dependent and P-selectin independent mechanisms and distinguish circulating monocyte-platelet aggregates from coincidental events. Monocytes were identified by expression of the lipopolysachharide receptor (CD14 BV421), while platelets were identified by expression of the glycoprotein Ib (CD42b APC). Differentiation of P-selectin dependent and P-selectin independent binding was achieved with AF488 labelled CD62P. Analysis of circulating and in vitro generated monocyte-platelet aggregates by conventional and imaging cytometry methods showed very strong correlation ( $r_2 = >0.99$ ,  $p < 0.01$ ). The Bland-Altman bias of -3.68 was not significantly different to zero. However, a greater bias (5.98) and lack of correlation ( $r_2 = 0.27$ ,  $p = n.s.$ ) for P-selectin negative monocyte-platelet events likely reflects better discrimination of coincidence events using imaging cytometry.



## P249. Validation of a hybrid anti-Xa calibration assay in unfractionated heparin (UFH) monitoring - A single centre experience

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### Aim

UFH remains the preferred anticoagulant in selected groups of patients. Anti-Xa assays offer a reliable alternative option to aPTT in monitoring UFH. At our institution, we use low molecular weight (LMWH) specific calibrator (STA<sup>®</sup> calibrator HBPM/LMWH) with a corrective factor (instead of UFH specific), and corresponding aPTT therapeutic range established through UFH spiked plasma samples. However, this may cause imprecision. A new, hybrid chromogenic anti-Xa assay (STA<sup>®</sup> Multi-Hep calibrator) enables the monitoring of UFH and LMWH anti-Xa activity with a single calibrator. Our study aims to validate the Multi-Hep assay and evaluate the therapeutic aPTT UFH range using *ex-vivo* samples.

### Methods

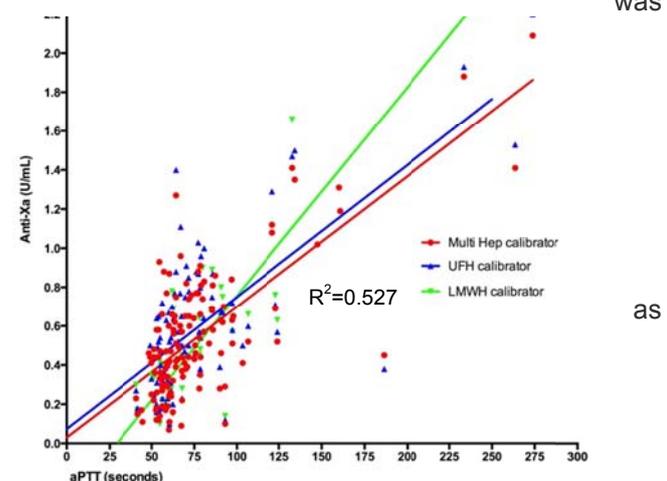
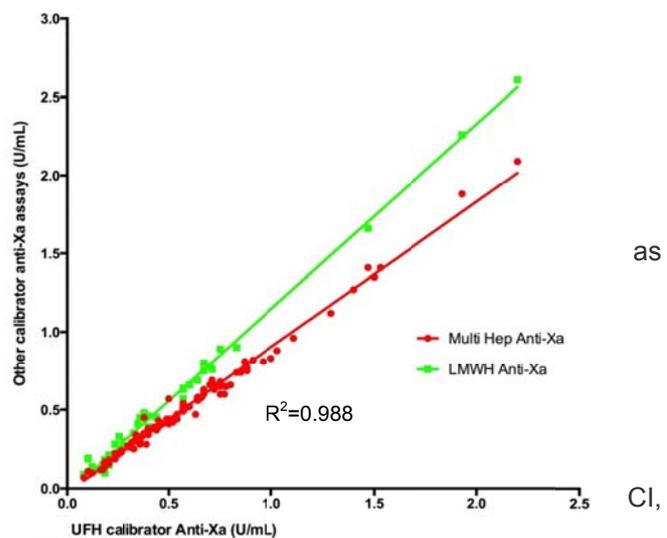
A retrospective, observational analysis was conducted for patients on UFH infusion between November 2013 and April 2014 at a tertiary hospital. Paired aPTT and anti-Xa measurements were performed using UFH specific anti-Xa assay (n=97), LMWH specific anti-Xa assay (with a corrective factor of 1.285, n=39) and Multi-Hep anti-Xa assay (n=138) on STA-R Evolution automated coagulation instrument (Diagnostica STAGO). Linear regression analyses were performed to investigate correlation between the different assays.

### Results

We demonstrated excellent correlations between the Multi-Hep assay and UFH specific ( $R^2=0.988$ , 95% CI, figure1) as well LMWH specific ( $R^2=0.994$ , 95% CI) anti-Xa assays. Using the established therapeutic anti-Xa range of 0.3-0.7 IU/mL, the corresponding therapeutic aPTT range for UFH was 52-94 seconds. However, poor correlation was demonstrated between the Multi-Hep and aPTT assays ( $R^2=0.527$ , 95% figure2). Only 60% of the aPTT and anti-Xa values were concordant; the discordance particularly evident in intensive care or septic patients.

### Conclusion

We validated Multi-Hep anti-Xa assay to monitor UFH and established therapeutic aPTT range of 52-94 seconds using *ex-vivo* samples. The Multi-Hep assay could be used



complementary testing to aPTT to dose UFH more accurately for selected groups of patients in whom aPTT measurement is unreliable.

## **P250. Prolonged APTT in a patient presenting with thrombosis unexpectedly identified as a Factor XII Deficiency**

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*Royal Hobart Hospital, Hobart, TAS, Australia*

### **Aim:**

An isolated prolonged APTT in a preoperative patient requires investigation due to possible increased risk of bleeding or thrombosis depending on the cause.

### **Method:**

APTT was performed on Stago STA-R Evolution using FSL Actin reagent and anti-Xa activity was performed using a chromogenic assay.

### **Results:**

We report a case of an isolated prolonged APTT in a 59 year-old male smoker who presented with an ischaemic left lower limb secondary to a thrombosis extending from his left superficial femoral artery endovascular graft (placed one month previously) to his left popliteal artery following mild trauma. On arrival the patient was receiving intravenous unfractionated heparin and the coagulation results were: APTT 182secs (27-37), PT 12.8secs (11.5-13.5), INR 1.0 (0.9-1.2) and anti-Xa activity was <0.1 U/mL (Target Range 0.3-0.7) indicating no heparin was present at the time of testing. A month earlier at the time of endovascular graft placement the APTT was also elevated (>201secs) and the patient was not receiving heparin. A lupus anticoagulant was suspected and the clinical team advised to monitor heparin therapy with anti-Xa activity. Mixing studies were performed and resulted in a 96% correction of the APTT which indicated a factor deficiency or inhibitor. Lupus anticoagulant screening was negative. As the patient did not have a clinical bleeding phenotype a Factor XII deficiency was suspected. Factor XII Assay result was <0.03U/mL (0.5-1.5) confirming deficiency.

### **Conclusion:**

Factor XII (Hageman factor) deficiency is a rare autosomal recessive disease. Epidemiological studies however have found no correlation between FXII deficiency and thrombosis. In John Hageman's and in this case additional factors relating to Virchow's triad would have predisposed to thrombosis.

## **P251. A preliminary comparison of a Chemiluminescence method for von Willebrand Factor Antigen and Ristocetin Cofactor with current methodology**

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### **Aim**

Laboratory diagnosis of von Willebrand disease (VWD) is dependent upon accurate measurement of VWF antigen (VWF:Ag) and ristocetin cofactor activity (VWF:RCo). This study compares the ACL AcuStar chemiluminescence assay for VWF:Ag and VWF:RCo with our standard testing (the STA-R Evolution, latex immunoassay assay for VWF:Ag, and platelet agglutination-based assay for VWF:RCo.)

### **Method:**

Citrated samples stored at -80°C from 50 patients (tested using the STA-R Evolution) were selected to include low, normal and borderline VWF levels. We had 50 episodes, of which 22 had a VWF:RCo <50IU/dL, and 28 with VWF:RCo >50IU/dL. These samples were double freeze-thawed, analysed on the AcuStar, and the results compared.

### **Results:**

Whilst there appeared to be encouraging correlation between the two test systems (*Table 1*) the Bland Altman analysis shows a systematic negative bias for the AcuStar for both the VWF:Ag and VWF:RCo, with the STA-R Evolution results being higher by 12.8% and 12.2%, respectively. This lowered the result below the 50IU/dL threshold in three samples, all of which were from paediatric patients. One adult patient had an isolated low VWF:RCo (STA-R Evolution), which may be artefactual. Several samples from a patient with Type 3 VWD following treatment with Biostate were included in the analysis.

