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001. A new level of control of thrombosis

Hogg P

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Platelet activation and coagulation are initiated by discrete cleavage of peptide bonds in platelet receptors and coagulation factors. Old and new anti-thrombotic drugs block these chemical events and effectively control unwanted thrombosis. It has become clear in the last few years that thrombosis is also regulated by cleavage of the next most common covalent bond linking the protein backbone – the disulphide bond. Disulphide bonds link the sulphur atoms of cysteine amino acids. Cleavage of certain disulphide bonds (known as allosteric disulphides) in platelet receptors and coagulation proteins has been found to be critical for initiation of thrombus formation in mice and likely also humans. Small molecules that target the factors that cleave the disulphide bonds are in clinical development. An overview of this emerging biology will be presented.

002. Karl Landsteiner was on to something

Wood E

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Over their long careers, Ruth Sanger and Karl Landsteiner each made many discoveries that improved the safety of blood transfusion around the world. In his Nobel Prize acceptance speech, Landsteiner said that “thorough study of cases with undesirable after-effects will help us to assess the significance of the suspected causes and perhaps reveal unknown causes, and thus finally virtually eliminate the slight risks which transfusion still involves.”

Although blood supplies in Australia and New Zealand are now very safe from many infectious and other hazards, our clinical transfusion practice can undoubtedly be safer than it currently is. Haemovigilance is a tool to identify, understand and quantify risks in order to prioritise efforts to reduce hazards from transfusion, to improve clinical outcomes, and to make best use of available resources. Before haemovigilance systems, public health reporting covered certain key infectious complications of transfusion, but there were limited measures for reporting and analysis of non-infectious hazards.

Over the past twenty years, international haemovigilance systems have described many complications of transfusion, including transfusion-associated circulatory overload, transfusion-related acute lung injury, bacterial contamination of blood components, and the frequent and potentially fatal consequences of human errors. Careful investigation of cases has helped to identify implicated products, procedures, and patient and donor groups at particular risk, and has informed research to understand aetiology and outcomes. Sharing experiences and results through haemovigilance reporting has led to international collaborative action to reduce risks. Many of the lessons from haemovigilance apply equally to other medical products of human origin, including cells, organs and tissues for transplantation.

Blood products cost Australia more than a billion dollars every year, but we need much better information on product utilisation and donor and patient outcomes, including complications and costs of transfusion.

Investments in haemovigilance, side-by-side with efforts in patient blood management, will improve the safety of transfusion into the future.

003. Curing acute promyelocytic leukaemia - blunderbuss, vitamins, and a poison chaser

Illand H

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It is not quite 60 years since acute promyelocytic leukaemia (APL) was first described as a distinct clinical and morphological entity within the broader spectrum of acute myeloid leukaemia. That distinction has subsequently been validated by the recognition of unique cytogenetic and molecular abnormalities that are now used to unequivocally establish the diagnosis.

The major manifestations of APL treatment failure include an extraordinarily high rate of early haemorrhagic deaths, and relapse after achieving complete remission. Although the early death rate has fallen significantly in the context of clinical trials as a result of improvements in haemostatic supportive care, population-based studies suggest that early death remains an unresolved problem. In contrast, the risk of leukaemic relapse has been dramatically reduced through the application of highly effective treatment targeted at the underlying pathophysiological molecular abnormality. Protocols that combine *all-trans* retinoic acid and arsenic trioxide to induce cellular differentiation and PML-RARA oncoprotein degradation now form the mainstay of initial APL therapy, with a limited role remaining for cytotoxic chemotherapy.

In this presentation I will review the improvement in APL outcomes that I have been fortunate enough to witness over the last 3 decades, including the contribution that the Australasian Leukaemia and Lymphoma Group has made to the current standard of care. The remaining challenges in the management of APL primarily involve the development of effective strategies to reduce early deaths, and the reconfiguration of consolidation protocols to reduce resource-intensity and improve the overall patient experience without sacrificing efficacy.

004. Use of ferric carboxymaltose in children

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Ferric carboxymaltose (FCM) is being used with increasing frequency as the IV iron preparation of choice due to its rapid administration, efficacy and safety profile. In Australia, it is only licensed by the Pharmaceutical Benefits Schedule for use in children aged 14 years or older. Evidence for its use in children less than 14 years is limited.

Aim

To review the safety and efficacy of FCM in children aged less than 14 years at the Royal Children's Hospital, Melbourne.

Methods

Retrospective review of all FCM infusions performed between September 2013 and May 2015 in children. Data were collected on: patient demographics, indication for infusion, dose, adverse effects, and efficacy as assessed by Hb, MCV and ferritin

Results

65 episodes of FCM administration to 60 children were analysed; 5 children received two infusions. Median age at administration 9.3 years [IQR 3.4 – 12.4] and youngest child to receive FCM was 6 months. Median dose was 19mg/kg [IQR 13 – 20].

The most common indication was gastroenterology disease 30/65 (46%), followed by renal disease 11/65 (17%), dietary iron deficiency anaemia (IDA) unresponsive to oral iron 8/65 (12%) and IDA secondary to menorrhagia 5/65 (8%).

No life-threatening complications were reported, 1 episode of extravasation was documented. Three infusions were associated with mild adverse effects, which may have related to IV iron or underlying clinical disease. Two children required multiple attempts to secure IV access.

IV FCM resulted in a significantly improved Hb and MCV ($p < 0.001$) whilst increase in ferritin did not meet statistical significance ($p = 0.06$).

	Pre-iron infusion Median, [IQR]	Post-iron infusion Median, [IQR]	Median Difference [IQR]	Significance (p value)
Ferritin (µg/L)	19 [8 – 82]	183 [88.5 – 324.5]	108 [13.5 – 263.75]	0.06
Hb (g/L)	100 [86 – 115]	121 [111 – 129]	16 [4.75 – 31.75]	<0.001
MCV (fL)	73 [66 – 80]	78 [74 – 83.5]	5 [0 – 9]	<0.001

Conclusion

Our study supports the safety and efficacy of FCM as an IV iron therapy for use in children under 14 years. Important benefits of this iron preparation including the rapidity of infusion, shorter hospital stay and less patient inconvenience.

005. Improving blood management in obstetrics: a practice improvement partnership

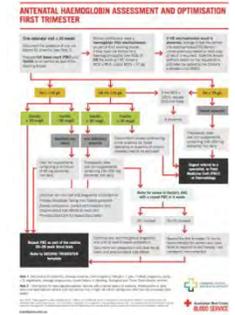
Sethna F¹, Flores C², Crispin P¹, Stephens B¹, Saxon B², Hong F², Spigiel T², Roberts T², Burgess M¹, Connors B¹

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Aim
2013 audit data from a tertiary obstetrics unit demonstrated 23% of women experiencing a postpartum haemorrhage received a transfusion; half of whom were anaemic on admission intra-partum. PBM guidelines in Obstetrics focuses on maximising red cell mass at the time of delivery and reducing the reliance on transfusion as a salvage therapy to treat blood loss. The aim of this project was to implement systems to improve antenatal detection and management of iron deficiency (ID) and anaemia (IDA).

Methods
In order to develop change strategies, reasons for intra-partum anaemia were identified following a quality improvement methodology. Education was delivered to all maternity healthcare providers and tools (Figure 1) were developed and trialled from December 2015. Follow-up audit and other hospital data were collected to measure outcomes.

Figure 1 CPI tools for maternity

<p>Antenatal, Intrapartum and Postpartum Haemoglobin Assessment and Optimisation Template (with unselective screening with ferritin)</p>	<p>Maternity patient handout on oral iron preparations for the treatment of iron deficiency anaemia</p>	<p>Guide on red cell transfusion for postnatal patients not actively bleeding</p>
 <p>ANTENATAL HAEMOGLOBIN ASSESSMENT AND OPTIMISATION FIRST TRIMESTER</p>	 <p>INFORMATION</p>	 <p>GUIDANCE ON RED CELL TRANSFUSION For postnatal patients not actively bleeding</p>

Results
The rate of anaemia intra-partum fell from 12.2% in 2013 to 3.6% in 2016 following the introduction of unselected ferritin screening and other interventions. 60%–70% of women screened each month had ID. The algorithms aided staff to become confident in blood test interpretation and management of ID and IDA. Women found the patient handout helpful, and attributed it for their persistence in taking supplementation despite a significant proportion reporting side-effects. IV iron use increased, with 89 women receiving an infusion. Ferritin screening detected more women who were ID in all three trimesters of pregnancy than detection by haemoglobin alone. Additionally over the project timeframe fewer blood transfusions were given in the postpartum period despite a similar PPH rate.

Conclusion
There have been positive practice changes that are in keeping with PBM guidelines, namely an increased detection of ID, an increased use of iron therapy, decreased rates of IDA at delivery; and, more appropriate use of red cells.

006. Development of laboratory assays for multiple parallel assessments of recipient immune responses for use in clinical studies of transfusion outcomes

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Background and Aims

Blood transfusion has the potential to modulate the recipient immune response leading to poor patient outcomes. We developed a suite of laboratory assays to assess transfusion-related immunomodulation (TRIM) using small blood volumes that would facilitate translation to clinical studies and align with the principals of patient blood management. As central regulators of the immune response, a number of assays focus on dendritic cell (DC) responses.

Methods

Small blood volumes were used to develop multiple assays to investigate potential mechanisms underpinning TRIM. Assays include:

- i) Assessment of haemodynamic parameters.
- ii) Quantification of immune cell subsets (Monocytes, Granulocytes, NK cells, B-cells, T-cells (subsets and Tregs) myeloid DC (mDC), plasmacytoid DC (Trucount Tubes).
- iii) Quantification of mDC specific costimulatory/activation/adhesion markers (Trucount Tubes).
- iv) Quantification of cytokines and chemokines in recipient plasma (Cytometric Bead Array; CBA).
- v) Assessment of mDC, monocyte and granulocyte activation markers, co-stimulatory markers and inflammatory response to bacterial stimulus (in-house assay).
- vi) RNA extraction from whole blood for assessment of changes in expression of immune regulator genes (32 genes).
- vii) RNA extraction from isolated mDC to assess changes in DC gene expression.

Results

We successfully optimised and validated the aforementioned assays using small blood volumes in order to facilitate translation into clinical studies. These assays can all be derived from only one 10mL blood sample for the assessment of multiple parameters associated with the development of TRIM.

Conclusions

We have developed a suite of assays for multiple parallel assessments of recipient immune responses for use in clinical studies of transfusion outcomes. In line with the “pillars of patient blood management” we optimised the assays to ensure withdrawal of minimal blood volume. These assays will now be used to investigate changes in the immune response in cardiac surgery patients following transfusion using only 10mL EDTA at five time-points (Admission, Theatre, ICU, Day3, Day5).

007. Epidemiology and outcomes of major obstetric haemorrhage requiring massive transfusion in Australia and New Zealand: Results from the Australasian Maternity Outcomes Surveillance System (AMOSS)

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Aims

To determine incidence, risk factors and outcomes following major obstetric haemorrhage (MOH) requiring massive transfusion (MT) in Australia and New Zealand (ANZ).

Methods

Prospective, population-based case-control study. All hospitalised women requiring MT (≥ 5 red cell units within 4h) for MOH at >290 eligible birthing services (>50 births/year) in ANZ were identified through AMOSS' monthly surveillance system from July 2014 to June 2015. Controls were the immediately preceding birth at the same maternity unit.

Results

133 cases and 133 controls were identified. Incidence of MOH requiring MT was 37.5 per 100,000 births. Complete data were available on 102 cases and 116 controls.

Compared to controls, cases had higher proportions of women aged ≥ 35 years (37% vs 24%, $p=0.035$); induction of labour (33% vs 23%, $p=0.015$) and births following assisted reproductive technology (ART) conception (16% vs 4%, $p=0.005$). Other risk factors (BMI, parity, prior caesarean section and multiple pregnancy) were not significantly different between cases and controls.

Causes of bleeding included uterine atony (36%), placenta accrete/increta/percreta (17%), retained placenta/membranes (10%) and genital tract trauma (9%).

Compared to controls, cases had higher rates of: hysterectomy (40% vs. 1%, $p<0.001$), intensive care unit (ICU) admission (71% vs 3%, $p<0.001$) and length of stay >5 days (92% vs 19%, $p<0.001$). There were no maternal deaths. There were 7 stillbirths among cases; and higher rates of preterm birth (26.1% vs 6.1%, $p<0.001$), neonatal ICU admission (17% vs 4%, $p=0.001$) and Apgar score <7 (11% vs 4%, $p=0.019$) compared to controls.

Conclusions

MOH requiring MT incidence is approximately 37.5 per 100,000 births and is associated with inferior maternal and neonatal outcomes. Older maternal age, ART associated pregnancy and induction were associated with increased risk. Further research to improve outcomes is needed.

008. Non Invasive Prenatal Testing (NIPT) for fetal RHD in twin pregnancies

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NIPT for fetal *RHD* in singleton pregnancies using cell free fetal DNA (cffDNA) is highly accurate, (>99.9%)¹. There are very few studies reviewing the accuracy for pregnancies involving monozygotic or dizygotic twins. Combining outcomes from two population studies, we reviewed the reliability for non-invasive prenatal fetal RhD assessment involving uncomplicated twin pregnancies.

Method

Maternal blood samples (n=1265) were collected and cell-free DNA isolated from 1mL plasma using either manual or automated methods. To detect fetal *RHD*, a minimum of two *RHD* exons (5 and 10) were amplified by quantitative PCR (qPCR). For 603(47.7%) samples, *RHD* exon 4 and *SRY* (male chromosome serving as internal control for presence of cffDNA) were also amplified. Outcome was measured by comparison of assay data with infant cord blood group and gender.

Result

Twin pregnancies were present for 26 (2.1%) enrolled participants. For these 2.1% the gestational age (GA) ranged from 11 to 36 weeks. Cord blood results were available for 22 of these with all outcomes matching the predicted result (12 RhD positive and 10 RhD negative) with the exception of one sample reported as *RHD* inconclusive due to a maternal RHD gene variant. All sets of twins expressed an identical RhD phenotype (either D-positive or negative). For all pregnancies the qPCR *RHD* signals were lower than predicted for that gestation age which indicates higher fetal cffDNA levels.

Gender prediction based on *SRY* signals was accurate for all 8 twin pregnancies. Three of these, one tested at 14 weeks GA, delivered a male and female infant. For these three pregnancies the NIPT detected *SRY* signals.

Conclusion

Fetal *RHD* and *SRY* predictions were accurate for all cases carrying twins.

We did not encounter an RhD positive/RhD negative twin scenario however *SRY* was informative in three mixed gender twins, signalling the ability to detect unique signals from an individual twin. In conjunction with ultrasound monitoring, it will be important to monitor performance of NIPT for multiple pregnancies to avoid false-negative reporting in fetal *RHD* screening programs.

009. Circulating free tumour DNA analysis demonstrates spatial mutational heterogeneity that coincides with disease relapse in multiple myeloma patients

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Aim

Mutational characterisation in multiple myeloma (MM) currently relies on bone marrow (BM) biopsy, which cannot capture the putative spatial and genetic heterogeneity of this multi-focal disease. An alternative is to analyse circulating cell-free tumour DNA (ctDNA) from the peripheral blood plasma (PL) that theoretically contains a representation of the entire tumour genome. Analysis of PL-derived ctDNA as an adjunct to BM biopsy for mutational characterisation was evaluated.

Method

Paired BM MM cell DNA and ctDNA from 18 relapsed [RR] and 10 newly diagnosed [ND] patients were analysed for KRAS, NRAS, BRAF and TP53 mutations using the OnTarget™ Mutation Detection (OMD) platform and validated with mutation-specific droplet digital (ddPCR). Sequential mutation-specific ctDNA quantitation with ddPCR for disease monitoring was undertaken for 2 patients.

Results

- OMD detected 85 mutations (PL=26, BM=28, both=31) with the presence (30.5%) and higher frequency of PL-only mutations in RR patients than ND (38.8% vs 10% respectively), authenticating the existence of spatial and genetic heterogeneity in more advanced disease.
- Activating RAS mutations were highly prevalent with 22 of 28 patients (79%) harboring at least one RAS mutation and 2 patients with >10 different RAS mutant sub-clones consistent with striking sub-clonal convergence on the RAS-MAPK pathway and a greater prevalence of RAS mutations than previously described with whole exome sequencing (WES) of BM.
- TP53 mutations were found exclusively in RR patients.
- Sequential PL tracking of ctDNA for specific mutant clones by ddPCR showed an increase in fractional abundance of certain clones coincident with relapse of the disease.

Conclusion

These data are the first, to the best of our knowledge, to demonstrate that ctDNA represents a medium for non-invasive therapeutic monitoring and comprehensive mutational characterisation of MM and confirm the presence of a more complex sub-clonal architecture in MM than demonstrated by whole exome sequencing based BM analyses.

010. The occupational and financial impact of Blood and Marrow Transplantation (BMT) on long-term survivors of BMT in NSW

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Aim

An increasing number of BMT recipients experience long term survival. Many, however, bear the burden of chronic illness and chronic GvHD. While the impact on quality of life is well-known, the financial and occupational impact on Australian survivors is less clear. We report the long-term impact of BMT on survivor's work status and income from a cross-sectional survey of BMT recipients from NSW.