	Correlation co-efficient	95% Confidence interval
VWF:Ag assay	0.9860	0.9754 - 0.9921
VWF:RCo	0.9636	0.9368 - 0.9792

*Table 1*

### **Conclusion:**

Our results showed that the AcuStar VWF:Ag / VWF:RCo assays were considerably less reliable at borderline (50IU/dL) levels. The effect of extra thawing on the AcuStar samples may be relevant. Further evaluation is required on samples run simultaneously on the two different test systems.

## **P252. Plasma fibronectin is a vital hemostatic factor in fibrinogen and coagulation deficiencies and is a unique self-limiting regulator in thrombosis**

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Plasma fibronectin (pFn) has long been suspected to play a role in hemostasis but direct evidence has been lacking. Here we demonstrated that pFn is vital for control of bleeding in fibrinogen deficient mice and in wild-type mice given anticoagulants. At the site of vessel injury, pFn rapidly deposits and initiates hemostasis even before platelet accumulation (the first wave of hemostasis). This pFn deposition is independent of fibrinogen, von Willebrand factor,  $\beta 3$  integrin, and platelets. Confocal and scanning electron microscopy reveals pFn integration into fibrin, which increases fibrin fiber diameter and enhances the mechanical strength of clots as determined by thromboelastography. Interestingly, pFn promotes platelet aggregation when linked with fibrin but inhibits this process when fibrin is absent. Therefore, pFn may gradually switch from supporting hemostasis to inhibiting thrombosis and vessel occlusion following the fibrin gradient that decreases farther from the injured endothelium. Our data established that pFn is a supportive factor in hemostasis, which is vital under coagulation deficient (both genetic and therapeutic) conditions. By interacting with fibrin and platelet  $\beta 3$  integrin, pFn plays a unique self-limiting regulatory role in thrombosis, suggesting pFn transfusion may be a potential therapy in controlling bleeding disorders, particularly in association with anticoagulant therapy.

## **P253. Platelet desialylation is a novel mechanism of Fc-gamma-R-independent platelet clearance and a potential diagnostic biomarker and therapeutic target in anti-GPIb-mediated thrombocytopenia**

Ni H<sup>1</sup>, Li J<sup>2</sup>, van der Wal D<sup>3</sup>, Zhu G<sup>4</sup>, Yougbare I<sup>4</sup>, Ma L<sup>3</sup>, Ruan M<sup>5</sup>, Zhu L<sup>5</sup>, Zeng Q<sup>5</sup>, Leytin V<sup>2</sup>, Freedman J<sup>1</sup>

<sup>1</sup> Canadian Blood Services; University of Toronto; St. Michael's Hospital, Toronto, ON, Canada, <sup>2</sup> St. Michael's Hospital; University of Toronto, Toronto, ON, Canada <sup>3</sup> St. Michael's Hospital, Canadian Blood Services, Toronto, ON, Canada; <sup>4</sup> St. Michael's Hospital, Toronto, ON, Canada <sup>5</sup> Anhui Medical University, Hefei, China

Autoimmune thrombocytopenia (ITP) is a common bleeding disorder caused primarily by autoantibodies against platelet GPIIb/IIIa and/or the GPIb complex. Current theory suggests antibody-mediated platelet destruction occurs in the spleen via Fcγ receptors (FcγR) on macrophages. However, we and others have demonstrated that, in contrast to anti-GPIIb/IIIa-mediated ITP, anti-GPIbα-mediated ITP is often refractory to therapies targeting FcγR pathways, but why remains unknown. Here, we developed a panel of murine monoclonal antibodies (mAbs) in gene deficient mice against both murine and human GPIIb/IIIa and GPIbα. Utilizing these mAbs and human ITP plasma, we found that anti-GPIbα induces not only platelet activation and apoptosis to a much greater extent than do anti-GPIIb/IIIa antibodies, but also significant surface expression of sialidase neuraminidase 1 and platelet desialylation. Utilizing inhibitors of platelet activation and desialylation, we found these two processes are not mutually exclusive, but rather exist in a positive feedback loop, leading to FcγR-independent platelet clearance in the liver via Ashwell-Morell receptors on hepatocytes. Furthermore, in a murine model of ITP, sialidase inhibitor treatment rescued platelet counts in anti-GPIbα-, but not anti-GPIIb/IIIa-mediated thrombocytopenia. These findings shed light on anti-GPIbα-mediated FcγR-independent platelet clearance and have important implications in both the diagnosis and treatment of refractory ITP.

## **P254. Australian Bleeding Disorders Registry – The evolution over 25 years of a national database for people with bleeding disorders**

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<sup>1</sup> *National Blood Authority, Canberra, ACT, Australia* <sup>2</sup> *Australian Bleeding Disorders Registry Steering Committee, Canberra, ACT, Australia*

### **Aims**

First established in 1988 as separate databases in individual haemophilia treatment centres (HTCs) across Australia, the Australian Bleeding Disorders Registry (ABDR) has continued to evolve over 25 years to support the treatment of people with bleeding disorders (PWBD) in the 16 HTCs in Australia.

### **Results**

The ABDR has evolved into a shared web based electronic summary record of the diagnosis, bleeds and treatments of all PWBD being treated in all Australian HTCs. Tight security, privacy and governance controls enabled staff from each HTC to have access to the full record of their patients, with staff at other HTCs limited to a summary containing the essential details of patients who their care transferred to an alternate HTC.

The ABDR also enables the NBA to access near-real time information on product use at an aggregated de-identified level to assist with national supply planning for the provision of clotting factors.

Launched in 2012, the fourth generation ABDR enhanced the functionality provided in previous versions including through the capture of enhanced data sets and reporting capabilities to enable HTCs to undertake practice improvement benchmarking and to collect and analyse data relating to overall activity within HTCs as part of the comprehensive care model.

The development of MyABDR, a patient interface using both web-based tools and smart-phone applications is the next evolution for the ABDR. MyABDR will enable PWBD to record bleeds and infusions into their ABDR record in real-time, enhancing the communication and sharing of essential clinical data with their HTCs in a systematic and coordinated manner.

### **Conclusion**

With a view to improving the availability of consistent and comparable data on haemophilia care worldwide, other countries may wish to consider the benefits of establishing a national haemophilia registry based on the ABDR model or code-base.

## **P255. Data and demographics from the Australian Bleeding Disorders Registry (ABDR)**

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<sup>1</sup> National Blood Authority, Canberra, ACT, Australia<sup>2</sup> Australian Bleeding Disorders Registry Steering Committee Canberra, ACT, Australia

### **Aim**

The Australian Bleeding Disorders Registry (ABDR) is a clinical registry for patients in Australia with bleeding disorders. It is used on a daily basis by clinicians in all Australian haemophilia treatment centres (HTCs) as a clinical tool to assist in managing the treatment of people with bleeding disorders and to gain a better understanding of the incidence and prevalence of bleeding disorders.

### **Method**

Aggregated de-identified data from ABDR and other ICT systems operated by the NBA is reported in the Annual ABDR Reports. The report presents an integrated view of current clinical and demographic information on people with inherited bleeding disorders in Australia and the resultant demand for clotting factor products.

### **Results**

There were 5,807 patients in the ABDR in 2012-13, with 2,391 patients with Haemophilia A (753 patients with severe HMA), 564 patients with Haemophilia B (106 patients with severe HMB), and 2,127 patients with von Willebrand Disease. Over 157 million IU of recombinant Factor VIII products were used by HMA patients, and over 25 million IU of recombinant Factor IX products were used by Haemophilia B patients in 2012-13. A total of \$202.2 million was expended by governments on clotting factor products in 2012-13. The most recent figures for 2013-14 will be integrated for discussion.

### **Conclusion**

The ABDR continues to be developed and the quality and timing of the data improves every year, while contributing to the work of all Australian HTCs. With the introduction of MyABDR in early 2014 and will provide real time data based on patient use.

**Note:** This abstract in part was submitted to WFH for 2012-13 data and is submitted again for interest of ASTH members and will include 2013-14 data.

## **P256. Use of Danaparoid in pregnancy complicated by Anti-Phospholipid Syndrome (APS) and possible Heparin Induced Thrombocytopenia (HIT)**

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**Background:** Anti-phospholipid syndrome is characterised by increased risk of thrombosis and recurrent miscarriage in pregnancy. Prophylactic anticoagulation with low molecular weight heparins (LMWHs) has been shown to decrease the rates of miscarriage, and has a low rate of fetomaternal complications. However, rarely heparins are contraindicated due to the development of HIT, in which antibodies to the Platelet Factor 4 (PF4)-heparin complex result in a high risk of thrombosis. In these cases, alternative anticoagulants must be used. Danaparoid is established in the treatment of HIT in non-pregnant patients, but its use in pregnancy has not been investigated in prospective studies.

**Aim:** We present a case of APS in pregnancy with a history of possible HIT successfully treated with Danaparoid, and to review the literature regarding its use in pregnancy, comparing with alternative anticoagulants.

**Methods:** The clinical notes of the case study were reviewed. A literature review was performed using a search of Medline for relevant publications.

**Results:** A 27 year old woman was referred to haematology with an obstetric history of G4P0, including 2nd trimester loss of twins secondary to twin-to-twin transfusion syndrome. She had high titre ANA antibodies with associated persistent lupus anticoagulant and weak anticardiolipin antibodies. She had been treated with Enoxaparin in her 3rd pregnancy, but treatment was stopped due to the onset of thrombocytopenia (nadir 58 x10<sup>9</sup>/L) associated with a positive HIT screen and miscarriage. Her 5th pregnancy was treated with Danaparoid 750mg bid and aspirin until 1 week prepartum, and resulted in delivery of a healthy boy without complications. The literature is reviewed including a series of 91 pregnancies treated with Danaparoid and compared to reports of the use of Fondaparinux and Argatroban.

**Conclusion:** The current case report and literature review suggest Danaparoid is safe and efficacious in pregnancy when LMWHs are contraindicated.

## **P257. Assessment of the safety and efficacy of anticoagulation and antiplatelet therapy in older adults with atrial fibrillation (AF)**

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**Background:** The prevalence of AF increases with age, reaching 10% by 80 years, with an associated stroke risk of 5x the general population. Anticoagulation reduces this risk, but a proportion of patients experience major haemorrhage or treatment failure. Determination an individual's risk of stroke versus haemorrhage before initiating anticoagulation would be of great benefit. However, assessment is difficult in older patients because, according to conventional clinical scores, the majority are at high risk of both haemorrhage and stroke.

**Aims:** To establish whether novel clinical and laboratory measures can predict adverse outcomes in older patients with AF.

**Methods:** Patients over 65 with AF were recruited and stratified according to anticoagulation. Baseline data included HAS-BLED (haemorrhagic risk) and CHA2DS2-VASC (stroke risk) scores. A clinical score assessing a cumulative deficit model of frailty, the Reported Edmonton Frail Score (REFS), was calculated for each patient and three global assays of haemostasis were performed: thrombin generation, fibrin generation and lysis (Overall Haemostatic Potential - OHP) and multiple electrode platelet aggregometry ('Multiplate'). Patient follow-up at 6 months was planned to record outcomes (haemorrhage, stroke or death).

**Results:** To date, 145 patients have been recruited (mean age 85). Interim analysis of 109 patients found 65 were assessed as frail and 44 non-frail by REFS. No difference was seen in HAS-BLED or CHA2DS2-VASC scores. However, using the OHP assay, the frail were significantly less hypercoagulable than the non-frail, with the most marked difference in the subgroup on no anticoagulants. Multiplate analysis revealed a trend to aspirin resistance in the frail. Interim follow-up revealed a low rate of adverse outcomes.

**Conclusion:** Amongst older patients with AF, a clinical frailty score (REFS) can discriminate patients with reduced fibrin generation and a trend to aspirin resistance. Final analysis including follow-up data will be presented to determine if these factors predict outcomes.

## **P258. Application of age-adjusted d-dimer cut off values for the diagnosis of venous thromboembolism in the elderly population**

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### **Aim**

Venous thromboembolism (VTE) is a commonly encountered clinical condition, especially in the elderly population. The D-dimer test is a validated tool in the exclusion of VTE but the level is known to rise with increasing age diminishing its sensitivity. The aim of this study was to apply validated age-adjusted cut-off values to a group of patients who were investigated for VTE and comparing the specificity of the test performance by patient age.

### **Methods**

An observational study of all patients who had a D-dimer requested for clinical suspicion of VTE from the Emergency Department at Royal Perth Hospital over a three month period. Age adjusted formula for patients over 50 years defined a positive d-dimer as levels  $> \text{age} \times 10\mu\text{g/L}$ .

### **Results**

420 patients were included with 19 events of VTE identified. For all patients, the sensitivity of the assay used at Royal Perth Hospital at a cut off value of 400 $\mu\text{g/L}$  was 100% with a specificity of 53.9%. Using a higher d-dimer cut off of 500 $\mu\text{g/L}$  improved the specificity to 65.3% at the expense of sensitivity which fell to 92%. Application of the age-adjusted cut-off values combined with the standard cut off of 500 $\mu\text{g/L}$  for those patients 50 years and under resulted in a sensitivity of 92% and specificity of 68.4%. Using the cut-off of 400  $\mu\text{g/L}$  combined with the age-adjusted rule resulted in a sensitivity of 100% with specificity 62.3%.

### **Conclusion**

Combining a d-dimer cut-off of 400  $\mu\text{g/L}$  with the age-adjusted rule for patients aged over 50 years produced the best combination of sensitivity and specificity in the diagnosis of VTE.

## P259. Case study of FXIII-A deficiency in Australian siblings

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Inherited Factor XIII (FXIII) deficiency is a rare, autosomal recessive bleeding disorder with an incidence of approximately 1 in 2 million live births, with 17 cases reported in Australia in 2012-2013. Genetic changes occurring in the *F13A1* or *F13B* gene cause a FXIII deficiency with mild to severe clinical manifestations with intracranial haemorrhage being the most common cause of mortality. Thus, FXIII deficient individuals tend to receive life-long prophylaxis. However, the timing and monitoring of such intervention remains a clinical and diagnostic challenge with little available supporting data. Here we report a case of two Caucasian, Australian born siblings aged 13 and 10 years old with known FXIII deficiency whose successful prophylaxis of four, then three weekly dosing was commenced at or soon after birth. The older female was diagnosed using functional tests when she presented with an umbilical bleed 10 days after birth, while the sibling brother underwent *in utero* genetic testing using a chorionic villus sample. Recently, further molecular analysis was undertaken by the Haemophilia Centre, Institute of Experimental Haematology and Transfusion Medicine in Germany and it was determined that both siblings shared the same point mutations in intron 5 (c.691-1G>A) and exon 15 (c.2111G>A, p.Arg704Gln) of the *F13A1* gene, as well as the common variant c.-19+12C>A in the promoter region of the *F13A1* gene. As such, these findings are consistent with the siblings' severe FXIII deficiency. Interestingly, despite the mother having a history of bleeding, her FXIII levels are normal. These siblings are the first two Australian patients to be added to the ETRO international registry of clinical, phenotypic and genotypic data for FXIII deficiency. The registry currently holds information about 104 patients (56 females and 48 males) out of 88 families from 14 countries. With the availability of both clinical and genetic information from a larger patient cohort, rare bleeding disorders will be advantaged by better treatment regimens and thus improved quality of life.

## **P260. Audit of heparin induced thrombocytopenia(HIT) testing at Royal Hobart Hospital**

**Sharma A**, Tegg L, Johnston A

*Royal Hobart Hospital, Hobart, TAS, Australia*

**Aim:** To review the appropriateness of HIT testing in Royal Hobart Hospital.

**Method:** We conducted a retrospective analysis of HIT testing from January 2010 to June 2014 . Data was extracted from Kestral PLS. Medical records of the patients were reviewed to assess the 4Ts score. The results of the HIT testing were correlated with 4Ts score. Based upon initial lack of confirmatory testing being offered, a change in our HIT testing policy has been implemented. This includes incorporation of 4Ts score in laboratory report, sample collection type and follow up of confirmatory testing by laboratory registrar.

**Results:** A total of 112 HIT tests were requested during the study period. Out of these 19 were positive using ELISA or particle gel immunoassay , 3 were weak positive and 88 were negative. For 4 cases the testing was not performed in consultation with the haematologist. Confirmatory testing was not performed in all the positive cases. The referral laboratory undertook platelet aggregation for confirmation. Seven samples that were referred for confirmation, all were negative for HIT antibodies.

**Conclusion:** Given the high false positive rate of serological testing for HIT appropriate patient selection for testing is important to avoid overdiagnosis. The results of this audit will determine the appropriateness of our current approach to HIT testing and will inform development of improved clinical and laboratory guidelines.

## **P261. Dysfibrinogenaemia associated thrombosis - a case report**

**Sharma A, Batt T, Robinson M, Tegg L, Prasad R, Mackie S**

*Royal Hobart Hospital, Hobart, TAS, Australia*

### **Introduction**

Dysfibrinogenaemia is characterized by structural abnormality of fibrinogen molecule resulting in altered functional activity. There may be discordance in the functional and antigenic assays. Patients might be asymptomatic or present with haemorrhagic or thrombotic episodes.