Method

BMT survivors aged over 18yrs and transplanted between 2000–2012 in NSW were eligible to participate. Survivors completed the Sydney Post BMT Study survey, FACT-BMT (V4), Chronic GvHD Activity Assessment Self Report, Lee Chronic GvHD Symptom Scale, DASS21, Post Traumatic Growth Inventory, and the Fear of Recurrence Scale.

Result

Of the 583 BMT survivors contacted, 441 (78%) completed the surveys. Respondents included 250 (57%) males and 191 (43%) females. Median age was 54 years (range 19-79years), and median age at time of transplant was 49 years (Range: 17-71).

The number of BMT recipients in full-time employment fell from 261 (64.2%) pre-transplant to 130 (32.2%) post-transplant ($P<0.001$). At the same time, the number of recipients who were retired increased from 5.4% pre-transplant to 18.8% post-transplant ($P<0.001$) while ill-health as the cause of not working increased almost 4-fold pre to post-BMT.

BMT also had a significant impact on household income, with 20.7% reporting low income status pre-transplant (up to \$AUD39,999) and 36.3% post-transplant ($P<0.0001$). And the number enjoying high income status falling from 44% pre-transplant (>\$AUD80,000) to 32.9% post-transplant (an absolute difference of -11.1%).

Conclusion

BMT has a significant impact on the work status of survivors and their household income. Many survivors are unable to remain in full-time work and retire due to ill-health and consequently experience a significant reduction in their household income. The impact of this is likely to be profound – exacerbating carer burden, social isolation and medication non-adherence. Our results suggest that the financial and occupational impacts of BMT should routinely be discussed pre-transplant and should be a focus of continuing education, counselling and support in the longer term post BMT.

011. ROAR: A Phase Ib trial of oral azacitidine in combination with lenalidomide and dexamethasone (Rd) for patients with relapsed and/or refractory (R/R) multiple myeloma who have failed a prior lenalidomide-containing regimen

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Aims

In R/R myeloma patients who previously failed a lenalidomide-containing regimen: to determine the maximum tolerated dose (MTD) of oral azacitidine in combination with Rd; to characterise safety/tolerability; to assess efficacy: overall response rate (ORR), progression free survival (PFS), overall survival (OS).

Methods

Phase Ib, single centre, 3 x 3 dose escalation study. Lenalidomide 25mg d1-21 and dexamethasone 40mg d1, 8, 15, 22 of 28 day cycle were combined with escalating doses of azacitidine: initial dose 100mg for d1-14, increasing by either 7 days or 50mg/cohort, to a maximum of 200mg d1-21. Dose limiting toxicity (DLT) was assessed during cycle 1. Treatment continued until toxicity/progression.

Results

22 patients commenced therapy (F=10, M=12), median age 67yrs (50-82yrs). Median prior lines of therapy: 5 (2-8), including 16 ASCT and 2 prior allogeneic transplant. All had failed lenalidomide, 10/22 received and failed pomalidomide. All had received bortezomib, 18/22 were both bortezomib and lenalidomide refractory. Azacitidine dose reached was 200mg d1-21, with no DLTs observed.

ORR (\geq PR) was 43% (9/21): 8 PR, 1 VGPR. Of the remaining patients, 2 achieved MR, 6 SD, and 6 PD.

Median time to best response in patients with \geq MR: 2.5m (1-3.7m). Median time on study: 3m; responders (\geq MR): 6.3m (2.5-15m), non-responders: 1.7m (0.9-8m)

3/6 patients treated with lenalidomide in prior 1-2 treatment lines responded (PR=2, VGPR=1), 1/6 had SD.

1/10 patients treated with pomalidomide in prior 1-2 treatment lines responded (PR), with 2 achieving MR and 3 SD.

Median PFS 3m, median OS 15m. One patient remains on study.

Conclusion

Oral azacitidine combined with Rd is well tolerated and effective with durable responses in heavily pre-treated R/R myeloma patients, including those who recently failed IMiD therapy, suggesting that azacitidine may overcome drug resistance. Trial is to be expanded to include other sites and less heavily pre-treated patients.

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

012. Point mutations in TKI-resistant Ph-like acute lymphoblastic leukaemia via In vitro modelling: - implications for targeted therapeutic approaches

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Aim

Treatment-resistant acute lymphoblastic leukaemia (ALL) remains a significant clinical issue. Recently, genomic profiling has identified a new subtype of high-risk ALL termed Philadelphia-chromosome-like (Ph-like) ALL. Ph-like ALL has a gene expression profile similar to Ph+ (*BCR-ABL1*+) ALL, characterised by the presence of fusion genes converging on kinase and cytokine signalling pathways targetable by tyrosine kinase inhibitors (TKIs). It is known from TKI-use in CML and Ph+ ALL that resistance is likely. Our study aims to model and understand mechanisms of TKI-resistance in Ph-like ALL, informing future therapeutic strategies that may avert or overcome resistance, potentially improving patient outcomes.

Methods

Three Ph-like ALL cell lines were generated via retroviral-transduction of fusions identified in patient cohorts into Ba/F3 pro-B cells – *RANBP2-ABL1*, *SSBP2-CSF1R* and *PAX5-JAK2*. Transformation was confirmed via IL-3 independent growth. Cells were tested for sensitivity to Abl and Jak inhibitors via Annexin-V/7-AAD flow-cytometry and western blotting of downstream effector proteins. Drug resistance was generated through exposure of cells to incrementally increasing concentrations of TKIs over 3-6 months. To determine if mutations had emerged, Sanger sequencing of the kinase-domain of the 3' partner gene of each fusion was performed.

Results

Ph-like Ba/F3 cell lines demonstrated sensitivity to TKIs at clinically-relevant concentrations, with exposure decreasing downstream signalling protein expression. Importantly, Ph-like TKI-resistant lines were found to acquire kinase-domain mutations including the clinically significant *ABL1* T315I mutation (*RANBP2-ABL1*), novel *CSF1R* L785M mutation (*SSBP2-CSF1R*) and a Y931C mutation in *JAK2* (*PAX5-JAK2*) (Table 1).

Conclusion

In vitro modelling of Ph-like ALL has enabled the identification of novel kinase domain mutations. Investigation of the downstream effect of novel point mutations underpinning TKI-resistance via mRNA sequencing is ongoing. Ultimately, modelling resistance has the potential to inform future clinical approaches including combinatorial therapy that may avert resistance and relapse in Ph-like ALL.

Table 1: Summary of drug sensitivity in Ba/F3 Ph-like ALL lines (Naïve vs TKI-resistant)

Fusion Gene	Drug-sensitivity in Control Cells	Drug-sensitivity in TKI-resistant Cells	Kinase-domain Mutation Identified
<i>RANBP2-ABL1</i>	~ 1 µM IM ~ 5 nM DAS	> 10 µM IM > 200 nM DAS	T315I (c.944C>T) mutation in IM & DAS resistant lines
<i>SSBP2-CSF1R</i>	~ 1 µM IM	> 10 µM IM	Novel L785M (c.2566C>A) mutation in IM resistant line
<i>PAX5-JAK2</i>	~ 1 µM RUX ~ 2 µM JAK2i	> 10 µM RUX > 50 µM JAK2i	Y931C (c.3286A>G) mutation in RUX & JAK2i resistant lines

IM: imatinib; DAS: dasatinib; PON: ponatinib; RUX: ruxolitinib; JAK2i: BMS-911543

013. Platelet development and bleeding phenotype is altered with mutation in different zinc finger domains of the GFI1B transcription factor

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Background

The H294fs mutation affects the GFI1B fifth DNA-binding zinc finger and causes bleeding, macrothrombocytopenia with a platelet function defect, as well as, reduced platelet α -granules and red cell anisopoikilocytosis. The phenotype of the C168F mutation affecting the first non DNA-binding zinc finger of GFI1B has not been characterised.

Aims

To compare the clinical and laboratory phenotypes of *GFI1B* C168F and *GFI1B* H294fs mutations.

Methods

Two unrelated families (n=7) with the C168F mutation were analysed and directly compared to our previously described family with the H294fs mutation using the ISTH-SSC bleeding assessment tool and laboratory data. Patient specific induced pluripotent (iPS) cells were generated to recapitulate aspects of observed phenotypes.

Results

Both the C168F and H294fs mutations cause thrombocytopenia with an autosomal dominant mode of transmission. The mean bleeding score of individuals with C168F mutations was significantly less than those with H294fs mutations (0.4 vs 3.8, P=0.03). Both mutations showed large platelets on the blood film and moderate thrombocytopenia. Platelets from C168F cases appeared well granulated and their red cells had normal morphology. Platelet surface CD34+ expression by flow cytometry was increased in primary cells and in vitro in iPS derived megakaryocytes. Individuals with C168F mutations lacked significant platelet functional defects, whereas, impaired platelet responses were seen, in particular to collagen, in individuals with H294fs mutations. Electrophoretic mobility shift assays show that both the C168F and H294fs variants disrupt GFI1B conformation causing altered DNA binding. This data is supported by luciferase experiments that suggest the C168F variant has less effect on gene transcription than the H294fs mutation.

Conclusion

The location of GFI1B mutation has important phenotypic implications with the C168F mutation producing a milder phenotype of thrombocytopenia without aggregation defects or granule deficiency. Increased CD34+ expression is common to both GFI1B mutations and may serve to identify GFI1B variants.

014. Validation of plasma miR-494-3p and miR-365a-3p expression levels as indicators of thrombotic risk

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Background and Aim

Expression of the microRNAs, miR-494-3p and miR-365a-3p were shown to be oestrogen responsive in HuH-7 human hepatoma cells, and directly downregulates Protein S (PS) and tissue factor mRNA expression, respectively, indicating a role for miRNAs contributing to oestrogen-mediated thrombotic risk. This study aims to determine if oestrogen-induced changes to miR-494-3p and miR-365a-3p levels observed in HuH-7 cells can be detected in plasmas from individuals with low and high circulating oestrogen concentrations. Findings from this study may be utilised for the identification of high risk individuals who may require prophylactic therapy to prevent thrombotic episodes during pregnancy, establishing the role of miRNAs in regulating coagulation.

Method

Blood samples were collected from women with low oestrogen concentrations (non-pregnant females not taking oral contraceptives), and women with high circulating oestrogen concentrations (females taking oral contraceptives and pregnant women >14 weeks gestation). The haemostatic capacity of all samples collected were determined by thrombin generation assays and free PS levels. Total RNA was extracted from plasma samples and relative levels of miR-494-3p and miR-365a-3p were determined by Taqman assays. The differences between groups were compared by one way ANOVA.

Result

Free PS levels were significantly lower in pregnant females compared to all other groups analysed. Thrombin generation assays showed that pregnant individuals also showed significantly lower lag times, higher peak thrombin levels and endogenous thrombin potential, indicating a higher haemostatic capacity and increased thrombotic risk. Levels of plasma miR-494-3p and miR-365a-3p were low in many plasma samples and not detectable by realtime qPCR.

Conclusion

The preliminary analysis suggests that oestrogen-mediated changes to miR-494-3p and miR-365a-3p levels may be tissue-specific and only detectable in the liver. Ongoing work is focused on increasing plasma miR-494-3p and miR-365a-3p detection in high throughput analyses to accurately quantitate changes in their expression levels in plasma samples.

015. Cancer Survivors: Who is responsible for Wellness?

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The most recent cancer statistics provide several key messages relevant to the clinical and social challenges we will face as oncology professionals and as human beings. Estimates for the life-time risk of developing cancer are staggering; one in two individuals when all age groups and all cancer sites are considered. When age, gender, and ethnicity are considered, the lifetime risk of selected cancers varies greatly. Progress has been made to effectively treat many cancer types offering hope for cancer survivors. Cancer survivors require ongoing health and wellness interventions including routine screening, health promotion, and management of co-morbid conditions. The complexity of health needs for cancer survivors together with a shift toward a chronic illness model presents unique challenges in providing these services. Wellness requires an individualized approach with a focus beyond the cancer diagnosis. The effective management of the cancer survivor will require a collaborative continuum based model of care which incorporates multiple disciplines, familiarity with current health maintenance and wellness strategies, incorporation of consensus guidelines for management of common co-morbidities, and promotion of active participation and self-management strategies by the patient and their caregivers. The focus of this lecture will be on how oncology professionals can facilitate LIVING in cancer survivors and their caregivers.

016. Optimising survivorship - The physicians perspective

Avery S

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Survivorship care as a distinct phase of the cancer trajectory is a relatively new construct. Advances in haemopoietic stem cell transplantation (SCT) have improved long-term survival of patients undergoing autologous or allogeneic SCT. With less early mortality and more widespread use of SCT, the number of long-term survivors will continue to grow. As patients survive longer, there is an increasing recognition that pre-, peri- and post-transplant exposures have the potential to compromise life expectancy and can contribute to the development of late complications and impaired quality of life. Mortality rates are up to 9 times higher than observed in an age-adjusted general population for at least 30 years after allogeneic SCT, resulting in an estimated 30% lower life expectancy compared with someone who has not been transplanted. These long term complications present a great diversity in respect to frequency, time of onset, risk factors, prevention strategies, treatment approaches and outcomes. The long-term side effects are usually a multifactorial and complex process and the natural history of such late effects will likely change in the future as transplant techniques have changed progressively and significantly over the past decades.

Among long-term survivors of SCT, the most common causes of excess deaths other than recurrent malignancy are chronic GVHD, infections, second malignancies, respiratory diseases and cardiovascular disease. Other comorbid health conditions are prevalent as well as psychological and social difficulties. The high burden of morbidity borne particularly by SCT survivors necessitates that strategies be developed for the prevention or early detection and management of these complications. Long-term follow up of these patients with continued care, beyond the acute treatment phase is now widely recommended to ensure that these issues are appropriately managed and that patient outcomes continue to improve.

017. Thrombocytopenia in pregnancy

McLintock C

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Pregnancy is associated with physiological and pathological changes in platelet numbers and function, which can be of clinical concern because of risks for maternal and fetal or neonatal bleeding. Thrombocytopenia in pregnancy is frequently encountered and may be due to increased platelet turnover and plasma dilution, immune mediated mechanisms, or a complication of a more severe underlying pregnancy-related disorder such as preeclampsia. Inherited defects in platelet function and number may also manifest during pregnancy with the risk of bleeding dependent on the underlying problem. In some women, the diagnosis of thrombocytopenia will precede pregnancy but in others, the problem is first identified when routine pregnancy blood tests are performed. An accurate diagnosis and risk assessment in the antenatal period are essential for developing specific plans for any antenatal interventions and for management of delivery and the postpartum periods, and the neonate.

019. Alloummunisation - what's new?

Savoia H

The Royal Children's Hospital, Melbourne, Australia

Abstract to come

020. Biology of multiple myeloma: Genetics and epigenetics

Spencer A

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Multiple myeloma (MM) is a presently incurable malignancy of terminally differentiated plasma cells that arises via a multi-step pathogenesis from a far more common clonal but benign disorder termed monoclonal gammopathy of undetermined significance (MGUS). MM is clinically a highly heterogeneous disease with marked variability in survival with contemporary therapies. This clinical heterogeneity is reflected in the complex genetic and epigenetic background found in the disease. At diagnosis MM patients manifest translocations involving the IgH gene and a hypodiploid background or a hyperdiploid picture characterised by trisomies of the odd numbered chromosomes, with studies demonstrating that these 'founder' lesions exhibit approximately the same prevalence in MGUS. The cause for the transition from MGUS to MM remains obscure but limited evidence demonstrates that intra-tumour genetic clonal heterogeneity may precede the development of symptomatic MM. Whole exome sequencing (WES) studies of the bone marrow in MM have demonstrated activating driver mutations most commonly of the RAS-MAPK pathway with single-cell genetic analyses confirming the mutational convergence on the RAS—MAPK pathway via both linear and branching tumour evolution. Moreover, recent data including ctDNA studies have confirmed not only clonal genetic heterogeneity but also clear evidence of spatial heterogeneity that questions the utility of present methods of disease evaluation. Abnormalities of DNA methylation are hallmarks of cancer and several limited studies of methylation in MM have shown a consistent picture of widespread hypomethylation coincident with the transition from MGUS to MM but with subsequent disease progression characterised by hypermethylation of selected genes including tumour suppressors. A more extensive genome-wide methylation analysis has in addition demonstrated hypomethylation of intronic enhancers leading to the decommissioning of gene sets related to B-cell differentiation consistent with a stem-cell like methylome. While the increased insight into both the genetic and epigenetic heterogeneity of MM is daunting it is likely that personalised therapeutic opportunities will arise from this increased understanding.