### **Case report**

A 83 year old female previously diagnosed with dysfibrinogenaemia presented with pseudotumour secondary to a hip joint prosthesis. At presentation she was noted to have extensive deep venous thrombosis.

She was diagnosed to have dysfibrinogenaemia 10 years before this presentation, when she was evaluated for post-operative bleeding. There was no family history of a bleeding or thrombotic disorder. There was no history of complications associated with pregnancy.

### **Results**

Immunological fibrinogen – 2.0g/L

Functional fibrinogen – 1.2 g/L

### **Management**

Management issues in this case were

Management of bleeding complications in the perioperative period.

Management of thrombosis

For the management of thrombosis an IVC filter was inserted and the patient was commenced on a therapeutic dose of enoxaparin with anti Xa monitoring. Patient was also treated with cryoprecipitate to maintain her fibrinogen >2g/L from day -1 to day 5. On day 7 cryoprecipitate was discontinued and she was commenced on therapeutic dose of enoxaparin. Dose requirement for enoxaparin was low than expected. An attempt was made to retrieve the IVC filter , however this was unsuccessful. In view of risk of bleeding , anticoagulation was ceased at 6 months.

### **Conclusion**

This case demonstrates complexity of management in a patient with dysfibrinogenaemia.

## **P262. Cytomegalovirus-associated immune thrombocytopenia (ITP) demonstrating response to eltrombopag.**

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<sup>1</sup> *St Vincent's Hospital, Darlinghurst, NSW, Australia,* <sup>2</sup> *Prince of Wales Hospital, Randwick, NSW, Australia*

### **Aim**

Symptomatic cytomegalovirus (CMV) infection in immunocompetent adults is rare. We present a case of CMV-associated ITP responsive to eltrombopag.

### **Result**

A 64 year old otherwise well, immunocompetent female presented with petechiae, epistaxis and platelet count of  $8 \times 10^9/L$ . Initial assessment was consistent with ITP. Platelet count increased to  $52 \times 10^9/L$  following oral prednisone and intravenous immunoglobulin (IVIG), however 18 days later was  $5 \times 10^9/L$ . Viral studies at diagnosis showed serological evidence of acute CMV infection with CMV viraemia, CMV IgM positive, CMV IgG negative, and CMV PCR titre of 7700U/L. Bone marrow biopsy showed normal megakaryopoiesis without CMV inclusions seen.

Prednisone was tapered, antiviral therapy was commenced with Valganciclovir and CMV hyperimmune globulin, and a second dose of IVIG was administered. Platelet count peaked at  $70 \times 10^9/L$ , before regressing to  $18 \times 10^9/L$  after a further nine days. Over the next three months, further doses of IVIG and CMV hyperimmune globulin were administered. Transient platelet responses were seen, however this was followed by gradual decline to platelet count of  $< 20 \times 10^9/L$  with occasional mild epistaxis. During this time, CMV viraemia level fell and remained at negligible levels.

With persistent severe thrombocytopenia, and concerns of immunosuppressive therapy and CMV illness, the patient was commenced on Eltrombopag in a clinical trial (SPRITE study). After 11 weeks of 50mg daily she has experienced an excellent platelet response count  $60-123 \times 10^9/L$ . The patient remains well with no evidence of CMV viraemia and no longer needing IVIG or Valganciclovir

**Conclusion.** In this case of CMV-associated immune thrombocytopenia, the observation that Eltrombopag was associated with a sustained platelet response in a patient previously refractory to conventional ITP and antiviral therapy is an interesting clinical finding which offers insight into pathogenic processes that mediate this uncommon condition.

## **P263. Prevalence of Coeliac and von Willebrand disease in women with menorrhagia and iron deficiency anemia**

**Sirdesai S** , Chunilal S

*Monash Health, VIC, Australia*

### **Aim**

The prevalence of coeliac disease (CD) in developed countries is approximately 0.5-1%. The prevalence of von Willebrand disease (vWD) is also approximately 1%, though few patients are appreciably symptomatic. However, in certain high risk groups - such as adults with iron deficiency anaemia (IDA) or women with menorrhagia - the prevalence of both conditions has been reported to be higher. Here we examine the prevalence of CD and vWD in women with IDA and menorrhagia.

### **Methods**

We audited the records of consecutive women who were referred to an outpatient clinic for IDA. From the clinical history we identified women with menorrhagia - defined as more than 80mls of bleeding per cycle. The majority of women were routinely screened for CD and vWD.

### **Results**

From 30 June 2012 to 23 May 2014 we identified 74 women with IDA and menorrhagia. Of these 74, 55 women were tested for CD and 58 for vWD. The average age of patients was 38 years. Mean haemoglobin, mean cell volume and ferritin were 96g/L, 70fL and 8ng/ml respectively. 5/55 (9%) women had serological or histological evidence of CD. Of these, 3 were new diagnoses ( $p = 0.1381$ , 2-tailed Fisher's exact test, assuming a background prevalence of 1%). A further 2 women had serology that was equivocal for CD. No cases of vWD were identified (0/58).

### **Conclusion**

IDA in the setting of menorrhagia is often attributed primarily to blood loss and CD is not routinely tested for. Our data suggest that, in spite of another readily evident cause for IDA, women with menorrhagia and IDA have a trend towards statistically higher than expected rates of CD. Therefore, testing all women with menorrhagia and IDA for CD should be considered. Conversely, we were unable to verify the previously reported prevalence of vWD in women with menorrhagia.

## **P264. Catheter Directed Thrombolysis (CDT) for prevention of Post Thrombotic Syndrome (PTS): A single centre experience**

**Tatarczuch M**, Ninkovic S, Quach H, Jhamb A

*St. Vincent's Hospital, Melbourne VIC, Australia*

### **Aim**

CDT is not routinely utilised in many centres for significant limb deep vein thrombosis (DVT). We aim to assess if CDT can prevent PTS for patients with a DVT.

### **Method**

Retrospective review of all patients who underwent CDT for DVT at St. Vincent's Hospital (Melbourne) during period of 08/2012-05/2014 (18 months). Upper limb (UL) and lower limb (LL) DVTs were included. Patients were identified through the interventional radiology database and data was extracted from clinical and radiology and databases.

### **Results**

Twelve patients identified

Patient characteristics: female: 9 (75%), median age: 43 (range 22-75)

DVT characteristics: location [UL: 4, LL: 8], side (R:L 3:9, UL: 2:2, LL: 1:7), occlusion (full: 10, partial 1, information not available 1 [NA]), provoked: 3, external vessel compression: 4 (May-Thurner Syndrome: 2, Paget-Schroetter Syndrome: 2)

Treatment: CDT/Thrombectomy: 12 (with angioplasty: 4, with angioplasty and stent insertion: 6), failed procedure: 2 (persistent clot: 1, abandoned procedure: 1), anticoagulation (warfarin: 11, rivaroxaban: 1), leg compression stockings: 3 (information NA: 3)

### Outcomes:

Symptoms resolved promptly, PTS not seen in any patients (median follow up: 10 months).

No serious procedural complications (urokinase allergic reaction: 1, stent occlusion: 2)

	Patients	Time from symptoms to procedures [days <sup>1</sup> ]	Time from imaging to procedure	Follow up [months <sup>1</sup> ]	Post thrombotic syndrome <sup>2</sup>
Upper limb	4	10 (1-16)	1 (0-9)	5 (3-7)	0
Lower limb	8	6 (3-156)	3.5 (1-6)	13 (3-21)	0
Entire cohort	12	7.5 (1-156)	2.5 (0-9)	10 (3-21)	0

### Key:

( )= range

<sup>1</sup>median

<sup>2</sup>Two lost to follow up

### **Conclusion**

In carefully selected patients, CDT is safe and reduces PTS. Factors to consider include: duration of thrombosis, extent of occlusion and risk of bleeding. Stenting should be considered, especially in those with external compression of the vessels.

## **P265. Thrombotic microangiopathy associated with intravenous abuse of the new tamper-resistant formulation of oral extended-release oxycodone hydrochloride (OxyContin)**

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### **Background**

In April 2014, in response to intravenous (IV) abuse of oral extended-release oxycodone hydrochloride (OxyContin), a new crush-resistant formulation with the intent to deter inappropriate tampering and abuse of the drug was released in Australia. Supply of the old formulation has been discontinued. We report a case of thrombotic microangiopathy (TMA) after IV abuse of the new tamper-resistant formulation that was successfully managed without plasma exchange.