021. Optimal treatment in de novo and first relapse myeloma

Facon T

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Treatment of de novo, or newly diagnosed, multiple myeloma (NDMM) is dependent on patient fitness. Fit patients who are transplant-eligible can receive induction therapy to improve response rate with minimal treatment-related toxicity while increasing time to progression, progression-free survival (PFS), and overall survival (OS). Common induction therapies include either lenalidomide-dexamethasone (Rd) alone or in combination with bortezomib (RVd), or bortezomib-dexamethasone (Vd) alone or in combination with doxorubicin, cyclophosphamide, or thalidomide prior to high-dose chemotherapy (melphalan) and autologous stem cell transplant (SCT; ESMO Guidelines, 2013; NCCN Guidelines v3.2016). SCT following induction improves outcomes, as demonstrated by the PFS benefit seen with SCT following RVd vs RVd alone (Attal et al., ASH 2015).

Since residual disease is likely to persist following SCT, maintenance therapy post-transplant can prolong the time to relapse. For example, lenalidomide maintenance after SCT significantly prolonged PFS and OS vs no maintenance or maintenance with placebo (Attal et al., 2012; McCarthy et al., 2012; Palumbo et al., 2014; Attal et al., ASCO 2016). While lenalidomide is currently considered the best candidate for post-SCT maintenance, other options include single-agent thalidomide or bortezomib, but associated toxicities may limit their utility. Transplant-ineligible patients typically receive treatment until disease progression or unacceptable toxicity. In these patients, frontline treatment regimens with melphalan-prednisone (MP), Vd, or Rd backbones have shown clinical benefit (Moreau et al., 2015; NCCN Guidelines v3.2016). For example, continuous treatment with Rd until disease progression (Rd continuous) significantly prolonged PFS and OS vs 12 cycles of melphalan, prednisone, and thalidomide (MPT) in transplant-ineligible patients with NDMM (Benboubker et al., 2014).

Despite significant improvements in patient outcomes after frontline therapy, this disease remains largely incurable so that patients will inevitably relapse, necessitating identification of optimal therapeutic and sequencing strategies. Rd is a common backbone for triplet therapies with agents from different therapeutic classes (ex, carfilzomib, elotuzumab). Ongoing translational and clinical research into the pathobiology of MM and mechanisms of drug resistance may further optimize and potentially personalize treatment strategies for improved patient outcomes.

022. The myeloproliferative neoplasms - JAK/STAT signalling and stem cell subversion

Green T

University of Cambridge, Cambridge, UK

The human myeloproliferative neoplasms (MPNs) are a spectrum of clonal haematological malignancies which arise in the haematopoietic stem cell compartment. These disorders are experimentally tractable, permit clonal analysis and provide a window on the earliest stages of tumorigenesis. A single somatic gain-of-function mutation in JAK2 is present in most MPN patients. This observation emphasised the significance of the JAK signalling pathway which plays a key role in stem cell biology and has now been implicated in many human malignancies. This talk will focus on the molecular and cellular normal and mutant consequences of JAK2 signalling. Highlights of general relevance for stem cell and cancer biology include unexpected insights into chromatin biology, clonal evolution and haematopoietic stem cell function. Most recently, we have demonstrated for the first time in any cancer, that mutation order influences stem/progenitor cell behaviour, clonal evolution, clinical presentation and response to therapy (Nanglia et al NEJM 2013).

In addition, we have made several observations which establish new paradigms for cytokine signalling. These include the demonstration that nuclear JAK2 functions as a histone kinase and regulates transcription (Dawson et al Nature 2009; Griffiths et al Nat Cell Biol 2011) and our recent identification of a genome-wide role for tyrosine-unphosphorylated STATs (Park et al EMBOJ 2016).

023. Clinical relevance of leukemia stem cells in AML

Dick J

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The cellular and molecular basis for intra-tumoral heterogeneity is poorly understood. Tumor cells can be genetically diverse exhibiting intra-tumoral functional heterogeneity. Often proposed as mutually exclusive, cancer stem cell (CSC) models postulate that tumors are cellular hierarchies created due to epigenetic programs that are sustained by CSC. I will focus on three lines of evidence showing these models are highly integrated. Gene signatures specific to AML LSC have revealed a common stemness program that is highly predictive of patient survival in clinical databases of >1000 samples. Thus, determinants of stemness influence clinical outcome of AML across a spectrum of mutations indicating that many genetic abnormalities coalesce around stem cell properties. Secondly, combined genetic and functional studies of the LSC from either B-ALL or AML point to commonalities between clonal evolution and CSC models of cancer. LSC originate genetically diverse subclones that are related through a complex branching evolutionary process, specific mutations influence LSC functions. Thus the clonal evolution models are highly relevant in cancer but need to be extended to adopt the concept that CSC are subject to clonal evolutionary forces. Finally, the combined genetic and functional analysis of both AML blast cells as well as the non-leukemic hematopoietic cells isolated from the same patient blood samples is revealing fundamental insights into the cell of origin, nature and biological consequences of initiating lesions and order of subsequent mutations; concepts that demonstrate how highly integrated the CSC and genetic evolution models must be. HSC, progenitor and mature cell fractions contained recurrent mutations but without other mutations present in AML blasts. These pre-leukemic-HSC were clonally expanded and survived chemotherapy, remaining present in remission and relapse samples. Detailed studies on 11 paired Dx/Rel samples followed by detailed genetic tracking in sorted blasts and progenitor subpopulations as well as in LSC derived xenografts allowed us to trace the origins of Rel. In all cases, the Rel clone was pre-existing in the Dx blood sample and often originating in rare LSC subclones distinct from the dominant blasts. Thus our findings indicate the future therapeutic strategies must account for and target rare genetic subclones, as well as the functionally important LSC and preL-HSC that are solely capable of clonal propagation.

024. Circulating tumour DNA reflects overall disease burden and clonal evolution in chronic lymphocytic leukemia

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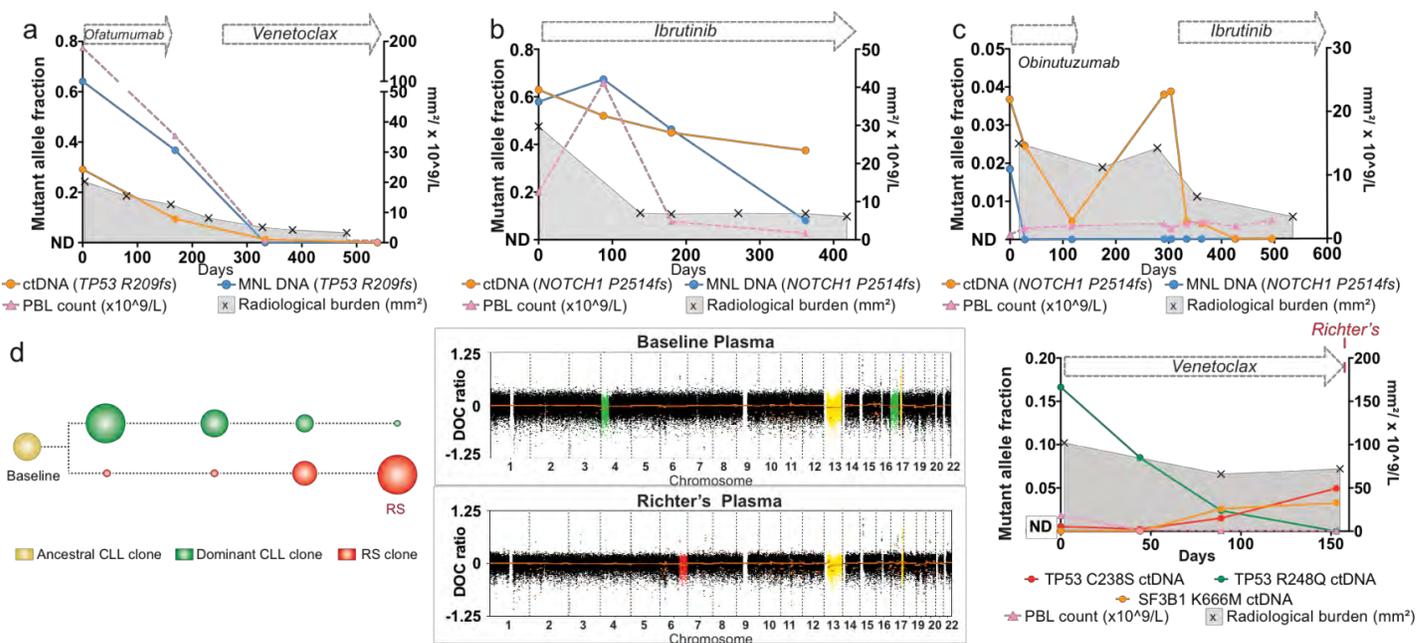
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Aim
Novel therapies are poised to change the natural history of chronic lymphocytic leukemia (CLL). Their increasing use highlights a need to comprehensively monitor disease and understand any genetic events that may occur during these therapies, especially in treatment failure. We explore the role of cell-free circulating tumour DNA (ctDNA) from plasma as a novel and minimally invasive molecular tool that can assesses CLL and overcome the limitations of traditional disease monitoring methods.

Method
Serial plasma and peripheral blood mononuclear layer (MNL) samples (n=111) were analysed from 32 patients with relapsed/refractory CLL commencing either ibrutinib or venetoclax. We tracked the dynamics of driver mutations and IgH re-arrangements using highly sensitive strategies including targeted amplicon next generation sequencing and digital PCR. Additionally, we performed a comprehensive assessment of mutational profile and copy number aberrations in 3 patients on therapy that progressed to Richter's syndrome (RS) by whole exome and low-coverage whole genome sequencing.

Result
ctDNA levels tracked with overall disease response (Figure a), even in cases of ibrutinib induced lymphocytosis, where monitoring peripheral blood lymphocyte (PBL) counts can be misleading (Figure b). In the absence of leukemic disease, ctDNA accurately reflected multiple disease sites (Figure c) and showed a stronger correlation with nodal disease by radiology ($r^2=0.71$, $p<0.0001$) than with PBL counts ($r^2=0.38$, $p<0.0001$). There was also complete concordance between ctDNA and flow cytometry (n=30). Finally, ctDNA was able to recapitulate and, in some cases, predict the clonal evolution seen in RS cases by detecting the emergence of mutations and chromosomal alterations (Figure d).

Conclusion
Here we provide the first report describing ctDNA detection in patients with relapsed/refractory CLL and demonstrate its unique potential to provide global disease assessment which can complement existing methodologies.



025. Interferon Lambda is a Critical Cytoprotectant in Bone Marrow Transplantation

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Aims

A protective role for type I Interferons (IFN) in experimental Bone Marrow Transplantation has been defined through inhibition of Th1 differentiation invoked by recipient CD8^{neg} dendritic cells. Type III/Lambda IFN (IL-28 and IL-29) is more recently described and signals through the unique IL-28R, primarily expressed on epithelial tissues and implicated in mucosal pathogen defence. Clinical use of type I IFN is associated with adverse neurological, haematological and constitutional symptoms where IL-28 is better tolerated yet still demonstrates potent anti-viral effects.

Methods

We used IL-28R^{-/-} and IFN α R1^{-/-} donors and recipients in murine models of GVHD and GVL to develop logical therapeutic strategies to improve transplant outcomes.

Results

IL-28R^{-/-} donors invoked similar GVHD and GVL to WT. However IL-28R^{-/-} recipients had accelerated acute GVHD (aGVHD) mortality and disease relative to WT with a phenotype intermediate to that and the hyperacute aGVHD seen in IFN α R1^{-/-} recipients (median survival WT 42 vs. IL28R^{-/-} 26 vs. IFN α R^{-/-} 6.5 days, $p < 0.0001$). IL28R^{-/-} recipients have augmented colonic GVHD histopathology early (*d* 7) after BMT (WT 7.111 \pm 0.6550 vs. IL28R^{-/-} 12.33 \pm 0.7993, $p = 0.0004$) and exaggerated inflammatory cytokine generation (*d*4 IFN γ WT 331 \pm 41.47pg/mL vs. IL28R^{-/-} 667 \pm 48.79 pg/mL, $p < 0.0001$ and IL-6 WT 61.25 \pm 10.91pg/mL vs. IL28R^{-/-} 91.99 \pm 11.23pg/mL, $p = 0.024$). IL-28R^{-/-} recipients have increased gastrointestinal permeability as measured by serum concentrations of FITC-Dextran after gastric lavage (WT 2.041 \pm 0.9842ng/uL vs. IL28R^{-/-} 4.028 \pm 6.950ng/mL, $p = 0.0273$). Re-transplantation of chimeras with WT or IL28R^{-/-} haematopoietic, non-haematopoietic tissue or combinations thereof demonstrated that IL-28 mediated protection required signalling through both compartments, putatively recipient antigen presenting cells and colonic epithelia. Treatment of mice with Peg-rIL-28m resulted in significant reduction of inflammatory serum cytokines when compared to PBS treatment (*d*4 IFN γ Peg-rIL-28m 59.79 \pm 7.272pg/mL vs PBS 227.4 \pm 20.99pg/mL, $p < 0.0007$).

Conclusion

IL-28 represents an attractive therapeutic to mediate cytoprotection within the GI tract and attenuate to GVHD in the peri-transplant period.

026. BCL2 selective inhibition with venetoclax induces apoptosis in high risk del(17p) and TP53-defective CLL in vitro and in patients leading to durable responses

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Aim

The BCL2 selective inhibitor, venetoclax (ABT-199), induces responses in 79% of patients with relapsed/refractory CLL in Phase I/II trials^(1,2). We sought to (1) define its mechanism-of-action *in vivo*, (2) assess whether del(17p) and/or TP53 mutations affected response and (3) ascertain durability of response in del(17p)/TP53-mutated (TP53mut) CLL.

Method

PB or BM CLL samples from patients on early Phase venetoclax trials were analysed before and after first exposure to venetoclax *in vivo* for markers of apoptosis. Cells were also assayed for *in vitro* sensitivity to venetoclax and nutlin-3a, del(17p) and TP53 mutation. Clinical outcomes among the 65 patients receiving venetoclax on trials were assessed objectively using iwCLL criteria; and then analysed according to whether the CLL was del(17p) and/or TP53mut (n=31) or neither (known normal TP53 n=23).

Results

The *in vitro* sensitivity of CLL cells with del(17p) &/or TP53mut was equivalent to those with neither abnormality, with LC₉₀ <1uM in all groups (p>0.1). Venetoclax was highly active in CLL cells that lacked detectable TP53 function. *In vivo*, phosphatidylserine exposure and caspase 3 activation were evident after the first dose of drug irrespective of TP53 status. Responses were observed in 58 pts (89%), with 17 complete remissions (26%). The depth of responses in PB, nodes and BM compartments was independent of del(17p) and/or TP53mut (p>0.1 for each). Objective responses were observed in 91%, 90% and 87% patients with del(17p), del(17p) &/or TP53mut and neither (normal TP53) respectively (ANOVA, p>0.1). Amongst 55 patients receiving ≥300-mg/day venetoclax (n=55), 60% were progression-free at 3 yrs, and was not significantly affected by presence of TP53 abnormalities.

Conclusion

Venetoclax kills CLL by inducing apoptosis in a TP53-independent manner, explaining the efficacy of the drug in patients with poor prognosis CLL, and suggesting a future role in treatment for other BCL2-expressing haematological malignancies carrying TP53 abnormalities.

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

027. Therapeutic blood dendritic cell vaccination for acute myeloid leukaemia

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Aim

Many acute myeloid leukaemia (AML) patients achieve a complete remission (CR) with chemotherapy but relapse is common. Allogeneic transplantation is used to consolidate CR by addressing residual disease but has a high morbidity and mortality. Therapeutic dendritic cell (DC) vaccination has the potential to provide immune control with limited toxicity. Previous trials using monocyte-derived DC have demonstrated modest clinical effects. We have developed a more practical, functionally superior vaccine composed of blood DC (BDC), purified using the human chimeric antibody CMRF-56. We determined the prospects for preparing a CMRF-56+ BDC vaccine from AML patients in CR.

Method

We established an extended flow cytometry panel to distinguish BDC from blasts in AML, and sorted them to establish their morphology. Whole blood subsets were counted in AML patients at diagnosis and post-chemotherapy (5-28 weeks). CMRF-56 antigen upregulation was monitored and CMRF-56 purification performed after culturing peripheral blood mononuclear cells from AML patients in CR. CMRF-56+ BDC potency was evaluated using *in vitro* autologous peptide antigen-specific T cell expansion assays.

Results

Reports of elevated BDC in AML diagnostic samples are misleading as AML blasts were not excluded. CD1c and CD141 BDC are depleted at diagnosis, but recover to 56% and 44% of healthy aged-matched controls during CR1. CD1c BDC from AML patients in CR upregulated the CMRF-56 antigen (primary AML blasts did not) similarly to healthy donors (n=5, p=0.4), enabling AML blast free, CMRF-56+ BDC purifications. CMRF-56+ BDC isolated from AML patients in CR expanded anti-viral and Wilms' Tumour 1-specific autologous CD8+ T cells *in vitro*.

Conclusion

This data suggests it is feasible to prepare a functional BDC vaccine from AML patients in CR using CMRF-56 immune selection. BDC vaccination may consolidate chemotherapy induced CR in AML by stimulating effective immune responses to control residual disease.

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

028. Germline variant detection by next generation sequencing in routine tumour sample analysis - mountain or molehill?

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Aim

Next generation sequencing (NGS) is routinely utilised in our diagnostic laboratory for diagnosis, prognostication and therapeutic decision making in haematological malignancies. These assays may result in the unintended detection of variants of germline origin in tumour samples, which may have significant implications for patients and their families.

We aimed to define the extent of this concerning outcome in our laboratory practice.

Method

The diagnostic laboratory at the Peter MacCallum Cancer Centre performs two 26 gene NGS amplicon panels for myeloid and lymphoid disorders. Genes analysed by these panels that are associated with an inherited syndrome include: TP53, RUNX1, GATA2, WT1, CSF3R, CBL and KRAS/NRAS (for JMML).

We reviewed reports issued for clinical use analysing at least one of the above genes over an 18 month period (Jan 2015 – June 2016 inclusive).

Result

Key findings on review of 538 clinical reports include:

- 1.5% of requests suggested the possibility of an inherited condition
- 17.3% of reports contained variants in genes associated with an inherited syndrome
- 5.8% of reports suggested confirmatory germline testing based on clinicopathological features

Where detection was incidental, samples were received for confirmatory germline testing in 25.8% of cases in which it was suggested. Of those successfully performed, 28.6% were confirmed to be of germline origin, representing 0.4% of all tests.

Conclusion

In our cohort, confirmatory germline testing was recommended in ~6% of reports but actioned in only a minority.

Despite the low rate of incidentally detected germline variants and the rarity of associated clinical syndromes, such findings can have major implications for both patients and their families.

Consequently, we strongly recommend routine pre-test counselling in this context to educate patients about the possibility of incidental germline variant detection.

029. Identifying downstream targets of IRF4 for therapeutic targeting in myeloma

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Interferon regulatory factor 4 (IRF4) is a critical transcription factor for plasma cells (PC). Deletion of IRF4 leads to a rapid loss of PC in mice¹ and cell death in all myeloma cell lines². Unfortunately, IRF4 has proven difficult to directly suppress. We aimed to identify key downstream mediators of IRF4 function with the aim of identifying targets for patients with myeloma.

Initial focus centred on Mcl-1 which is known to be critical for PC survival³. Mcl-1 mRNA and protein levels in IRF4 heterozygote PC were reduced compared to wild-type PC. Additionally, ChIP-sequencing data suggested that IRF4 and its binding partner PU.1 had binding sites upstream of the Mcl-1 gene⁴. These results suggested that IRF4 may control *Mcl-1* transcription in normal PC. Next we used CRISPR/Cas9 to delete IRF4 in mouse and human PC lines. IRF4 deletion caused cell death in all cell lines. Interestingly, amounts of Mcl-1 protein levels did not change with IRF4 deletion. These results indicated that in cell lines there may be an altered mechanism of Mcl-1 regulation with cell death following IRF4 deletion via an Mcl-1 independent mechanism. To investigate these processes further we generated IRF4-floxed-ER2-Cre mice (allowing inducible deletion of *IRF4* in vivo) crossed to *Bcl2* transgenic mice with the expectation that over-expression of *Bcl2* would allow prolonged PC survival. Indeed, the presence of the *Bcl2* transgene allowed a proportion of PC to survive IRF4 deletion not seen in its absence. RNA sequencing was performed on these PCs and compared to IRF4 intact PC. Additionally, RNA sequencing was performed to analyse IRF4 deletion in MPC-11 (mouse) and MM1S (human) cell lines. These RNA signatures will provide us with a detailed transcriptional profile of IRF4-dependent genes. We anticipate identifying key genes regulated by IRF4 that contribute to PC cell death on deletion and which could be therapeutically targeted.

030. Novel agents - who needs them?

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Follicular lymphoma is the second most common lymphoma in the United States, and although it remains incurable, we have seen marked overall survival improvements for patients with follicular lymphoma in the past decade. The median overall survival for patients presenting with advanced stage follicular lymphoma now exceeds 20 years. The majority of patients, therefore, with follicular lymphoma do very well with currently available therapies. However, there is a subset of patients with follicular lymphoma who have inferior outcomes. At present, these patients are not easy to definitively identify at diagnosis, and that remains a key research goal moving forward. We have recently made the observation that patients with follicular lymphoma who progress within 2 years of standard chemoimmunotherapy have significantly inferior overall survival, with a median of only 5 years. These represent 20% of patients being treated with chemoimmunotherapy, and this finding is reproducible across several data sets. Insights from gene expression profiling and mutation analyses may assist in describing the unique biology of these patients who have inferior outcomes, and ultimately provide rational targets for therapy. We have also identified low vitamin D levels in serum at diagnosis as a marker for inferior overall survival. In this talk, currently available prognostic markers will be reviewed, including the imperfect M7 FL-IPI score, to define a group of patients with follicular lymphoma needing a novel therapeutic approach. We will then review the landscape of novel agents to define rational choices to study given these prognostic findings, with a goal of ultimately moving toward a precision therapy approach to the treatment of follicular lymphoma.

031. Genomic based treatment advances in Waldenstrom's Macroglobulinemia

Treon S

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Using whole genome sequencing, we identified highly recurrent activating mutations in MYD88 and CXCR4 in patients with Waldenström's Macroglobulinemia (WM). Using highly sensitive AS-PCR assays, we and others confirmed the presence of MYD88L265P mutations in 95% of untreated WM patients which occur from a C>T transversion at position 38182641 at 3p22.2. Recent studies by us and others have shown that other activating MYD88 mutations including S243N, M232T, L265RPP can also occur in WM patients. In contrast to diffuse large B-cell lymphoma where non-L265P mutations make up a quarter of all MYD88 mutations, non-L265P mutations are rare in WM. By in vitro modeling, we have shown that MYD88 mutations trigger BTK, IRAK 4/IRAK1 dependent survival signaling in WM cells, which can be abrogated by exogenous administration or endogenous lentiviral transduced expression of peptides which block MYD88 homodimerization. MYD88 can also trigger HCK, a SRC member which can further propagate survival signaling through PI3K/AKT and ERK1/2. Both BTK and HCK are targets of ibrutinib. Mutations in the C-terminal domain of CXCR4 are present in 40% of untreated WM patients, and are almost always present in MYD88 mutated patients. CXCR4 mutations are subclonal in most WM patients supporting their acquisition after mutations in MYD88. Multiple mutations including compound heterozygous and homozygous CXCR4 mutations may occur within individual patients suggestive of targeted CXCR4 genomic instability in WM patients. CXCR4 mutations attenuate ibrutinib activity in WM cells through AKT/ERK signaling. Unsupervised clustering and principal component analysis of RNASeq data from WM patients demonstrate that MYD88 and CXCR4 mutation status are principal determinants of gene expression in WM. MYD88 and CXCR4 mutation status also impact bone marrow disease burden, serum IgM levels, and extra-medullary disease. Patients with mutated MYD88 exhibit longer overall survival (>10 years) versus those with wild-type MYD88 (4.7 years), and also demonstrate higher overall and major responses to ibrutinib. CXCR4 mutation status is associated with slower response kinetics to ibrutinib, and lower response activity in WM patients. The findings highlight the importance of MYD88 and CXCR4 mutations in the pathogenesis and clinical outcome of patients with WM.

032. A Frailty Scale Predicts Outcomes in Transplant-Ineligible Patients With Newly-Diagnosed Multiple Myeloma (NDMM) Treated in the FIRST Trial With Continuous Lenalidomide Plus Low-Dose Dexamethasone (Rd)

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Aim

To examine efficacy outcomes in patients in the FIRST trial based on a recently described frailty scale (Palumbo et al, 2015).

Methods

Patients with NDMM received Rd until progression (Rd continuous), Rd for 18 cycles (Rd18), or melphalan, prednisone, and thalidomide (MPT). Patients were categorized as fit, intermediate, or frail using the frailty scale. Baseline characteristics included in the algorithm were age, Charlson Comorbidity Index score, and self-care and usual activities from the EQ-5D questionnaire. PFS and OS between treatment arms within each frailty group were evaluated (data cutoff March 3, 2014).

Results

Of 1517 evaluable patients, 51% were frail, 17% fit, and 30% intermediate. Frail patients vs fit or intermediate were older and had higher ECOG PS, higher International Staging System (ISS) stage, higher LDH levels, and worse renal function. OS was significantly improved in both fit (hazard ratio [HR] 0.42; $P < .0001$) and intermediate (HR 0.62; $P < .0001$) vs frail patients. Rd continuous vs MPT prolonged PFS and OS and reduced risk of progression or death by 44% (fit), 39% (intermediate), and 22% (frail). Grade ≥ 3 hematologic adverse events (AEs) were similar in all groups. Fit patients had a lower risk of grade ≥ 3 non-hematologic AEs compared with frail patients ($P = .0021$). Across all groups, Rd continuous vs MPT had similar rates of grade ≥ 3 non-hematologic AEs and grade ≥ 3 hematologic AEs were less common with Rd continuous. Combining ISS stage with frailty classification further improved PFS and OS prognostic assessment.

Conclusions

Our data support the use of the frailty scale for predicting risk of death in patients with NDMM. PFS and OS improved with Rd continuous vs MPT across all frailty levels especially in fit patients. We confirm Rd continuous therapy as a standard of care for patients with transplant-ineligible NDMM.

The authors acknowledge the financial support for this study from Celgene Corporation

033. 'Real-world' Australian experience of pomalidomide for relapsed/refractory myeloma

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Background

Pomalidomide and low-dose dexamethasone has been shown to prolong progression-free survival, with an acceptable safety profile, in patients with multiple myeloma who have relapsed or progressed following lenalidomide and bortezomib. We aimed to evaluate the outcomes of a 'real-world' cohort of Australian patients treated with pomalidomide on a compassionate access program.

Patients and methods

Patients were retrospectively identified from the national compassionate access program (Pomalidomide Named Patient Program) and demographic, treatment and outcome data were recorded.

Results

Eighty-six patients were identified with sufficient information for analysis. At the time of pomalidomide commencement, median age was 64.5 years (range 36.6 – 85.4 years) and 65% were male. Patients had received a median of five prior lines of therapy including upfront autologous stem cell transplantation in 59%. 76% and 90% were refractory to bortezomib and lenalidomide respectively, and 69% were refractory to both. 32.9% achieved a PR or better, with a further 34.1% achieving a minor response or maintaining stable disease. The median number of cycles received was 3.5 (range 1-33 cycles) and the median time to best response was 2 months.

The median progression-free survival (PFS) and overall survival (OS) were 3.39 and 7.5 months respectively. In patients who achieved PR or better, median PFS was 12 months. On univariate and subsequent multivariate analysis, significant factors adversely affecting PFS were age <65 years, male gender, haemoglobin <100g/L, and thrombocytopenia <100x10⁹/L (all p<0.05). Prior refractoriness to thalidomide, bortezomib and/or lenalidomide was not statistically significant; nor was the number of prior lines of therapy. Patients aged <65 years demonstrated inferior response rates (PR 23% vs. 45%; p=0.03), and were more likely to be anaemic (HR 2.47, 95%CI 1.43-4.30) and thrombocytopenic (HR 2.47, 95%CI 1.41-4.34).

Conclusions

The "real-world" Australian cohort of relapsed/refractory myeloma patients demonstrated similar response rates and PFS compared to the published phase III MM-003 study. The worse OS likely reflects the differences between "real world" and "clinical trial" populations, and in particular in patients aged <65 years likely reflects a higher prevalence of more advanced/refractory disease.

Celgene Australia provided data management support for this research, but had no role in analysis of the data or writing of the abstract.

034. Myeloma outcomes in New Zealand: Increasing regional variation

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Myeloma outcomes have improved though access to new drugs. In New Zealand most centres claim have a similar approach to transplant eligible myeloma; CyBorD induction, transplant and VTD consolidation. Transplant ineligible patients are treated with a Velcade based regimen; CyBorD or VMP with some centres using VTD consolidation. However, the number of cycles in older patients and the upper age limit for using Velcade based therapy may vary.

Aims

We wanted to see if there were regional differences in outcomes. Identification of regions who underperform should prompt review of practice in that region. Outperforming areas also need to be identified so differences in care can be examined to allow dissemination of best practice.

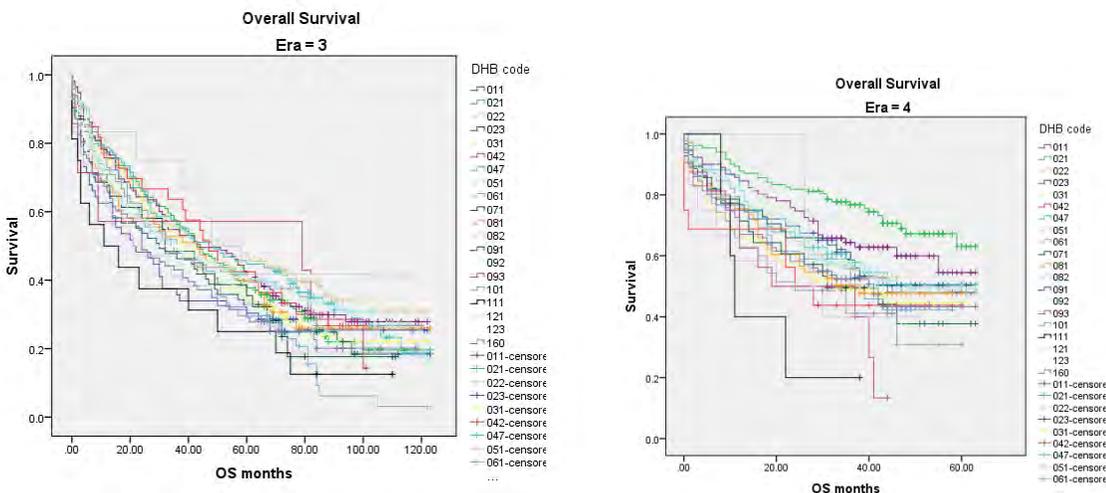
Methods

We extracted data on new cases of myeloma (ICD-10 code C90.0) from the New Zealand Cancer Registry from 1994 to 2013. Deaths were extracted from the national death registry. District Health Board (DHB) was as listed in the cancer registry. Patients were grouped into 4 eras 1) <2000, 2) 2001-2005, 3) 2006-2010, 4) >2011

Results

There was a trend for regional variation in era 1 ($p=0.075$) 2 ($p=0.045$) and 3 ($p=0.133$) but in the most recent era regional variation was greater ($p=0.003$) Log Rank (Mantel-Cox).

The best DHB had a 4 yr OS of $67\pm 5\%$ vs Rest Of New Zealand $46\pm 2\%$ in the latest era ($p<0.00001$). The difference was greater in >65 yr patients $62\pm 6\%$ vs $32\pm 2\%$ ($p<0.00001$), than in those <65 yrs 77 ± 8 vs $71\pm 3\%$ ($p=0.089$). There was no difference in outcomes between DHB's who had <100 , $100-200$ or >200 cases of myeloma since 1993, or between the North and South Islands, when DHB 21 was excluded.



Conclusion

While there has been an improvement in myeloma outcomes, a significant regional variation has developed in the last 5 years. This is most marked in patients aged >65 years. The reasons for this need to be explored so best practice can be shared and adopted.

035. Early autologous stem cell transplant associated with improved overall survival in multiple myeloma: findings from a population based study

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Background

The use of early autologous stem cell transplant (SCT) in multiple myeloma (MM) remains the standard of care for eligible patients. We identified patients objectively eligible for SCT, of whom only 40% underwent early SCT within 12 months of diagnosis.

Aim

To evaluate whether early SCT in MM is associated with a survival advantage.

Method

Retrospective analysis of new MM cases notified to the Victorian Cancer Registry 2008-9. MM cases were identified by age and comorbidity to be theoretically eligible for SCT and then divided into having received SCT within 12 months of diagnosis or not. Overall survival (OS) was determined as the time from diagnosis to death from any cause, determined by linkage with the Victorian Deaths Registry (complete to the end of 2014). Median follow-up was 5.2 years. OS was estimated by the Kaplan-Meier method while the log-rank test used was to test survival differences between SCT and no SCT groups. Cox proportional hazards regression was used to test associations with survival adjusting for age at diagnosis, sex, residential location, location of initial treatment, comorbidities, stage, renal related organ/tissue impairment (ROTI), type of chemotherapy (novel vs traditional agent), and high risk cytogenetic abnormality. Co-variables were included in a final multivariate model if $p < 0.05$ on initial analysis.

Results

Data were obtained for 225 MM cases deemed eligible for SCT of which 123 underwent early SCT. Median OS for SCT and no SCT groups were 5.3 and 4.5 years, respectively. Age >60 years, the presence of renal ROTI and high-risk cytogenetic abnormalities were associated with reduced OS in the multivariate model. Neither presence of moderate or high comorbidity, location of residence, location of initial treatment nor type of chemotherapy prior to SCT were associated with OS in the multivariate model. Reduced OS for the no SCT group remained significant (HR 1.60, $p=0.02$) after adjustment for covariates.

Conclusion

Early SCT is independently associated with improved OS, regardless of the use of novel therapies in initial MM treatment. Early SCT in MM should remain the standard of care for eligible patients.

036. Transplantation in FLT-3-ITD+ AML with normal karyotype is associated with improved overall survival

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Introduction

FMS-like tyrosine kinase 3 – internal tandem duplication (FLT3-ITD) positive acute myeloid leukaemia (AML) is associated with increased relapse risk and reduced survival compared to wild-type FLT3 AML.