### **Case Report**

A 56 year old Caucasian male presented with a three day history of peri-umbilical abdominal pain. He admitted to IV abuse of OxyContin over a period of months, however for the five weeks prior to presentation had been injecting the new tamper-resistant formulation due to inability to access the discontinued crushable form.

The patient had a blood pressure of 154/85, and mild peri-umbilical tenderness. Laboratory investigations showed his haemoglobin was 87 g/L, platelets  $53 \times 10^9/L$ , lactate dehydrogenase 769 U/L, unconjugated bilirubin 34  $\mu\text{mol/L}$ , reticulocytes 168, haptoglobin 0.04g/L (0.36-1.95g/L) and serum creatinine 66  $\mu\text{mol/L}$ . 3% of red blood cells were fragmented and polychromasia was present, consistent with microangiopathic haemolytic anaemia. ADAMTS13 activity was 70% (40-130%). We elected to manage conservatively without the use of plasmapheresis, steroids or anti-platelet agents. Spontaneous resolution of the microangiopathic haemolysis followed and subsequent outpatient review showed his parameters continued to normalise.

### **Discussion**

We are not aware of any reports of TMA after IV abuse of reformulated OxyContin. In August 2013, the US FDA and CDC issued warnings regarding reformulated oral extended-release oxymorphone hydrochloride (Opana ER; not available in Australia) after a link between IV Opana ER abuse and TMA was identified. Initial cases of reformulated Opana ER-induced TMA were treated with plasma exchange, however subsequent reports have demonstrated that plasma exchange is unnecessary.

We report the first case of reformulated OxyContin-induced TMA and demonstrate that conservative management is a valid approach.

## **P266. Eculizumab is effective therapy for atypical Haemolytic Uraemic Syndrome (aHUS): A case series of Australian patients**

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### **Aim**

To report on Australian patients with aHUS who have received the complement inhibitor, eculizumab, on compassionate grounds.

### **Background**

aHUS is an ultra-rare, genetic, life-threatening disease associated with high rates of end-stage kidney disease and premature death. Chronic, uncontrolled complement activation causes systemic thrombotic microangiopathy (TMA), acute kidney injury and multi-organ system damage. Prior to the advent of eculizumab, 33-40% of patients with aHUS died or reached ESKD with their first manifestation of the disease.

### **Methods**

Patients with a clinical diagnosis of aHUS who have received compassionate eculizumab therapy have been included in this case series.

### **Results**

10 patients with aHUS began treatment with eculizumab between 2010 and 2013. 80% of patients were female with presentation at 7 months to 40 years of age. The median duration of treatment was 12 months (range 4-42 months). All patients had haematological evidence of TMA and organ damage, had ADAMTS-13 activity of >5% and were negative for STEC. Five patients demonstrated multiple progressive extra-renal complications including severe hypertension, cardiomyopathy and neurological complications including headaches, fatigue, drowsiness, blurred vision, poor balance and seizures.

Eculizumab was well tolerated and all patients remain on drug with median follow-up of 18 months. All 9 surviving patients had a rapid haematological response with resolution of TMA and discontinued plasma exchange/infusions (PE/PI). Five patients (50%) were dialysis-dependent at initiation of eculizumab. Treatment with eculizumab was able to eliminate dialysis in 4 of these 5 (80%) patients. All patients experienced improvement or stabilisation of extra-renal manifestations.

### **Conclusions**

aHUS is a severe multi-system disease and eculizumab treatment is a promising therapy that allows for preservation of renal function and elimination of dialysis and PE/PI.

## **P267. Errors in laboratory measurement of fibrinogen in a patient with dysfibrinogenaemia**

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We describe a 26 year old female, seven weeks pregnant, who presented to the PAH emergency department with a possible pulmonary embolism. A coagulation profile and D Dimer were performed on an ACL TOP 700 optical coagulation analyser. The APTT, PT and D Dimer results were normal (reference interval APTT 24-39s, PT 9-13s, D Dimer cutoff <0.23), however the derived fibrinogen (FibD) gave a low result of 0.9g/L (reference interval 1.7-4.5g/L). A claus fibrinogen (FibC) was performed but no fibrinogen could be detected using Haemosil IL (Instrumentation Laboratory) thrombin reagent. A reptilase time (11s, reference interval <20s) and thrombin clotting time (TCT) (19s, reference interval 12-17s) were also performed. An aliquot of the sample was sent to another laboratory for a FibC to be performed on a StaR analyser which uses mechanical clot detection and a normal FibC was obtained (2.8g/L, reference interval 2.0-4.0g/L).

These results were unusual as the optical analyser could not detect the clot in the functional FibC assay while the mechanical clot detection of the StaR analyser could. Although not the typical presentation, these results pointed to type II dysfibrinogenaemia and further protein and DNA testing were performed on the patient and her parents. Fibrinogen B $\beta$  chain mass spectrometry and DNA sequencing showed the patient and her father were heterozygous for a B $\beta$ 166Arg $\rightarrow$ Cys mutation in the coiled coli region of the molecule. This causes the formation of S-S linked dimers and trimers of fibrinogen to form in circulating plasma. The presence of these fibrinogen multimers disrupts the ordered lateral stacking of the fibrin monomers that form after thrombin activation. And because lateral stacking and final fiber thickness determine the optical response in turbidity based polymerisation assays the B $\beta$ 166Arg $\rightarrow$ Cys mutation compromises the result.

**P268. To evaluate the effectiveness of a chlorhexidine gluconate transparent dressing in reducing rates of catheter related bloodstream infections in Haemato-Oncology patients with central venous catheter in a regional hospital in Hong Kong**

**Choy W**

*Pamela Youde Nethersole Eastern Hospital, Chai Wan, Hong Kong*

**Background**

Immunocompromised haemato-oncology patients with long term central venous catheters (CVC) susceptible to infection, the concern for catheter related bloodstream infections (CRBSI's) are ever present in haemato-oncology care. CRBSI rates are continuously monitored as a sensitive nursing Indicator. Chlorhexidine (CHG) dressing was introduced as one of new option used in some Haemato-Oncology patients in Pamela Youde Nethersole Eastern Hospital, Hong Kong.

**Method**

A 12-months prospective follow-up study of 58 Haemato-oncology patients with CHG impregnated dressing was performed. The subjects were recruited by convenience sampling. Treatment outcomes in terms of

- i. Incident rate of CRBSI
- ii. Local site allergies

Allergies included exit site redness, skin rash and blister.

To reduce CVC associated infections, the skin is treated with 2% CHG with alcohol before insertion, application of steri-strips and dressings.

**Result**

The rate of CRBSI was significantly reduced from (31% to zero incidence,  $p \leq 0.003$ , unpaired t-test) after the intervention. Severe contact dermatitis was seen in 10% (3/29) of patients treated with a CHG impregnated dressing while 0% in conventional group.

**Conclusion**

1.CHG-Impregnated dressing on CVC is effectively reducing the rates of CRBSI in our haemato-oncology patients in PYNEH.

2.CHG did not produce an immediate inflammatory response in this clinical population.

3.The difference in CHG group and conventional group has a significant higher infection rate when compared to historical data of 5.5% (from 2007 to 2011). Nevertheless our early experience suggests its beneficial effect in decrease CRBSI.

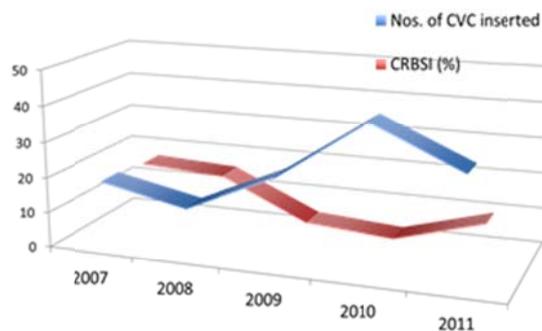


Fig.1 Incidence of CRBSI in 2007-2011

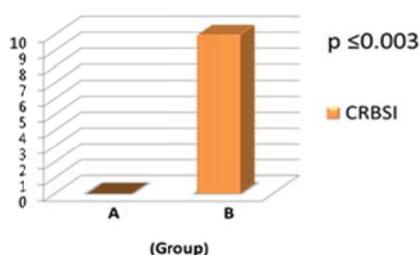


Fig.2 Incidence of CRBSI in 2012-2013

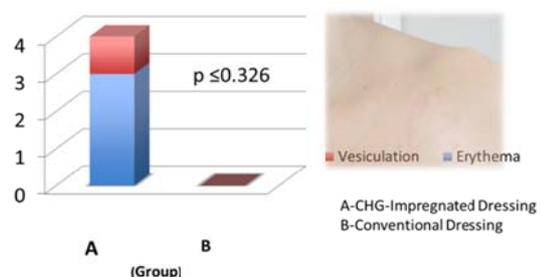


Fig.3 Change in skin integrity

## **P269. Are the myeloma population computer literate?: Squashing the snail mail**

**King H, Burgess J, Houdyk K**

*Myeloma Australia, Richmond, VIC, Australia*

Myeloma Australia (MA) is a not-for-profit organisation providing information and support to the myeloma community. Information is distributed in the form of books, fact sheets and a quarterly magazine. As a disease of the elderly, most people who are seeking information are from a generation educated before the computer age.