Aim

The aim of this study was to assess the impact of allograft on survival for FLT3-ITD+ AML with normal cytogenetics.

Methods

A database search was conducted to identify all cases of FLT3-ITD + AML diagnosed in the Pathology Queensland Laboratory since 2008. A retrospective review of patient charts, pathology, pharmacy and clinical databases was conducted. 71 patients were eligible, who were treated at five centres throughout Queensland. Data was collected on patient characteristics including cytogenetic and molecular risk profile and FLT3-ITD ratio, treatment and response, transplant status, relapse and overall survival.

The primary endpoints for this study were overall survival (OS), event-free survival (EFS), and remission duration (RD).

Results

Median age at diagnosis was 51.4 years. The CR rate after one or two cycles of induction was 64/71 (90%). There were four (6%) induction deaths.

Of the 64 patients with who achieved CR, 34 (53%) relapsed.

46 (65%) patients died during the study. The median OS was 12.98 months. EFS and RD were 7.4 and 8.3 months respectively.

33 patients were allografted in CR1. A further 7 were allografted in CR2 or with secondary refractory disease. 11 (28%) patients who were allografted died from progressive disease and 11 (28%) died from non-relapse mortality.

Of 24 patients who achieved CR but were not transplanted, 18 (75%) died from progressive disease.

The OS for patients allografted in CR1 was significantly better than those who were not (50.1 mths vs. 8.5 mths) ($p < 0.0001$). EFS was 33.1 months for those transplanted in CR1 compared to 5.5 months for those who were not ($p < 0.0001$).

Conclusion

Our study supports the role of allograft in first remission for patients with FLT3-ITD+ AML. Further prospective studies are required to determine the optimal management strategy for these patients.

037. The Prognostic Impact Of Early Donor Chimerism After Allogeneic Stem Cell Transplantation Is Dependent On Disease Risk Index

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Background

The efficacy of alloSCT after reduced-intensity (RIC) or non-myeloablative (NMA) conditioning relies on the graft-versus-tumour (GVT) effect. This requires robust donor immunological engraftment, reflected by T-cell chimerism. The degree of T-cell chimerism required for disease control is likely to depend on disease characteristics including disease risk index (DRI).

Aim

To investigate the impact of day 30 T-cell chimerism (D30chim) on post-transplant relapse in patients with haematological malignancies stratified by DRI.

Method

Patients who underwent alloSCT from sibling or matched-unrelated donors with RIC/NMA conditioning at Royal Melbourne Hospital from 2001-2015 were analysed. D30chim was measured on peripheral blood. Patients who died or relapsed before day 30 were excluded.

Results

207 patients were analysed with median follow-up 44.0 months (Table 1). 57% of patients achieved complete donor T-cell chimerism, defined as D30chim \geq 95% (Table 2). On univariate and multivariate analyses, DRI and complete D30chim were significantly associated with relapse (DRI high/very high vs low/intermediate HR 1.94, p=0.017; complete vs incomplete D30chim HR 0.51, p=0.009). Complete D30chim was significantly associated with increased NRM on univariate but not multivariate analysis (HR 2.70, p=0.067), and was not associated with RFS or OS. In low/intermediate DRI patients, complete D30chim was associated with relapse on univariate (HR 0.35, p<0.001) and multivariate analyses (HR 0.40, p=0.014). In comparison, complete D30chim was not significantly associated with relapse in patients with high/very high DRI. Our cohort of 42 patients with high/very high DRI had 82% power to detect a HR 0.35 for relapse, suggesting that our findings were unlikely to be due to lack of statistical power.

Conclusion

Complete D30chim is associated with reduced relapse in patients with low/intermediate DRI but not high/very high DRI. This suggests that robust early donor T-cell engraftment is insufficient to control disease in patients with high/very high DRI, and these patients may benefit from novel approaches to enhance the GVT effect.

Table 1. Demographics

Characteristic	Value
Age (median years, range)	54 (19-69)
Disease (n, %)	
AML	77 (38.3)
MDS	14 (7.0)
MPN/CML	10 (4.8)
NHL/CLL	64 (30.9)
HL	13 (6.5)
MM	22 (10.9)
Other	7 (3.5)
Disease Risk Index (n, %)	
Low	22 (10.9)
Intermediate	143 (71.1)
High	38 (18.9)
Very high	4 (2.0)

Table 2. Donor T-cell chimerism

DRI category	T-cell chimerism (median %, IQR)
All patients	96% (87-100%)
Low/Intermediate DRI	96% (87-100%)
High/Very High DRI	100% (88.3-100%)

038. Biopsychosocial Outcomes of Victorian Allogeneic Stem Cell Transplant (alloSCT) Late Effects (LE) Program: Metabolic Syndrome (MetS), Quality of Life (QOL) & Returning to Work

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Alfred Hospital (AH) and The Royal Melbourne Hospital (RMH) / Peter MacCallum Cancer Centre (PMCC) provide the majority of alloSCT service including LE programs to Victoria and Tasmania. In mid-2014 these services established a coordination of LE data collection. AlloSCT recipients 2 or more years following transplant are assessed in a multidisciplinary LE clinic. Biopsychosocial outcomes with strong potential for modification for improved health are presented.

Method

Prospective data from individuals presenting to their first late effects clinic from May 2008 (AH) and November 2014 (RMH/PMCC) to May 2016 was analysed. Data included patient demographic, disease & treatment details, anthropometric & biochemical measures. Standardised incidence ratios for MetS risk factors (Table 1) were calculated from Australian Bureau of Statistics population data. The Functional Assessment of Cancer Therapy-BMT (FACT-BMT) measured self-reported QOL.

Result

Data from 346 patients was analysed with the following demographics: 46% female; median age at clinic 47 (range 18-70) years, age at alloSCT 41 (range 1-67) years, time since transplant 5 (range 2-27) years; 66% myeloablative; 52% acute leukaemia; 80% ≥ 2 prior therapies. Both males and females had significantly higher prevalence of MetS risk factors including hypertension, increased fasting glucose and triglycerides and diagnosis of MetS compared with Australian population data (Table 1). Males and females had significantly higher self-reported social wellbeing and males higher functional wellbeing but lower other QOL measures compared with Australian normative data (Table 2). 93% of women and 94% of men were working or in active study at the time of their diagnosis or treatment compared to 47% and 50% respectively at their first LE clinic assessment.

Table 1: Risk factors for MetS and diagnosis of MetS for males and females at LEC clinic. Standardised incidence ratio (SIR) derived from Australian population data

MetS risk Male (M) Female (F)	↑Waist ≥94 cm M ≥80 cm F	↑fGlucose ≥5.6 mmol/L	Hypertension ≥130 mmHg / or ≥85 mmHg	↓fHDL chol <1.03 mmol/LM, <1.29 mmol/LF	↑fTrig ≥1.7 mmol/L	MetS ≥3 risk
M (n=189)	88 (47%)	41 (22%)	114 (60%)	38 (20%)	72 (37%)	40 (21%)
SIR; 95% CI	0.8; 0.84,1.76	3.4; 3.2,3.6	1.8; 1.7,2.0	1.05; 0.99, 1.0	2.1; 1.9,2.2	1.14; 1.08, 1.2
F (n=157)	118 (75%)	22 (14%)	82 (52%)	42 (28%)	50 (32)	35 (22)
SIR; 95%CI	1.15; 1.1,1.2	3.7; 3.5, 3.9	1.8; 1.7, 2.0	1.0; 0.95, 1.0	3.6; 3.4,3.8	1.2; 1.14, 1.26

Table 2: Quality of life well being domains for males and females

QOL domain	Physical	Social	Emotional	Functional	QOL	Aus Population
M (n=187) T score	45.9	56.9	46.1	54.3	50.1	50
p value	<0.01	<0.01	<0.01	<0.01	0.8	SD = 10
F (n=157) T score	45	53.3	47.9	46	49.2	50
p value	<0.01	<0.01	<0.01	<0.01	0.3	SD = 10

Conclusion

This study has highlighted a number of priority areas for intervention for survivors of alloSCT. These include improvement in MetS risk factors, assistance with returning to work and identification of factors reducing health-related QOL. A coordinated multicenter approach to alloSCT LE provides a strong model to develop excellence in survivorship care for individuals following intensive haematological treatment.

039. Early acute kidney injury post allogeneic stem cell transplant is associated with increased risk of GVHD, higher mortality and reduced survival

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Aim

To define rates, risk factors and clinical significance of early renal failure in allogeneic stem cell transplantation (SCT).

Methods

Consecutive allogeneic SCT performed at our centre between January 2008 and December 2013 were identified from an institutional database. Clinical characteristics and outcomes were then retrospectively determined by review of individual patient records. Baseline serum creatinine was defined by day 0 value, with maximum relative increase in creatinine before day 14 graded using the Kidney Disease Improving Global Outcomes score. Non-relapse mortality (NRM) was defined as any non-relapse related death; PFS and OS were calculated from day 0 till relapse or death respectively.

Results

A total of 479 consecutive transplants were reviewed; median follow-up for the whole cohort was 69mths (range 36-108mths). Of these patients, 57 (12%) developed \geq grade 2 acute kidney injury \leq day 14 (early AKI cohort), including 41 patients with grade 2 (9%) and 16 (3%) with grade 3 AKI. Patients who developed early AKI were significantly more likely to be female and have undergone ABO-minor mismatched and unrelated donor SCT. Clinical outcomes were significantly inferior in patients who developed early AKI, with increased incidence of grade III-IV acute GVHD (72% vs 52% respectively; $p=0.004$), increased NRM (40% vs 21% respectively; $p=0.001$) and reduced OS (median 21mths vs 118mths respectively; $p=0.0001$); PFS was similar irrespective of development of early AKI or not (4yr PFS 67% vs 69% respectively; $p=0.7$). Multivariate analysis revealed early AKI, development of grade III-IV acute GVHD and unrelated donor transplantation as independent predictors of mortality. For patients with early AKI, requirement for dialysis and modification of immunosuppression prior to day 14 were both associated with significantly inferior OS

Conclusion

Early AKI is not uncommon post allogeneic SCT and is associated with significantly reduced OS. Novel immunosuppression approaches for management of these patients are required.

040. Inhibition of glucosylceramide synthase induces apoptotic cell death in multiple myeloma cells

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Aim
Ceramide is an apoptotic sphingolipid which is often elevated in cells by chemotherapy and radiotherapy, and contributes to cancer cell death induced by these therapies. Some cancers, however, are able to convert this ceramide to relatively non-apoptotic glucosylceramide via glucosylceramide synthase (GCS), reducing the effectiveness of therapy. Indeed, increased glucosylceramide often correlates with drug resistance. However, there is no published data about the role glucosylceramide plays in the incurable haematological cancer multiple myeloma (MM). This is despite the fact that patients with the sphingolipid storage disorder Gaucher disease, which is treated with a GCS inhibitor, are more prone to developing MM. Thus, we aimed to determine whether GCS is a viable target in MM.

Method
GCS activity was measured using a fluorescence-based, HPLC enzyme assay. The GCS inhibitor used was D-threo-PDMP. Genetic knockdown was achieved using an inducible lentivirus expressing GSC shRNA. Cell viability and caspase-3 cleavage were measured using multi-parameter flow cytometry.

Results
The enzyme activity assay showed that GCS is highly active in many MM cell lines. When these cells were treated with D-threo-PDMP, cell death was induced within 24 hours, with an IC₅₀ between 50 and 80µM for the majority of cell lines. Furthermore, treatment with D-threo-PDMP also sensitised MM cells to bortezomib, a current clinically-employed drug in MM therapy. Genetic knockdown of GCS also induced cell death, with a 45% reduction in viability 3 days after shRNA induction, and a 65% reduction in viability after 7 days. This was accompanied by a matching increase in the number of cleaved caspase-3 positive cells, which strongly suggests apoptosis is the mechanism of cell death. Knockdown of GSC was confirmed by qPCR, which showed ~70% reduction in GCS mRNA after 3 days.

Conclusion
Targeting GCS causes apoptotic cell death in MM cells, and sensitises MM cells to bortezomib.

041. Application of EuroFlow for minimal residual disease detection of multiple myeloma following tandem autologous-NMA allogeneic stem cell transplantation

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Aim

Use of the 8-colour EuroFlow MM MRD assay has not been studied post-allogeneic transplantation. We retrospectively reviewed our Euroflow-MRD experience to identify the relationship between MRD status and outcome for patients following tandem transplantation.

Method

Since May 2008 selected newly-diagnosed high risk (HR) and relapsed MM patients have undergone tandem ASCT-NMA AlloSCT at our centre. HR patients had at least 2 of 5 HR features including ISS-score 3, adverse-cytogenetics, elevated LDH, plasma-cell leukemia or induction-failure(<PR) with bortezomib or IMiD-based induction and underwent 'upfront' tandem transplantation. Patients relapsing following conventional treatment received 'deferred' tandem transplantation. The 8-colour EuroFlow-MM MRD assay panel was introduced in November 2014 for post-transplant bone marrow monitoring.¹ Euroflow-MRD analysis was performed post-alloSCT, quarterly in first year and yearly thereafter. Clinical response was defined as per IMWG criteria.² Survival was from the time of alloSCT.

Results

34 patients (20 upfront, 14 deferred) (median age=55.5yrs; median follow-up (MFU) 31.3months) had sequential EuroFlow-MRD performed until Feb.2016 (Figure 1). 12 patients had sibling donors and 22 unrelated donors (VUD) (p=0.03). 4 distinct patterns of disease behavior post-transplant were identified. 18 patients were MRD negative (MRD-) and didn't progress; 3 MRD- patients developed isolated extra-medullary disease (EMD); 9 progressed in the context of MRD positivity (MRD+); and 4 with sustained MRD+ received IMiDs and remained progression free (MFU 23months). Median PFS of MRD+ patients was inferior to MRD- patients (735 days vs 2540 days; p<0.0001) (Fig 2) but OS was similar (p=0.2). PFS was different between MRD- and MRD+ patients after adjusting for significant covariates (disease-status at transplant, donor-type, ISS-stage and transplant-timing). Delayed conversion of MRD+ to MRD- on subsequent testing consistent with an ongoing graft-versus-myeloma effect occurred in 4 patients.

Conclusion

MRD estimation by Euroflow is a reliable predictor of outcome after tandem transplantation but does not predict EMD relapse.

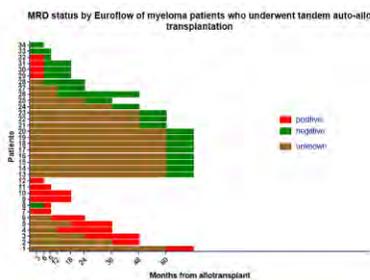


Figure 1

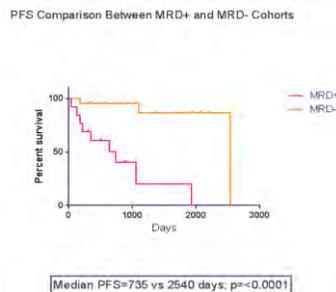


Figure 2

042. TOP2A a New Predictive Marker of Response to Carfilzomib in Multiple Myeloma

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Aim

Microarray was used to determine if a genetic signature associated with multiple myeloma (MM) resistance to carfilzomib, a second-generation proteasome inhibitor (PI) could be identified.

Methods

Nine human myeloma cell lines (HMCLs) were treated with carfilzomib and categorised as sensitive or resistant. Subsequently, the gene expression profiles (GEP) of untreated resistant versus sensitive HMCLs were compared. GEP identified differences were validated on a panel of 17 HMCL with q-RT-PCR and siRNA. TOP2A immunohistochemistry was performed on a panel of fully annotated trephine biopsy specimens acquired prior to treatment with PI.

Result

206 genes were differentially expressed between the sensitive and resistant HMCLs. Gene ontology analysis identified two pathways that were significantly different: pathogenic E.Coli infection (p=0.002) and lysosome (p=0.006). Eight GO terms were also enriched. TOP2A, an enzyme that controls and alters the topologic states of DNA during transcription and is involved in cell cycle and proliferation was overexpressed in resistant HMCLs. It functions as the target for several anticancer agents and a variety of mutations in this gene have been associated with the development of drug resistance. TOP2A has also been noted to be overexpressed in the 'proliferation cluster' of published MM GEP profiles and associated with poor outcome. Following suppression of TOP2A by siRNA, carfilzomib-resistant HMCLs were resensitized to carfilzomib, moreover, the combination of carfilzomib with topoisomerase inhibitors, known to target directly TOP2A, demonstrated synergistic cytotoxic effects against HMCLs. Trephine-derived TOP2A protein expression levels were shown to be higher in patients treated with PIs including bortezomib, carfilzomib, ixazomib, marizomib failing to achieve a response when compared to responding patients. Finally, logistic regression analysis confirmed that TOP2A protein expression was a highly significant predictor of response to PI (AUC 0.764, p=0.048).

Conclusion

TOP2A status may be used as a predictive factor for patient response to PI including carfilzomib.

043. Overcoming innate resistance to a new beta-catenin inhibitor by manipulating the autophagic pathway in Multiple Myeloma

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Introduction

Chemoresistance is a major challenge in the development of new therapies in multiple myeloma [MM]. Inhibition of autophagy has been shown to restore chemosensitivity in several tumors. We have previously validated a beta-catenin inhibitor (BC2059) which targets the Wnt/beta-catenin signaling pathway.

Aim

In the present study we aim to overcome innate resistance to BC2059 by manipulating the autophagic pathway.

Methods

Autophagic flux was estimated by measurement of LC3II/LC3I in the absence and presence of hydroxychloroquine [HQ] by Western blot [WB]. Induction of autophagy was measured by the increase of LC3II/LC3I by WB, and concomitant drop of p62 expression by Flow Cytometry. Combination Indices [CI] were calculated using CalcuSyn software.

Results

BC2059 induces apoptosis in a dose-dependent manner by induction of both the intrinsic and extrinsic apoptotic pathways, (increase of active -caspase-8, -caspase-9 and -caspase-3) in all human myeloma cell lines [HMCL] tested. All HMCL tested have significant autophagic flux at baseline. Chemical inhibition of autophagy has an anti-proliferative effect, decreasing the relative cell numbers from 40% (NCI-H929) to 23% (KMS12BM) at 24hr. In parallel BC2059 is able to induce autophagy in a dose dependent manner. Induction of autophagy is BC2059 specific as treatment with melphalan or bortezomib at relative equal anti-proliferative doses did not increase LC3II/LC3I. Further autophagic inhibition by HQ was synergistic for all HMCL with CI of 0.7-0.3 (CI<1.1 indicates synergy). Interestingly, inhibition of autophagy halved the LD₅₀ of BC2059 in a resistant HMCL (LP1). Other autophagy inhibitors (3-MA, bafilomycin A1 and NH₄Cl) were also synergistic with BC2059 in LP1.

Conclusion

BC2059 exerts cytotoxicity mainly by induction of apoptosis but also induces cyto-protective autophagy. Autophagy inhibition was able to overcome innate resistance to the drug, ameliorating its cytotoxic effect. This study warrants further investigation.

044. The Delayed Diagnosis of Philadelphia Negative Myeloproliferative Neoplasms (MPN) is Common and Results in a High Incidence of Potentially Preventable Thrombotic Complications

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Background / Aims

Patients with MPNs are at significantly increased risk of thrombotic and haemorrhagic complications which are a major cause of morbidity and mortality. Prior to diagnosis of MPN the incidence of thrombotic events is high and prior arterial and venous thromboses have been shown to be highly predictive for subsequent events. Earlier diagnosis and treatment of MPNs may reduce the incidence of thrombosis. We performed a study to determine if delayed diagnosis was common.

Methods

Medical records of patients diagnosed with MPN from January 2010 until June 2016 under the care of haematologists at our centre were examined. We determined time from first appearance of a blood count abnormality consistent with the diagnosis of MPN until their formal diagnosis. We recorded thrombotic and haemorrhagic complications that occurred during this time.

Results

135 patients were diagnosed with MPN; 34 with PV, 65 with ET, 24 with PMF 12 with MPN-U. Patients with PV had average delay till diagnosis of 704 days (range0-2650), median 358 days and 9.4% had potentially preventable thrombotic events. Patients with ET had average delay till diagnosis of 1420 days (range0-8731), median 825 days and 21.5% had potentially preventable thrombotic events. PMF had average delay till diagnosis of 782 days (range0-3684), median of 256.5 days and 12.5% potentially preventable events. In MPN-U the average delay till diagnosis was 1272 days (range42-4255), median 1391.5 days and 33.3% had potentially preventable adverse events.

Conclusions

Delay to diagnosis of MPN results in potentially preventable adverse events. Earlier recognition of abnormalities consistent with MPN and their investigation is may prevent adverse thrombo-haemorrhagic events and reduce the incidence of subsequent thrombotic events. New reporting guidelines for the interpretation and reporting of the full blood count are essential to increase awareness of MPN and prompt further investigation and specialist review.

045. Immune Responses in Chronic Myeloid Leukaemia Patients Following Cessation of Tyrosine Kinase Inhibitor

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For chronic myeloid leukaemia (CML) patients with a deep molecular response an attempt at treatment free remission (TFR) leads either to prolonged remission or early molecular relapse. We hypothesise that immune responses promote sustained TFR and that immunological markers may predict response following TFR attempt.

We studied 54 CML patients on TKI (minimum 24 mo MR^{4.5}) and following TKI discontinuation from ALLG CML 8 and 10. Effector immune responses of NK cells were characterised by flow cytometry and cytotoxic T lymphocyte (CTL) responses to leukaemia-associated-antigens (LAAs) WT1, BMI-1, PR3 and PRAME by interferon-gamma ELISPOT.

CD56^{dim}CD16^{bright} cytolytic NK cells as a proportion of lymphocytes were increased in patients with TFR-success (n=22) (73.3% ± 3.8) compared to TFR-failure (n=23) (57.0% ± 3.5, p=0.002) at baseline (TKI cessation timepoint). TFR-success patients displayed increased CD57⁺ NK cells at baseline (74.5% ± 2.2) vs TFR-failure (66.3% ± 2.7, p=0.02). NKG2D activating receptor expression was significantly increased in NK cells in TFR-success patients (baseline= 56.8% ± 3.8, 3 mo= 61.3% ± 5.0, 6 mo= 50.1% ± 5.8) compared to TFR-failure (baseline= 44.2% ± 3.7 (p=0.02), 3 mo= 42.2% ± 5.5 (p=0.02), 6 mo= 22.0% ± 8.3 (p=0.01)), Figure 1.

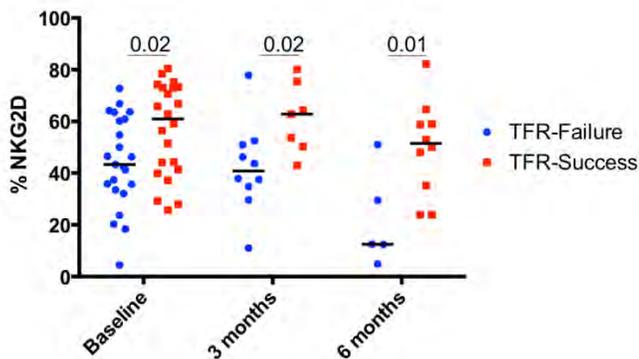


Figure 1. CD3⁺CD56⁺ NK Cell NKG2D⁺ expression

KIR2DL2/DL3/DS2 expression increased in TFR-failure patients at 3 and 6 months compared to TFR-success. (TFR-failure; 3 mo= 44.8% ± 4.6, 6 mo= 48.8% ± 4.9. TFR-success; 3 mo= 31.5% ± 4.0 p=0.05, 6 mo= 31.1% ± 2.1, p=0.001). Functional CTL immune responses were observed in both TFR-success and TFR-failure patients. BMI-1-CTL responses were increased at baseline in TFR-success patients (23%) compared to TFR-failure (9%). PR3-CTL responses were not detected in TFR-success patients at baseline, 3 mo or 6 mo (0%) compared to TFR-failure (baseline= 18%, 3 mo= 50%, 6 mo= 50%).

Enhanced immune effector responses may promote sustained TFR; methods to enhance these may increase TFR-success rates.

046. Mitochondrial DNA mutations at diagnosis are linked to response in TKI treated chronic myeloid leukaemia patients

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Aim

Genomic instability in *BCR-ABL1*-positive chronic myeloid leukaemia (CML) is postulated to be related to increased generation of reactive oxygen species (ROS). The mitochondrial (mt) genome is susceptible to ROS-induced mutations due to the high oxidative stress in the mitochondrion and limited DNA-repair mechanisms.

Therefore, we investigated the acquisition of mtDNA somatic mutations, and whether these mutations could identify heterogeneity in the leukemic clone.

Methods

We performed targeted-next-generation-sequencing on mtDNA from 38 CML patients enrolled on the ALLG-CML9 trial at diagnosis and after 12 months of tyrosine kinase inhibitor (TKI) therapy. Patients were classified as good (20) or poor (18) responders based on achievement of major molecular response (MMR, *BCR-ABL1*^{IS} ≤ 0.1%) at 12 months. LowFreq software was used to identify mtDNA somatic mutations at diagnosis by comparison with follow-up samples at 12 months. Treating the number of mutations as quantitative predictive factor, we dichotomized patients by high (≥3) or low (>2) mutational burden at diagnosis and we generated Kaplan-Meier survival curves.

Results

The most abundant substitution found in our samples was the G/C→A/T transition supporting the hypothesis of the damage induced by ROS.

We identified 114 mtDNA somatic point mutations (mean 5.7 per patient, range 0-21) in 18 (90%) good responder patients, compared to 18 somatic mutations (mean 1 per patients, range 0-3) in 11 (61%) poor responder patients at diagnosis ($p=0.0004$) (Figure 1).

A higher mutational burden at diagnosis (≥3) was associated with better MMR ($p=0.0036$) and MR4.5 (*BCR-ABL1*^{IS} ≤ 0.0032% or undetectable disease in cDNA with >32,000 *ABL1* transcripts; $p=0.015$) achievement at 24 months.

Conclusions

This study demonstrated that CML patients who respond well to TKIs have a greater number of mtDNA mutations at diagnosis. The reasons for this are still to be elucidated, but one postulation is that mtDNA damage may promote susceptibility to pro-apoptotic signals leading to cell death with TKI treatment.

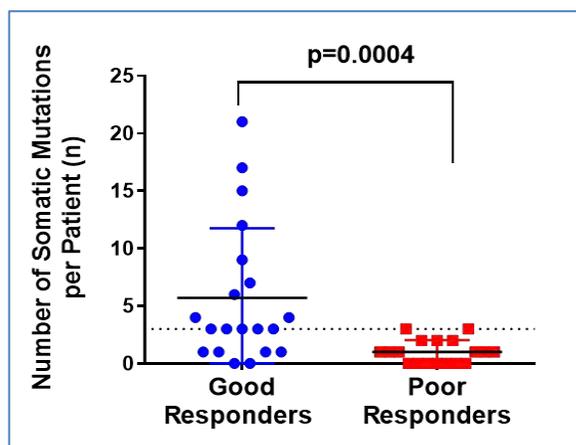


Figure 1. Number of mtDNA somatic point mutations per patient in good and poor responders at diagnosis.

047. Targeted amplicon sequencing combined with selected extended sequencing efficiently establishes diagnoses in conventional and triple-negative BCR-ABL negative myeloproliferative neoplasms in the molecular diagnostic laboratory

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Background and Aim

Assessment of multiple genes is required to diagnose myeloproliferative neoplasms (MPN) and is typically performed using serial allele specific molecular methods. We aimed to describe molecular lesions in MPN referred for diagnostic testing using comprehensive next generation sequencing (NGS) in order to determine the effectiveness of this approach for patient diagnosis.

Methods

All MPN referred for testing at the Molecular Haematology Laboratory (Peter MacCallum Cancer Centre) from December 2014 to June 2016 were sequenced using a diagnostic amplicon-based NGS panel targeting hotspot regions of *JAK2*, *MPL*, *CALR*, *SF3B1*, *CSF3R*, *KIT* and *ASXL1* plus 19 genes frequently mutated in myeloid malignancy. Capture-based extended sequencing (including all coding exons of *JAK2*, *MPL*, *CALR*, *THPO*, *SH2B3*, *EGLN1*, *EPOR*, *EPAS1*, *VHL*) and copy number assessment was performed on selected cases.

Results

Of 366 patient referrals tested using the amplicon panel, 134 had canonical mutations including *JAK2*V617F (n=91), *CALR* (n=36), *MPL*W515L (n=5) and dual *JAK2*V617F/*CALR* (n=2). Three *JAK2*V617F mutations were detected in patients previously designated negative by outside laboratories. Of the remaining samples (n=232), non-canonical mutations were identified in six patients (2 novel *JAK2*delins, 3 novel *MPL*delins in exon 10 and a *MPL*P106L). 10% of negative cases had mutations in at least one additional gene therefore establishing the presence of a clonal myeloid neoplasm.

Extended capture-based sequencing of 20 mutation-negative cases which otherwise met MPN clinicopathological criteria (i.e. "true triple negative-MPN") identified previously described mutations in *MPL* (S204P) and *SH2B3* (E208Q) along with novel variants of uncertain significance in *CALR*, *SH2B3* and *EPOR*. Non-canonical mutations detected in MPN by next generation sequencing

<i>JAK2</i>	c.1849_1853delinsTTTTA	p.Val617_Cys618delinsPheTyr
<i>JAK2</i>	c.1849_1851delinsTTT	p.Val617Phe
<i>MPL</i>	c.1543_1544delinsAA	p.Trp515Lys
<i>MPL</i>	c.1492_1513delinsTGCGCCGTCCTGGGCCTGCTGT	p.Leu498_Ser505delinsCysAlaValLeuGlyLeuLeuCys
<i>MPL</i>	c.1543_1544delinsGC	p.Trp515Ala
<i>MPL</i>	c.[317C>T];[317C>T]	p.Pro106Leu
<i>MPL</i>	c.610T>C	p.Ser204Pro
<i>SH2B3</i>	c.622G>C	p.Glu208Gln
<i>SH2B3</i>	c.1210A>G	p.Thr404Ala
<i>CALR</i>	c.269C>T	p.Thr90Met
<i>EPOR</i>	c.1261G>C	p.Ala421Pro

Conclusion

We have demonstrated that NGS (i) efficiently and sensitively detects canonical MPN mutations (ii) identifies disease causing non-canonical mutations missed by allele specific methods (iii) can provide a clonal diagnosis in canonical mutation negative cases. Extended sequencing may diagnose a minority of cases and should be considered on a case-by-case basis.

048. Biology and Therapy of Waldenstrom's Macroglobulinemia

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Waldenström's macroglobulinemia (WM) is a B-cell neoplasm manifested by the accumulation of clonal IgM secreting lymphoplasmacytic cells. MYD88^{L265P} and CXCR4 WHIM-like somatic mutations are present in >90%, and 30-35% of WM patients, respectively, and impact disease presentation, treatment outcome, and/or overall survival. Familial predisposition is common in WM. Asymptomatic patients should be observed. Patients with disease related hemoglobin <10g/L, platelets <100x10⁹/L, bulky adenopathy and/or organomegaly, symptomatic hyperviscosity, peripheral neuropathy, amyloidosis, cryoglobulinemia, cold-agglutinin disease or transformed disease should be considered for therapy. Plasmapheresis should be used for patients with symptomatic hyperviscosity, and pre-rituximab for those with high serum IgM levels to pre-empt a symptomatic IgM flare. Treatment choice should take into account specific goals of therapy, necessity for rapid disease control, risk of treatment-related neuropathy, immunosuppression and secondary malignancies, and planning for future autologous stem cell transplantation. Frontline treatments include rituximab alone, or combined with alkylators (bendamustine, cyclophosphamide), proteasome-inhibitors (bortezomib, carfilzomib), nucleoside-analogues (fludarabine, cladribine) and ibrutinib. BTK and HCK are both transactivated by mutated MYD88, and are targets of ibrutinib. CXCR4 WHIM-like mutations transactivate AKT and ERK, and promote in vitro drug resistance. Responses to ibrutinib are impacted by MYD88 and CXCR4 mutation status, with lack of major responses observed in wild-type MYD88 patients, while slower response kinetics and lower levels of response are seen in those patients who have CXCR4 WHIM-like mutations. Secondary resistance to ibrutinib in WM patients is associated with acquired mutations in BTK, PLCG2, and CARD11, and include multiple BTK mutations within individual patients progressing on ibrutinib. In the salvage setting, an alternative frontline regimen, ibrutinib, everolimus, or stem cell transplantation can be considered. Investigational therapies under development for WM include agents that target MYD88, CXCR4, and BCL2 signaling, and novel proteasome inhibitors.

050. Single hit lymphoma

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The negative prognostic impact of aggressive B-cell lymphomas harbouring a cytogenetic 'double-hit' (DH) for switch translocations of the *cMYC* and *BCL2* loci is well recognised as an area of unmet clinical need. However, the prognostic impact of 'single-hit' (SH) *cMYC*-translocated or *cMYC*-protein expressing disease is less clear in the case of diffuse large B-cell lymphoma (DLBCL), with conflicting reports in the literature. Indeed, some investigators have reported *improved* prognosis of SH lymphoma with chemo-immunotherapy. Moreover, *cMYC* itself has inherent tumour-suppressor properties which counter-regulate its oncogenicity, most notably pro-apoptotic and pro-senescent effects. While this seems to contradict the common assertion that *cMYC*-expression is an adverse prognostic factor in lymphoma, the explanation is that it is not a *cMYC*-break *per se* which defines disease biology, rather the co-operative oncogenic landscape in which a *cMYC*-break resides. In reality, there is no such thing as a 'SH DLBCL' as this disease is genetically complex and assessing *cMYC* in isolation of co-operating events (e.g. translocation partner gene and acquired somatic mutations) and cellular contexts (e.g. 'cell of origin') is inherently flawed. Improvements in diagnostic sophistication, particularly genomic characterisation, may well improve our understanding and ability to prognosticate *cMYC*-positive disease via identification of co-operative dependencies (some of which may be directly 'actionable'). Concurrently, advances in drug development mean that *cMYC* can no longer be considered an 'undruggable oncogene'. Although no effective '*cMYC*-inhibitor' has progressed to the clinic, a range of novel agents can antagonise the transcriptional outputs of *cMYC* including histone deacetylase inhibitors, cyclin dependent kinase-9 inhibitors (e.g. dinaciclib), second generation thalidomide analogues and BET-bromodomain inhibitors. An emerging hypothesis built upon pre-clinical modelling is that in the absence of *BCL2* overexpression, *cMYC*-driven lymphoma relies on *MCL1* to evade apoptosis. Thus the *MCL1* inhibitors undergoing early clinical evaluation in haematological malignancy may have a future role in SH lymphoma. In conclusion, *MYC* is a tumour suppressor as well as an oncogene, its overexpression is not always bad, there's no such thing as a 'single hit' lymphoma and its not so 'undruggable' after all.