### **Aim**

To establish what proportion of members would be capable of receiving electronic information and how they would prefer resources to be distributed.

### **Method**

MA members completed an anonymous hard-copy survey with 15 questions, 13 questions multiple choice and 2 questions short answer. Ethics approval was granted by the Royal Melbourne Hospital HREC, Quality Assurance Sub-Committee. The data was then collected, collated and analysed by MA.

### **Results**

256 participants completed the survey of which 44% were male and 56% were female. The mean age group was 61-65 with the majority residing in metropolitan areas (68%) in Victoria, NSW and SA corresponding to the states of MA's largest presence. 59% of respondents were patients, with carers (25%), family (13%) and friends (3%) the remainder.

Only 11% of participants did not use computers, and of these 75% were over 65 years of age (equal gender representation). Of this group, the geographic location was spread in the same proportions as the whole sample. The majority of this group did not use computers because they either do not know how to use (57%), or do not own (43%), one.

Of those that use computers (89%), 82% have accessed myeloma information on the internet and 93% check their emails at least weekly.

70% of all participants prefer their information delivered electronically via email, website download or USB stick.

### **Conclusion**

These results suggest the majority of MA members are computer literate and prefer their information delivered in an electronic format.

## **P270. Can Triple Lumen (TL) Peripherally Inserted Central Catheters (PICCs) be used in the Haematology setting? A single unit's experience**

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**Aim.** Compare and evaluate TL PICCs with CVCs for haematology patients and evaluate the development of a nurse-led insertion team.

**Background.** Patients with acute haematology malignancies require central venous access (CVA) to safely and effectively manage their treatment. Historically CMN has used short-term, non-tunnelled CVCs. The availability of TL PICCs at CMN provided the opportunity to evaluate an alternate CVADevice.

**Methods.** Eligible patients received a TL PICC over 12 months (n=8). Data was compared to a retrospective cohort of patients receiving CVCs (n=27). Line dwell-time, number of lines required and reasons for removal were used as the evaluating comparators. Additionally, the impact of a nurse-led insertion team was evaluated using a satisfaction survey and reduced need for out of unit referrals for insertion.

**Results.** Eight (n=8) patients received a TL PICC, 4 (50%) completed all cycles of chemotherapy, 1 (12.5 %) removed before completion of chemotherapy due to suspected line related infection, 1 (12.5%) due line related thrombus, 1(12.5%) due to exit site infection and 1 (12.5%) removed on completion of first cycle of chemotherapy (safety reasons).

In the retrospective cohort of twenty seven (n=27) patients, there were 22 CVC insertions for 18 patients, 9 other patients had either a DL or SL PICC. Of the patients with CVCs no patient had the same line for all cycles of chemotherapy, 11(50%) removed after completion of first cycle of chemotherapy, 3 (14%) died with line insitu, 3 (14%) removed with suspected line related infections, 2 (9%) removed due to lumen blockage, 2 (9%) removed with exit site infections and 1 (4%) transferred to another facility.

Turnaround time for line referrals was evaluated to be less than 48 hours.

**Conclusion.** TL PICCs are an alternate form of CVAD for haematology patients with at least equivalent benefits to traditionally used CVC at CMN. The nurse-led insertion team provided a useful alternative with at least equal efficiency to medical staff insertion. TL PICCs and the nurse-led insertion team continue to be used for haematology patients at CMN as a result of this study.

## **P271. Integrative literature review of instruments used to assess informational and practical needs of acute leukaemia and lymphoma survivors**

**Taylor K**<sup>1,2</sup>, **Monterosso L**<sup>2,3,4</sup>

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### **Aim**

To identify validated measurement instruments that will assess informational and practical concerns of leukaemia and lymphoma survivors. Haematology cancer nurses have the potential to lead the way in providing excellent post treatment survivorship care for the increasing number of haematology survivors. An important element of care is assessment of haematology survivors' perceived needs for the provision of appropriate resources and support. Unlike other cancers, haematological cancers are highly variable in disease type and treatment.

### **Method**

This Integrative literature review utilised a search of electronic databases (CINAHL, Medline, PsychInfo, PubMed, EMBASE, PsychArticles, Cochrane Library) for eligible articles published between 1970 and 2014. Articles were included if they described an instrument to assess informational and practical concerns of leukaemia and/or lymphoma survivors.

### **Results**

Ten full text articles were identified that described cancer-specific instruments used to assess informational and/or practical needs of the haematology cancer survivor. There was variation in use of cancer survivor-specific instruments and generic quality of life cancer instruments. Most studies reported instruments to measure ongoing concerns around cancer recurrence and screening, and the necessity to identify patients at higher risk of unmet needs along the cancer survivor continuum.

### **Conclusions**

No identified instrument was haematology-survivor specific. It is therefore difficult to determine the best instrument to use with haematology survivors. The development of a reliable and validated haematology survivor instrument that assesses supportive care needs and survivors' desire for support and resources is warranted. This could be used in conjunction with nurse-led survivorship clinics.

## P272. Patient outcomes for ViP, a home-based injection program for patients treated with azacitidine.

Whitelaw K

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**Aim:** Explore whether the Vidaza Injection Program (ViP), a home-based injection program for patients with myelodysplastic syndromes (MDS) treated with azacitidine (AZA), improved patient-reported outcomes.

**Method:** AZA was administered to patients with MDS in the hospital for their early cycles. Those deemed appropriate by the treating haematologist for home administration were enrolled into ViP for subsequent cycles. Injection 1 was completed in hospital (together with a full nurse assessment) and all subsequent injections (2–7) were home-based, administered by a nurse.

Patient reported outcomes were captured in survey using a visual analogue scale (VAS, 1–10). All data reported as mean±standard error.

**Results:** Figure 1 illustrates all individuals involved in ViP.

To date, a total of 9 patients with MDS have participated in ViP, of which 7 responded to the survey. Patients were aged  $67\pm 4.1$  years received  $5.9\pm 2.3$  cycles prior to ViP commencement (range: 1–19).

All patients acknowledged that the treatment schedule of ViP was 'easy' (VAS 1) with a  $7.1\pm 0.7$  point improvement in their VAS versus pre-ViP. All patients were also 'extremely satisfied' with ViP (VAS 10) and reported they could return to regular daily activities, including general household (n=4) and social (n=3) aspects. Home-based injections saved the patients a total of  $3.4\pm 0.4$  hours each day ( $\approx 23.6$  hours/week, approximately an extra day per week).

**Conclusion:** A pilot model of care has been established to manage AZA-treated patients. AZA was administered as successfully in the home environment as it was in the hospital environment. ViP improved the QOL and extended the AZA treatment duration for patients who may have ceased therapy whilst in response.

*This study and ViP are funded by Celgene.*

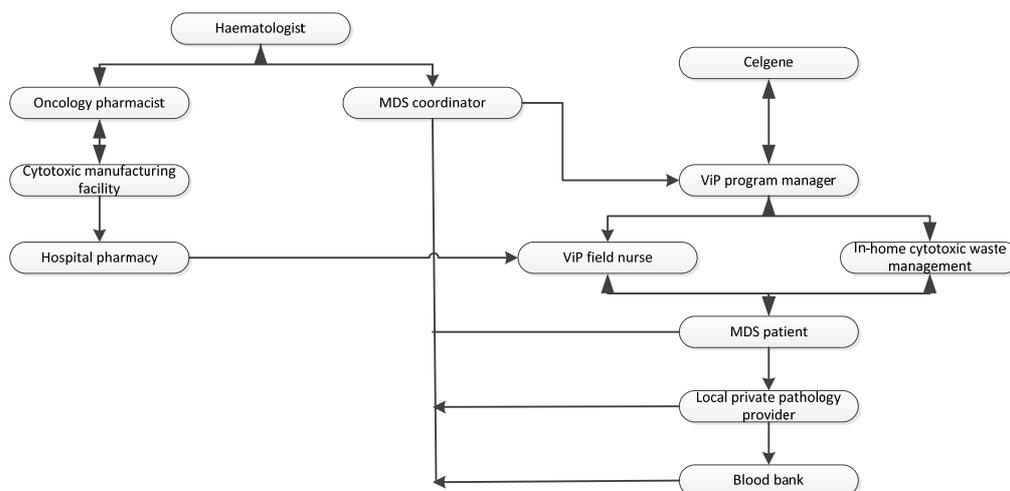


Figure 1

## **P273. Case study – Nursing management of haploidentical transplant patients**

**Williams E, O'brien S, Collin E**

*Canterbury District Health Board, Christchurch, New Zealand*

Pre-haploidentical transplants offer an important alternative for patients requiring transplant who do not have a sibling match or matched unrelated donor. A haploidentical transplant uses a donor who is a partial HLA match. This type of donor is often more accessible as they can be a parent, sibling or child. 90% of patients have a haploidentical family member.