051. Ann Arbor Staging vs. Total Metabolic Tumour Volume. Time for a change?

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Lymphoma staging aims to facilitate communication and exchange information, and to provide a guide to prognosis and assist in therapeutic decisions (Carbone, Cancer Research, 1971). The former is best achieved with data as condensed as possible, the latter when the greatest amount of information is collected.

The Ann Arbor system developed for HL in 1971 was revised in Cotswold in 1988. It designated nodal involvement based around the capacity to encompass radiation fields given that radiotherapy was the dominant therapy at the time. In 2007 there was recognition that PET-CT is central to staging of most FDG-avid histologies. Nonetheless, multiple other surrogates for tumour bulk and proliferation: LDH; B2M; LoDLIN; number of extra-nodal sites; bone marrow involvement; are included in various lymphoma-specific prognostic indices. The recent international staging criteria (Cheson, Barrington, JCO 2014) noted the significant (~20%) stage migration with the more sensitive PET-CT scanning. While still separating lymphomas into localised or advanced stage it was recognised that distinguishing nodal vs. extra-nodal status and uni-dimensional measurement of bulk was of limited use.

More recently, PET studies across a range of lymphomas suggest that calculation of the baseline Total Metabolic Tumour Volume (TMTV), may more accurately quantify tumor burden for determining prognosis. With standardisation of PET acquisition and software packages to assist with measurement of TMTV we may be getting closer to providing a single staging parameter across all histologies. However, we must remain committed to systematically addressing the challenges of volume calculation, and the appropriate choice of TMTV software algorithms to provide reproducible measurements of TMTV across multisite studies.

With widespread use of multi-agent systemic therapies the Ann Arbor staging is no longer fit for purpose. TMTV has the potential to provide the single most efficient and relevant means of informing clinicians and patients of their disease burden. With reliable software and consensus, TMTV may in time replace the Ann Arbor system and become the new standard to convey prognosis and rationale for tailored therapy to our patients.

052. Hodgkin lymphoma gone bad

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Approximately 75% of patients with Hodgkin Lymphoma (HL) are cured with first line therapy. For relapsed/refractory HL (RR-HL) standard therapy consists of salvage chemotherapy followed by high dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT), however, still 50% of patients are either refractory or relapse. There is no obvious superior salvage therapy among the commonly used regimens such as DHAP, ICE, IGEV and GDP; maintaining dose intensity is important for optimal responses. Functional imaging using FDG-PET scanning after salvage chemotherapy and before ASCT is a critical predictor of outcome; the goal of salvage should be a negative PET scan. Either a second line of salvage, or a tandem ASCT, may benefit some patients with residual FDG avidity post-salvage. RIC-allogeneic SCT may provide a GVL effect and durable responses in some patients with HL relapsing or progressing after ASCT. New agents such as Brentuximab vedotin (BV) or PD-1 inhibitors have shown substantial activity in RR-HL particularly among those patients relapsing after ASCT. In a recent 5-year update of BV use in 102 patients relapsing after ASCT, the overall and progression-free survival rates were 41% and 22%, respectively for the entire group, and, 64% and 52% for the 34% of patients achieving a CR. Since the majority of BV-treated patients experience disease progression, long term survival prognosis also depends upon whether BV can be a bridge to a RIC-alloSCT. For HL patients failing both ASCT and BV therapy, two recent trials of PD-1 inhibitors Nivolumab and Pembrolizumab have yielded tantalising results with ORR rates of 66% and 64%, respectively. The major challenges concerning PD-1 inhibitor use in RR-HL relate to whether they can be used as a bridge to a RIC-alloSCT, and, how they can be combined with other agents in order to improve the overall and complete response rates.

053. The 2016 revision of the WHO classification of myeloid neoplasms & acute leukaemia

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The 2016 revision of the WHO classification of myeloid neoplasms & acute leukaemia incorporates into the 2008 classification the many advances that have occurred in the past few years particularly in the molecular area. The aim is to improve the diagnostic & prognostic relevance of the classification. Only a select few significant changes are highlighted below.

In the 2016 revision, some new entities have been added, for example AML with BCR-ABL and B-lymphoblastic leukaemia/lymphoma, BCR-ABL like. In AML cases with >50% erythroblasts, the myeloblasts are now counted as a percentage of total marrow (not non-erythroid) cells & thus many cases which would have been previously classified as acute erythroid leukaemia (erythroid/myeloid) would now be classified as MDS usually with excess of blasts. The myelodysplastic syndrome terminology has been revamped & the terms refractory anaemia with excess of blasts 1 & 2 have been replaced with myelodysplasia with excess of blasts 1 & 2. Presence of CSF3R T6181 or other activating CSF3R mutation has been added to the diagnostic criteria for CNL. The Hb cut-off levels for diagnosis of polycythemia vera have been lowered to prevent under diagnosis. The diagnostic criteria for ET now include CALR & MPL mutation in addition to the JAK2 mutation. Mastocytosis has been taken out of the MPN category. Refractory anaemia with ring sideroblasts with thrombocytosis (RARS-T) is now termed MPN/MDS with ring sideroblasts.

These & other changes in the 2016 classification will be discussed.

054. Update on molecular diagnostics of acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS)

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Molecular characterisation of aggressive myeloid malignancies is central to therapeutic decision making. Moreover, the importance of genomic characterisation is maintained throughout the disease course from upfront risk stratification through assessment of minimal residual disease and finally to identifying targeted therapy options in relapsed/refractory disease.

Until recently, the ability to provide rapid turnaround and cost-effective genomic characterisation was not possible. However, high-throughput sequencing is now being translated into molecular diagnostic laboratories and is able to provide extensive molecular characterisation of myeloid malignancy in the diagnostic setting. This presentation will review the utility of molecular characterisation in acute myeloid leukaemia and myelodysplastic syndromes from a molecular diagnostic laboratory perspective including the unique challenges the translation of novel molecular strategies such as next generation sequencing pose to the molecular diagnostic laboratory.

055. Alpha Thalassaemia and Hb E/Beta Thalassaemia-clinical course and disease modifiers

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Non transfusion dependent thalassaemia (NTDT) encompasses a group of thalassaemia patients who need no or minimal blood transfusions in order to survive. Although NTDT is present world-wide, its prevalence is highest in Asia, the Middle East, and the Mediterranean region. In Asian population, two types of NTDT; haemoglobin E (HbE)/ β -Thalassaemia (Hb E/ β -thal) and alpha-thalassaemia; Hb H disease (both deletional and non-deletional forms) are dominant in the clinics. HbE/ β -Thalassaemia is the genotype responsible for approximately 50% of all β -Thalassaemia cases worldwide and affects at least 1 million people worldwide. Early clinical recognition of NTDT and close clinical monitoring is essential to prevent affected children to appropriately tailor management plan for individual patients and prevent some patients from being mistakenly placed on lifelong transfusion therapy.

The diagnosis of NTDT is challenging and must be performed carefully. Due to the broad spectrum of clinical presentations that reflect the various associated genotypes as well as the influence of genetic modifiers, diagnosis of NTDT requires comprehensive haematology, haemoglobin study, and DNA analysis. Three levels of genetic components are responsible for NTDT phenotype; globin defects, ameliorating factors (mutations in globin counterpart such as alpha-thalassaemia in patients with α -thalassaemia disease or Hb E/ α -thalassaemia and quantitative trait locus (QTL) for α globin genes) and deteriorating factors (other genes including UGT1A1, vitamin D receptor, genetic haemochromatosis etc.). Thus, the diagnosis of NTDT is primarily based on the clinical evaluation of the physician. Due to the lack of a pathognomonic symptom or sign, laboratory evaluations need to be combined to confirm diagnosis. Anaemia is one of the key criterion to confirm the presence of NTDT, together other information such as the family history, regional prevalence of thalassaemia, and NTDT associated morbidities such as haemolysis, splenomegaly, gall stones, and iron overload status (serum ferritin > 800 ng/ml, LIC > 5 mg Fe/dL) must be taken into account when diagnosis of NTDT would be made. In the case of Hb E / β -thalassaemia - anaemia of variable severity (3-13 g/dL) with the presence of both Hb F ($\alpha_2\alpha_2$) and Hb E ($\alpha_2\alpha_2^E$) are the disease hallmarks. However, Hb E trait or even homozygous Hb E (Hb EE) have a rather mild phenotype and need no medical management although these individuals have variable degree of hypochromic microcytosis. Therefore, it is crucial to distinguish Hb E disorders diagnostically because of the marked difference in clinical course. In patients with alpha thalassaemia, their clinical presentation could be variable as well. Most patients are transfusion independent in particular those with a classical Hb H disease due to three alpha globin genes missing from four normal alleles (deletional Hb H disease). In this syndrome, they usually have their baseline haemoglobin (Hb) of 8-10 g/dL and do not require frequent blood transfusion. In a less common form, non-deletional Hb H disease due to mainly an interaction of deleted two linked alpha globin genes on one allele and a missense or non-sense mutations on another allele, they frequently have a more severe phenotypes since 30% of this interaction requires more blood transfusion due to acute haemolytic crises and splenectomy. It is of important to follow these patients since childhood and provide appropriate care in order to avoid acute anaemia due to infection and possibility to get complications related to anemia such as growth retardation, cardiac arrhythmias etc.

Using peripheral blood smears, microspherocytes, RBC fragments and nucleated RBC are usually typical blood pictures of all NTDT. In addition, haemoglobin analysis by HPLC and capillary electrophoresis (CE) show a high percentage of HbA2/E, Hb F and other variants including Hb H (α_4) are of important to suggest the genotype of both NTDT conditions. Finally, DNA analysis is useful and might necessary for genetic counselling in several cases.

056. Global Strategies for Sickle Cell Disease

Ware R

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The prognosis of sickle cell disease (SCD) has greatly improved over the past 40 years due to advances in diagnosis, education, prevention, and treatment. Children born with SCD in developed countries are typically identified early in life, and then receive comprehensive care that includes infection prophylaxis and prompt management of acute vaso-occlusive events, along with ready access to transfusions and hydroxyurea as disease-modifying therapy. In contrast, children who are born with SCD in low-resource settings have a different fate. Over 75% of affected births occur in sub-Saharan Africa, and according to the World Health Organization, 50-90% of these children will die by the age of 5 years, often without ever establishing the correct diagnosis. Several African countries have begun pilot screening projects to determine their sickle cell burden, as a first step toward establishing neonatal screening and improving medical care. In addition to financial challenges for the development and sustainability of such programs, numerous programmatic challenges exist such as selecting the optimal methodology for screening; deciding whether new point-of-care devices can supplement standard electrophoresis techniques; providing proper education, preventive therapy, and screening; ensuring blood transfusions can be administered safely; and determining the feasibility and effectiveness of hydroxyurea therapy. Over the next 5-10 years, major advances in SCD will likely occur in low-resource settings. The creation of successful academia-industry-pharma-government partnerships represent a crucial component of effective national SCD strategies. The global burden of SCD should be considered a top priority among the new UN Sustainable Development Goals that focus increased attention on non-communicable diseases.

057. Towards novel therapies for the hemoglobinopathies

Jane S

Australian Centre for Blood Diseases

Disorders of hemoglobin production are the commonest genetic diseases worldwide, affecting a staggering 10% of the global population, and consuming 30% of the donated blood supply. They include the devastating illnesses sickle cell disease (SCD) and β -thalassemia, which are both associated with significant morbidity and early mortality. Very rare SCD and β -thalassemia patients, who inherit a second mutation that results in prolonged expression of their fetal (γ) globin genes after birth, exhibit a dramatic amelioration in the clinical severity of their disease, often being transfusion independent, and having a normal life expectancy. Studies in these patients, and in mouse models, have demonstrated that γ -globin expression levels of only 20% are sufficient to completely abolish the clinical sequelae of SCD and β -thalassemia. In SCD, this is due to the anti-sickling effects of fetal hemoglobin (HbF), and the reduction in expression of the sickle (β^S) allele through competitive silencing by the γ -gene. These observations have prompted the search for therapeutic strategies to re-activate fetal globin gene expression as a cure for SCD and β -thalassemia patients. To date, these strategies have primarily focused on agents that induce changes in "generic" epigenetic modifications associated with γ -gene silencing, such as inhibitors of DNA methylation and histone de-acetylation. Although several of these agents (decitabine, butyrate) are already in clinical use, they suffer from lack of efficacy in more than half of compliant patients. More targeted therapies are dependent on identification of the specific epigenetic modifiers involved in silencing of the γ -genes. This talk will focus on advances in research areas that are defining the molecular mechanisms underpinning silencing of γ -globin gene expression, and progress in targeting these mechanisms for therapeutic reactivation of fetal globin gene expression.

058. Aplastic anemia: Treating the hematopoietic stem cell

Young N

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Aplastic anemia treated with supportive care only is a historically fatal blood disease, often affecting young persons. Clinical results have provided important insights into the pathophysiology of aplastic anemia. Transplantation of the hematopoietic (and immune) systems is curative. Most patients also respond to immunosuppressive regimens, with horse antithymocyte globulin and cyclosporine now standard. For the 2/3 of patients whose blood counts improve after hATG, survival is excellent. Immune destruction of hematopoietic cells can be modeled in the mouse, and new therapies tested in animals. However, a substantial minority of patients do not respond to hATG, or blood counts increase only modestly, and relapse occurs in about 1/3. Extremely low residual stem cell numbers has appeared to be limiting of non-replacement therapies. Eltrombopag, a thrombopoietin synthetic mimetic, was surprisingly effective in about 405 of patients with refractory severe aplastic anemia, leading to durable, robust, and trilineage responses. We combined eltrombopag with standard immunosuppression in a prospective trial in treatment-naïve patients. In >100 patients enrolled to date, overall response rates approach 90% and complete response rates 40%. Early institution of eltrombopag appears optimal. Toxicity has been moderate and the rate of “clonal evolution” to myelodysplasia and aplastic anemia as anticipated. That a drug that stimulates early progenitor proliferation is effective indicates existence of a stem cell reserve in severe marrow aplasia. Success of stem cell therapy in aplastic anemia has broad implications for the treatment of bone marrow failure syndromes.

059. Objectives and progress of the Australian Aplastic Anaemia Registry

Firkin F

St Vincent's Hospital, Melbourne, Australia

Aplastic Anaemia has been a typically chronic and eventually fatal disorder until substantial advances in treatment were made in transplantation and by carefully structured clinical trials with therapeutic agents. It is, however, a sufficiently rare disorder to limit the rate of accrual of newly diagnosed patients for most single or multicentre prospective randomised trials on therapeutic agents, where the results may not be strictly applicable to patients with medical or other issues that represent exclusion criteria in clinical trials. The aim of the Registry is evaluation of the efficacy of varied management practices dictated by clinician choices and associated patient modifying factors in an all-inclusive population to identify approaches that yield optimum clinical outcomes under the range of circumstances encountered in an overall patient population.

The Registry has currently recruited 44 centres with a major involvement in management of aplastic anaemia from all states to participate in a reporting network estimated to register at least 90% of newly diagnosed patients in Australia. Data is recorded at diagnosis and thereafter at regular intervals. The rate of reporting has progressively increased with increasing site registration to now exceed that usually attained in single or multiple centre trials, to provide the prospect of creating a sufficiently large body of data for meaningful statistical analysis.

Details of the course of the 60 patients enrolled at this time are sufficiently mature in 43 to indicate similarities and differences between responses to a variety of treatment approaches dictated by clinical circumstances, including pregnancy, comorbidities associated with advanced age, medication intolerance, and poor or transient responses to initial or subsequent treatment. Initial treatment is most commonly equine anti-thymocyte globulin (ATG) plus cyclosporin, but comparable initial response rates have occurred in smaller numbers of patients with one or other of these agents, and with rabbit ATG. Successful application of anabolic agent treatment after limited and transient responses to both forms of ATG has been reported, as well as successful and uneventful use of rabbit ATG following fulminant hepatotoxicity after one dose of horse ATG. The objective is to determine the extent to which meaningful depth and duration of response to different single or multiple medication combinations can be sustained in the various patient subpopulations, with the aim of providing guidance to the most effective approach to achieve an optimal outcome under defined circumstances. A more detailed presentation and analysis of the findings of the Registry is to be presented at this meeting.