The disadvantages associated with haploidentical transplant are: increased risk of graft versus host disease, graft rejection and delayed recovery of the immune system making the patient more susceptible to infectious complications.

We present the case of Mr Smith, a 49 year old male, diagnosed with CML in acute phase. Following high dose chemotherapy of CHOEP, Hyper CVAD and radiotherapy the plan was to proceed with a sibling allogeneic stem cell transplant. Unfortunately no full sibling match or matched unrelated donor was found and he proceeded with haploidentical stem cell transplant with his brother.

Post transplant, Mr Smith's major complications were mucositis, neutropenic sepsis, diarrhoea and severe hiccups. Two months post transplant Mr Smith unfortunately developed transverse myelitis causing a complete paraplegia from T9.

This case study will discuss nursing management of these complications. Mr Smith is the second patient in the South Island to have a haploidentical transplant. Although the principals of nursing care are similar to that of allogeneic transplant, the identification of risk factors and early effects is important to ensure best interventions are carried out.

## **P274. Audit of the implementation of a structured needs assessment in the malignant haematology outpatient setting.**

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### **Introduction**

Many cancer patients experience confusion and lack of information in their dealings with cancer care services and they are often unable to access appropriate care in a timely manner. In Western Australia, the Cancer Nurse Coordinator (CNC) position was implemented in 2006 as both a strategic and clinical role to facilitate continuity of care and access to appropriate resources for cancer sufferers. A key component of this role is a comprehensive assessment to ensure the appropriateness of onward referral. Whilst this assessment activity is currently being undertaken it is not always in a formal structured manner or at key points within the patients care pathway. A key strategic goal is to implement a formal assessment process to facilitate uniformity and promote patient empowerment.

### **Aim**

This paper will discuss the findings from an audit examining the process of implementing a validated structured needs assessment within the adult haematology setting as a component of care coordination. This service improvement initiative is being undertaken in collaboration with other tumour specific CNC services.

### **Method**

The assessment will be undertaken by asking newly diagnosed adult haematology patients within the outpatient setting to complete the SPARC (Sheffield Profile for Assessment and Referral for Care) assessment tool, followed by a phone follow-up and face to face consult. An action plan is then formulated and given to both patient and treating team outlining the key referrals and management strategies for the issues identified by the patient. Audit of the process will be undertaken by surveying approximately 30 patients as well as the health care professionals involved in patient care, to identify their experience with the action plan in communicating care within the context of care coordination.

### **Results**

Findings from the audit will be identified and future service improvements will be discussed.

## **P275. Haematopoietic Progenitor Cell (HPC) proficiency testing**

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### **Aim/Background**

In the complex process of assessing a laboratory's and its staff competence, reviewing performance in proficiency testing (PT) has a significant place as a meaningful and helpful indicator. Proficiency testing supplements the internal quality control system, providing a means of assessment of its testing and measurement capabilities. The proficiency test provides the opportunity to investigate any outlier results especially when compared to consensus results from other laboratories (where applicable), identify the root cause(s) of the problem and improve the performance where needed.

### **Methods**

PT test performed by staff includes the CFU Assays and CD34 Enumeration on each frozen sample using the StemCell Technologies QC – Human bone marrow, on a monthly basis by 2 of the 4 staff involved on the alternative basis, such that the end of 12 months testing period all staff would have performed six tests spread throughout the year.

### **Results**

The CD34+ cell enumeration parameters showed no marked outlier results compared to the group result, indicating that all scientific staff can produce results for CD34+ cell enumeration consistently and reproducibly. The results for the CFU-GM enumeration showed a couple of outliers, especially for the in-house method, however, due to the subjective nature of the colony counting this will have to be monitored and reviewed in the long term to be able to form a baseline in order to benchmark the results. Using the CV results from the Stemcell Technologies Frozen Bone Marrow Proficiency Testing Program as a benchmark, the average CV reported for total colony enumeration from over 130 worldwide laboratories was 47%. When we use this in comparison to our individual scientific staff results there are no marked outliers.

### **Conclusion**

The results in this report indicate that the scientific staff can reproduce consistent results for CD34+ cell enumeration.

## **P276. "A pilot to guide the way": Functional and phenotypic characterisation of haematopoietic progenitor cell-apheresis product for infusion using pilot vial material, a correlation analysis**

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### **Background and Aim**

Cryopreservation is often used to store Haematopoietic Progenitor Cell – Apheresis (HPC-A) product in the treatment of haematological malignancies. A pilot vial (PV) is usually cryopreserved and stored under the same conditions as HPC-A to assess HPC-A post-thaw viability prior to infusion. However, whether PV material can be used to assess other functional or phenotypic stem cell markers in HPC-A material following cryopreservation is unknown. We aimed to assess whether markers of viability, early apoptosis, DNA damage signaling and oxidative damage, together with CD34 subset analysis performed similarly using PV and HPC-A material following cryopreservation.

### **Method**

Twenty matched pairs of HPC-A and PV were identified. HPC-A and PV were simultaneously thawed to 37 °C. CD34 viability was assessed by 7-aminoactinomycin D (7-AAD); mitochondrial membrane potential was assessed using DiIC<sub>5</sub> staining as a marker of early apoptosis, intracellular aldehyde dehydrogenase (ALDH) activity by the ALDEFUOR Assay kit and stem cell subsets including CD133 and CD38. DNA damage was assessed using  $\gamma$ H2AX, intracellular Reactive Oxygen Species (ROS) level were assessed using 2', 7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCFDA). All assays were performed by flow cytometry.

### **Result**

There was a significant and linear correlation between HPC-A and PV material in all markers assessed. All markers, except viability, are expressed as fractions of viable CD34. For HPC-A vs PV, viable CD34 % was 1.77 (0.02-3.96) vs 1.77 (0.02-3.30) ( $p < 0.005$ ,  $R = 0.94$ ), % DiIC<sub>5</sub> staining was 35.46 (2.8-67.56) vs 50.62 (4.59-77.45,  $p < 0.005$ ,  $R = 0.72$ ), % ALDH activity was 49.98 (3.41-76.41) vs 62.67 (5.25-81.36,  $p < 0.005$ ,  $R = 0.87$ ), %ROS activity was 65.1 (16.4 - 87.6) vs. 64.05%(21.1 - 96.2)%, and %  $\gamma$ H2AX was 4.71(1.1-91.7) vs. 9.7 (1.6 - 91.8) ( $p < 0.005$ ,  $R = 0.96$ ). CD133+ and CD38-% were 67.33 (25.38-88.61) vs. 64.80 (18.48-90.52) and 35.4 (4.3-64.5) vs 35 (12.1-58.7) ( $p < 0.005$ ,  $R = 0.90$  and 0.72 respectively).

### **Conclusion**

Cryopreserved HPC-A and PV material appears to behave similarly when assessing functional and phenotypic stem cell markers in addition to viability assessed by 7-AAD. PV material is a suitable quality and research surrogate for HPC-A.

## **P277. StemLab Implementation of a database system at CTTWA**

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StemLab is a tissue and cell therapy Database Management System (DBMS) which assists cell therapy facilities with quality control, managing inventory, generating reports and traceability, including the ability to correlate product manufacture with clinical outcomes. Cell & Tissue Therapies WA (CTTWA) is a state and federally funded manufacturing facility within Royal Perth Hospital that currently manufactures a range of products for clinical transplantation including a full range of haemopoietic stem cells. Our facility is licensed by the Australian regulator, the Therapeutic Goods Administration (TGA).

To assist in the quality and traceability of our products as well as addressing national and international accreditation organisations such as FACT/JACIE and TGA a DBMS system was implemented. Stemlab provided the customization required for a cell and tissue based therapies while allowing further expansion for future clinical manufactured products. The role out of the software was detailed to cover areas such as client selection, production and test environments, software/reports construction and staff allocation. Once all aspects of software construction and software environment were completed the software was validated using Installation Qualification, Operational Qualification and Performance Qualification prior to end user input. The software was successfully implemented and now fills the needs of an expanding biotherapeutic manufacturing sector incorporating all Haemopoietic cell and autologous serum eye drop manufacture.

## **P278. Stem cell expansion studies for the treatment of leukemia**

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Stem cells (SCs) are a powerful tool in regenerative medicine because they may potentially provide an unlimited supply of many types of highly specialised cells for purposes of stem cell therapy and tissue engineering applications. SCs have more recently attracted interest in scientific research and are increasingly emerging in many therapeutic fields and applications. Leukemia patients desperately require healthy stem cell samples for transplantation after undergoing chemotherapy and radiation. There is an enormous shortage in the number of donors and the chances of finding a distinctly matching blood type are slim.

Alongside the huge shortage in donors, human ESCs are difficult to obtain with respect to strict ethics and policies governing their legal procurement and use. Hence, growing and culturing them in scalable quantities is of significant interest. Established techniques for growing ESCs are mostly based on 2D growth culture. The growth of these cells in 3D suspension culture (i.e. bioreactors) has recently emerged. This has several key advantages, including achievable high cell densities, ease of scale-up and control, as well as homogeneity particularly with respect to nutrient and dissolved gas distributions.

In this paper, we focus on effective methods and protocols for the rapid expansion of stem cells in 3D culture while maintaining their pluripotency, in minimum time. Several parameters will be investigated (such as CO<sub>2</sub> levels, pH, and feeding patterns).

**Keywords:** Stem cells, 3D culture, bioreactor.

## P279. Challenges associated with depletion of TCRαβ+ T cells and CD19+ B cells using the Miltenyi CliniMACS Device

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T cell depletion has been demonstrated to reduce the incidence of acute and chronic GVHD following allogeneic transplantation using alternative donors. Clinical systems for T cell depletion include the enrichment of CD34+ cells, the depletion of CD3+ T cells and CD19+ B cells, and more recently, the depletion of TCRαβ+ T cells and CD19+ B cells using the Miltenyi CliniMACS device. The Cellular Therapy Laboratory at the Royal Brisbane and Women's Hospital has performed two TCRαβ+ / CD19+ depletions of HPC, Apheresis collected either from a matched voluntary unrelated donor for a recipient with Fanconi anaemia (Patient 2347) or a haploidentical donor (Patient 163) for a recipient with X linked SCID.

The results of the TCRαβ+ T cell / CD19+ B cell depletions are tabulated in Table 1.

**Table 1 - Cell Recoveries Post TCRαβ+ T cell / CD19+ B cell Depletion on the Miltenyi CliniMACS**

	Patient UPN 2347 A500314000133				Patient UPN 163 A500313000293			
	HPC, Apheresis	Pre Selection	Post Selection	Infusion	HPC, Apheresis	Pre Selection	Post Selection	Infusion
Viable nucleated cells per kg (10 <sup>8</sup> )	150.6	91.0	60.6	13.1	108.5	80.1	46.3	15.4
Viable CD34+ cells per kg (10 <sup>6</sup> )	299.4	179.3	166.0	35.8	93.9	70.4	59.9	20.0
Viable TCRαβ+ T cells per kg (10 <sup>6</sup> )	2838	1859	0.23	0.05		2152	0.15	0.05
Viable TCRδγ+ T cells per kg (10 <sup>6</sup> )	9.3	6.1	7.4	2.0		85.6	44.25	14.7

The infusion of TCRαβ+ / CD19+ depleted HPC, Apheresis was limited to a maximum of 5 x 10<sup>4</sup> TCRαβ+ T cells per kg recipient weight (6.5kg and 7.2kg respectively) with cryopreservation of the excess TCRαβ+ / CD19+ depleted HPC, Apheresis. Both patients engrafted rapidly (ANC > 0.5 x 10<sup>9</sup>/L and Plt > 50 x 10<sup>9</sup>/L) at day 9 (ANC and Plt - Patient 2347), and day 11 (Plt - Patient 163) and 14 (ANC - Patient 163) post infusion with minimal acute GVHD. Patient 163 had reactivation of CMV post transplant.

Challenges for the processing and testing laboratory include the time taken for processing and cryopreservation of excess cells (non-depleted and depleted but not infused) (range - 14.5 to 17h for 2 - 3 scientific staff), staff training and complex flow cytometric analysis for low numbers of viable

TCR $\alpha\beta$ + and viable TCR $\delta\gamma$ + T cells necessitating the analysis of large numbers of cells per tube ( $>1 \times 10^6$  viable CD45+ cells).

It is envisioned that the depletion of TCR $\alpha\beta$ + T cells and CD19+ B cells using the Miltenyi CliniMACS device will become a routine laboratory procedure in the future with an additional two, and possibly three procedures scheduled for the Cellular Therapy Laboratory within the next month.

## **P280. Do Nucleated Cell and CD34+ Counts performed at the collection centre correlate with those performed at the Transplant Centre?**

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### **Background**

The use of voluntary unrelated donors for allogeneic transplantation necessitates the collection of HPC at centres within Australia and overseas. The participation of laboratories testing and processing HPC in accreditation programs such as FACT and NATA, together with the increasing standardisation of CD34 analysis to the single platform ISHAGE protocol, should result in greater consistency between results obtained for nucleated and CD34 cell counts at collection and transplant centres.

### **Aim**

To compare the results of nucleated and CD34+ cell counts performed at the collection centre with those performed at the transplant centre.

### **Results**

The total nucleated and total CD34<sup>+</sup> cell counts on reports issued by the collection centre for non-cryopreserved HPC, Apheresis and HPC, Marrow collected from voluntary unrelated donors between 01 January 2013 and 29 July 2014 was compared with the results obtained by the Cellular Therapy Laboratory. During this period 111 HPC products collected from voluntary unrelated donors were received in the laboratory. Thirteen HPC products were received from voluntary unrelated donors collected at the Royal Brisbane & Women's Hospital for recipients at the Royal Brisbane & Women's Hospital or Royal Children's Hospital and these results were excluded from the analysis. In addition, some results were missing from the initial collection centre reports and the final report containing the missing data had not been forwarded to the laboratory.

The analysis included 82 HPC products collected from voluntary unrelated donors of which 7 were HPC, Marrow and 76 were HPC, Apheresis. There was a good correlation between nucleated cell counts and CD34+ cell counts performed at the collection versus the transplant centre ( $r^2 = 0.981$  and  $r^2 = 0.957$  respectively). Reasons for poor correlation between individual results were apparent errors on reports generated by the collection centre.

### **Conclusion**

Minimal variation in nucleated cell and CD34+ cell counts obtained at the collection centre versus the transplant centre is observed. However apparent errors in the reporting of some these results at the collection centre is observed. This emphasises the need for transplant centres to repeat the testing of all HPC products obtained from voluntary unrelated donors collected at collection centres both within Australia and overseas.

## **P281. Autologous serum eyedrops: Patient survey of efficacy**

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Autologous Serum Eyedrops (ASE) are manufactured on request of medical staff for patients with various ocular conditions. CTTWA has manufactured ASE since 2005, with demand dramatically increasing in recent years. To manage the increased demand, manufacturing practices have been streamlined to provide a 3 month supply for each request. Even though ASE are being widely prescribed in our and other jurisdictions, there is little published studies assessing their efficacy. The aim of this study was to survey patients using ASE to determine the usefulness of these eyedrops.

Serum eyedrops are prepared from 100mL whole blood, clotted and serum obtained. The serum is diluted to a final concentration of 20% with saline, sterile filtered and dispensed as 2mL aliquots into 3mL sterile dropper bottles. Mandatory infectious disease screening is performed on patients and products are tested for microbial contamination. An expiry date of 3 months is assigned provided that the bottles are kept frozen until required for use. Patients receive approximately 100 bottles per request.

Patients receiving ASE between 2012–2013 were surveyed to rate the effectiveness of the eyedrops. Patients were asked to rate their symptoms and the effect (worse - much better) of ASE. The responses from 31 patients, aged 34–80 years, with graft versus host disease (7), corneal ulceration (3) dry eyes/Sjögren's syndrome (12) or other conditions (9) were evaluated. No adverse side effects related to the use of ASE were reported. Seventy percent of patients reported a "better" or "much better" overall change to their eye symptoms. Sixty four percent reported that the ASE were superior to other commercial tear products. Even though 27% of patients reported an overall change of "a bit better" they continued to have ASE prescribed.

In conclusion, most patients reported an overall beneficial therapeutic effect with the use of ASE. The manufacture and supply of ASE is a simple, safe and an inexpensive process.