060. Update on 2016 WHO classification of lymphoid neoplasms

Lade S

Peter MacCallum Cancer Centre, Melbourne, Australia

The current 4th edition WHO classification of tumours of haematopoietic and lymphoid tissue was published by IARC in the bluebook format in 2008. With the rapid advances in the world of molecular testing and biological therapies since 2008, an update had become a matter of some urgency. Negotiated with the publishers in 2014, this will be in the form of a hard copy and eBook, and available, on current estimates, sometime early in 2017. It will not be, however, a true 5th edition of the WHO monograph, but an updated 4th edition. This distinction is significant because it has placed constraints on the scope of the changes allowed. New provisional entities have been accepted, some provisional entities have been accepted as definite entities, but no definitive new entities have been added.

The updated classification has been published and the major changes have been discussed in broad terms in a couple of recent pre-emptive articles and presentations in advance of the bluebook publication. Full discussion of these is beyond the scope of this presentation, but the more substantial changes, particularly with regards classification of some of the more common entities and their variants, such as lymphoplasmacytic lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, high grade B-cell lymphomas, angioimmunoblastic T-cell lymphoma, as well as some of the more common new provisional entities, will be outlined.

061. A review of the RCPA EQA program for paroxysmal nocturnal haemoglobinuria

Sartor M

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder characterized by a somatic mutation in the PIGA gene, leading to a deficiency of proteins linked to the cell membrane via glycosphosphatidylinositol (GPI) anchors. While flow cytometry is the method of choice for identifying cells deficient in GPI-linked proteins and is, therefore, necessary for the diagnosis of PNH, to date there is no standardised method to identify these cells.

In this session, I will present a review of the RCPA Quality Assurance Program for the detection of PNH cells, discuss the Australian guidelines in conjunction with published literature.

062. The science behind Bcl2 inhibition

Huang D

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Interactions between the proteins of the BCL2 family are the critical for determining whether a cell lives or dies by apoptosis. The key mediators of this process are BAX and BAK which act to permeate the outer mitochondrial membrane triggering the release of pro-apoptotic factors such as cytochrome c into the cytosol. The pro-survival members of the family - BCL2 itself and its close relatives such as BCLxL and MCL1 normally hold BAX and BAK in check. However, damage or stress signals flip on the switch for cell death by activating the BH3-only proteins that which to neutralize pro-survival BCL2 proteins thereby driving cell death. As impaired apoptosis can promote tumor formation and confer chemoresistance, there has been great interest in targeting pro-survival BCL2 or its relatives for cancer therapy. One promising approach is to mimic the action of the BH3-only proteins with small organic compounds, the BH3 mimetics, thus flipping on the death switch in tumor cells. Preclinical studies and early phase clinical studies with BH3 mimetic compounds such as ABT-737 and a compound closely related to it, navitoclax (ABT-263), show promising results and raise important questions about how these drugs act. More recently, a compound that is selective for BCL2 (venetoclax; ABT-199) has been approved by the US FDA for treating patients with high risk CLL. Understanding the precise mechanism by which these agents kill leukemic cells, and why they fail, will be important for their optimal clinical utility.

063. Bcl2 inhibition in lymphoproliferative disease

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BCL2 is ubiquitously over - expressed in many B cell malignancies, most particularly CLL and FL. It is a recognised driver in the development of these conditions as well as a significant predictor of chemo – resistance. It has thus long been seen as an attractive target in therapy for these diseases, however early attempts at inhibiting BCL2 for clinical purposes using antisense oligonucleotides (oblimersen), and pan – BCL2 family inhibition (obatoclox and AT - 101) were associated with limited efficacy with overall response rates less than 10% in both CLL and NHL.

Lessene et. al. describe four criteria for a true BH3 mimetic: (1) the drug induces apoptosis via BAX and BAK with associated mitochondrial dysfunction; (2) the drug binds at least one BCL2 family member with high affinity; (3) the drug's activity must correlate with the expression of relevant BCL2 family members; and (4) relevant biomarkers should be affected by the drug in animal models.

Applying the above criteria the first true BH3 mimetic was ABT – 737, which showed promising pre – clinical efficacy in a variety of B lymphoproliferative disorders. This drug inhibited BCL2, BCLx_L and BCLw with high affinity. The orally available analogue navitoclax demonstrated a 35% ORR in phase I clinical trials of CLL and 22% ORR in NHL early phase trials with median PFS of 25 and 16 months respectively. The clinical potential of this drug may not have been realised due to the on target DLT of thrombocytopenia (due to BCLx_L inhibition in circulating platelets). This led to the development of the BCL2 selective inhibitor, Venetoclax.

In CLL venetoclax demonstrated 79% ORR among relapsed and refractory patients in first in human phase I studies with a 20% CR rate. Similar response rates were noted among patients with ultra high risk factors including fludarabine refractory disease, deletion 17p and IGVH unmutated status. The median duration of PFS was 25 months. To date the results in NHL patients have only been published in abstract form however the preliminary results are more heterogeneous among the NHL group with ORR in MCL of 75% (21% CR) in contrast to the DLBCL population with an ORR of 18% (12% CR). Among the 106 NHL patients as a whole the ORR was 44% (13% CR rate). Accordingly the median PFS was variable amongst this group ranging from 14 months in the MCL population to 1 month in the DLBCL population (overall PFS 17 months).

Venetoclax is even more promising as it is well tolerated by most patients with the only DLT being TLS.

However a number of unanswered questions remain pertaining to the clinical use of venetoclax including:

Why do some patients who initially respond then progress at a median of 2 years?

What are the biomarkers both for response and also for relapse on drug?

What is the optimal clinical use of the drug e.g. upfront vs. reserved for relapse and what combinations with conventional therapies or other novel agents achieve the best results with least toxicity?

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

064. Therapeutic progress in targeting apoptosis pathways in AML

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Despite decades of clinical investigation, therapeutic progress in AML has been limited. The complexity and plasticity of the genomic landscape poses potential barriers to the success of targeted therapies. Induction of apoptosis by chemotherapy revolves around the activation of pro-apoptotic TP53 and BH3-only proteins and the neutralization of pro-survival BCL2 family members. Advances in the development of BH3-mimetic drugs to target BCL2, such as venetoclax, have culminated in positive proof-of-concept clinical trials for a number of B-cell lymphomas. Drugs able to directly activate TP53 via MDM2 inhibition are in clinical development as are additional BH3 mimetics able to target other members of the BCL2 family.

Although venetoclax monotherapy studies in AML have produced modest clinical response rates in advanced AML, early results from studies combining venetoclax with demethylating agents or low-dose chemotherapy as frontline therapy in elderly patients with AML appear highly promising.

In this session, challenges and questions relating to the clinical optimization of venetoclax in the treatment of AML will be discussed, along with the potential mechanisms responsible for the enhanced activity of venetoclax combinations in AML. The growing therapeutic landscape surrounding the development of other BH3-mimetics and their potential role in the future of AML therapy will also be presented.

067. Constitutional bone marrow failure syndromes manifesting in adults

Young N

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Underlying genetic lesions affecting DNA repair predispose to bone marrow failure. Traditionally recognized in early childhood and by pediatricians, subtle presentations of constitutional marrow failure in adults is not infrequent and will increase with routine application of next generation sequencing panels. Particularly important for the internist are the telomere diseases and GATA2 deficiency. Telomeropathies can often be diagnosed from careful personal and family history and physical examination. The telomere diseases are phenotypically variable and penetrance can range widely, even within a pedigree. Genetic defects in telomere repair, inherited mutations in TERT, which encodes telomerase, and TERC, which encodes the RNA template, are most common. Telomere length of peripheral blood leukocytes can be measured in commercial laboratories. Therapies that target the molecular lesion can improve blood counts. Sex hormones upregulate TERT gene expression. In a prospective clinical trial, danazol, a synthetic androgenic sex steroid, improved blood counts and led to telomere elongation. These results have implications for the treatment of patients with genetic telomere defects and also more generally for telomere modulation as a strategy to prevent malignant transformation due to accelerated physiologic telomere attrition.

Telomeres, repeated short nucleotide sequences and associated shelterin proteins, constitute the termini of linear chromosomes. Telomeres protect chromosomes from recognition as double stranded DNA breaks. Telomeres shorten with every cell division, and attrition is partly abrogated by the telomerase repair complex. Critical telomere shortening accounts for the Hayflick phenomenon, and critically short telomeres cause genomic instability. Telomerase mutations are etiologic in the telomeropathies, organ failure syndromes that include bone marrow failure, pulmonary fibrosis, and liver disease. Telomere content of peripheral blood leukocytes can be measured by a variety of assays, some now commercially available. The phenotype and penetrance of telomeropathies is highly variable. Dyskeratosis congenita in childhood is associated with mucocutaneous findings, telomeropathies in adults with early hair graying. Mutations in TERT (the gene encoding the reverse transcriptase) cause liver disease, mutations in TERC (encoding the RNA template), and pulmonary fibrosis. Accelerated telomere attrition is the major risk factor for malignant clonal evolution in SAA; chromosomal aberrations can be observed in tissue culture months to years preceding clinical MDS/AML. Sex hormones upregulate TERT expression and danazol in a prospective NIH clinical protocol has been effective in improving blood counts in telomeropathy patients and elongating telomeres.

068. 99m Technetium-hydroxy-diphosphate Tracer (99mTcHDP) Bone Scintigraphy: A Non-invasive Diagnostic Tool for Cardiac Amyloidosis

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Aim

Cardiac amyloidosis is a serious and progressive disorder, worsened if correct diagnosis and/or treatment are delayed. The two subtypes are AL and transthyretin (ATTR), which can be difficult to distinguish on immunohistochemistry. While chemotherapy is used in AL, it is futile in ATTR so confirming the amyloid subtype is crucial.

Gillmore *et al* demonstrated bone scintigraphy with 99mTc-DPD tracer can reliably diagnose ATTR, avoiding endomyocardial biopsies to confirm the subtype in most cases.^[1]

99mTc-HDP is a tracer similar to 99mTc-DPD and studied in USA for this indication with encouraging results. This tracer is readily available in Australia. We sought to examine experience at Victorian and Tasmanian Amyloidosis Service (VTAS) of 99mTcHDP bone scintigraphy, to confirm these scans can reliably diagnose cardiac amyloidosis and distinguish AL and ATTR subtypes.

Methods

20 patients with confirmed ATTR or AL who had 99mTcHDP bone scintigraphy were analysed. Results were correlated with histology, NTProBNP, Troponin-T, free light chains, cardiac MRI and echocardiography. Grading was conducted with Perugini scoring.^[1]

Results

13 patients with ATTR and 2 AL had positive scans. Patients with ATTR have higher Perugini scores (2 or 3) while AL have scores 0 or 1. 6 of 7 patients with histology-proven ATTR (3 endomyocardial biopsies) had positive scans, the negative patient had bladder-isolated disease.

All 6 patients with amyloid features on MRI had positive scans. 2 patients who have no amyloid features on MRI had negative scans, meaning bone scintigraphy correlates 100% to MRI scans.

Mean NTproBNP values for ATTR and AL were 609pmol/L and 1396pmol/L respectively. The two AL amyloidosis patients with positive bone scans had higher NTproBNP values.

Conclusion

Bone scintigraphy with 99mTcHDP tracer is an easily accessible, non-invasive method of diagnosing cardiac amyloidosis. There is increasing evidence that these scans can distinguish between AL and ATTR subtypes, thus improving time to diagnosis and treatment.

069. Rates of upfront autologous stem cell transplantation (ASCT) in newly diagnosed multiple myeloma (NDMM): a report from the MRDR on behalf of the MRDR steering committee

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Aim

Despite clear evidence for the use of front-line ASCT utilisation rates are lower than expected. We reviewed the utilisation of ASCT and maintenance therapy in age-eligible patients registered on the Myeloma and Related Diseases Registry (MRDR) and its impact on outcome.

Method

We conducted a retrospective review of adult patients registered on the MRDR, a prospectively maintained database from 18 sites across Australia (16) and New Zealand (2). Patients aged ≤ 70 with NDMM from June, 2012 to June, 2015 were eligible for analysis. Baseline characteristics, therapies and outcomes were compared between recipients and non-recipient using appropriate tests. Survival analysis was used to estimate time to disease progression.

Result

354 patients met eligibility criteria and by June, 2015 212 (60%) had received an ASCT. Patients who did not receive an ASCT were older (median age 62.9 vs 58.3 years $p < 0.001$), had a higher ISS (ISS 3 42% vs 25% $p = 0.004$), higher ECOG (ECOG 2/3 (27.5% vs 8.6% $p < 0.001$)). Patients not receiving an ASCT were less likely to have been treated with bortezomib-based induction (85% vs 94.3%, $p = 0.12$) but more likely to be treated with melphalan or thalidomide-based induction (7.6% vs 0%, $p < 0.001$ and 9.5% vs 4.2%, $p = 0.062$ respectively). Patients who did not receive an ASCT had a shorter progression free survival (PFS) (median 19.8 vs 32.6 months, $p < 0.001$). Maintenance therapy was used in 124 (58.4%) of patients post ASCT with thalidomide containing therapy most frequent (67%).

Conclusion

ASCT is a highly effective therapy in MM but is currently under-utilised in Australian/New Zealand. ASCT is utilised less frequently in less fit patients (older, higher ECOG) and is associated with a poorer PFS. Consideration of an ASCT may benefit patients in this group.

070. Monoclonal antibodies in systemic AL amyloidosis - when it rains, in pours

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Aim

We sought to examine the current literature and trial data about the efficacy of monoclonal antibodies, including NEOD001, the anti-SAP monoclonal antibody and daratumumab, in systemic AL amyloidosis and clarify their current and expected availability in Australia

Methods

A review of the reported literature of monoclonal antibodies in systemic AL amyloidosis was undertaken, as well as reviewing the available trials of these agents currently open in Australia. We reviewed our current experience with these agents, and highlight the expected trials to open in 2017 and beyond.

Results

The Prothena VITAL study is currently open in three centres in Australia. This is a randomized double-blind placebo-controlled Phase III trial for newly diagnosed systemic AL amyloidosis, using the novel compound, NEOD001 which targets a cryptic epitope on amyloid fibrils. Phase I/II data is encouraging with early renal, cardiac and nerve responses above those expected from chemotherapy alone. The PRONTO study of the same antibody may soon be open, targeting those patients with stable cardiac disease.

The anti-SAP monoclonal antibody, as reported by Richards et al (NEJM, 2015) is about to undergo a Phase II trial in the UK and USA. Phase I data in 15 patients with hepatic amyloidosis demonstrated significant clearance of amyloid deposits in the majority of patients. Like NEOD001, this monoclonal antibody appears to be very well tolerated. We are hopeful that Australia will access this compound in 2018 in a Phase III trial. Daratumumab may soon to be trialled in Australia for upfront treatment of newly diagnosed systemic AL amyloidosis. Experience in the USA of this compound has demonstrated good tolerance and impressive haematologic responses, as seen in multiple myeloma.

Conclusion

Monoclonal antibodies offer significant hope of improved survival and organ function to patients with systemic AL amyloidosis. Further studies will hopefully confirm Phase I/II data of these compounds, with some agents already available in Australia.

071. A 2-stage phase II study of panobinostat consolidation in Multiple Myeloma (MM) patients with < CR Following High-Dose Chemotherapy (HDT) conditioned Autologous Stem Cell Transplantation (ASCT) as part of first line therapy

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Aim

To determine rate of conversion to CR/VGPR, to evaluate safety, toxicity and to document PFS/OS in MM patients treated with panobinostat consolidation post-ASCT.

Methods

Phase II, open label, single arm study. Newly diagnosed MM patients commenced panobinostat (45mg 3x per week, alternate weeks) 8-12 (median 10) weeks after a single MEL200 ASCT. Panobinostat continued until toxicity/relapse/progression. After 6m of therapy, patients were restaged and only continued if depth of response improved (conversion to CR/VGPR).

Results

24 patients (F=12, M=12), median age 58yrs (44-69), commenced panobinostat. Diagnosis ISS stage: I/II: 16, III: 8. 20/24 had diagnostic cytogenetics/FISH: 5 were poor risk (t(4;14), t(14;16), +1q21).

After a median of 24m following panobinostat initiation (6-43m), 6 patients remain on therapy. Discontinuation was due to failure to improve depth of response (8), progression (6), non-compliance (2) and toxicity (2). 11 patients progressed, 2 died. Estimated 2 year PFS 65% (median PFS 25m, median OS NR).

22/24 patients completed at least 6m of therapy, 2 were not evaluable. 12/22 improved depth of response: 4 from VGPR→CR, 7 from PR→VGPR, 1 from PR→CR: 4 patients within 0-3m, 8 patients within 3-6m. Two patients (one each from 0-3m and 3-6m groups) demonstrated a further reduction in tumor burden with maximal response occurring after 11m and 29m. Median time to achieving maximal response was 4.6m (1.2-29).

One patient who discontinued after 6m due to failure to improve depth of response demonstrated further reduction in paraprotein (at 12m post C1D1) from 5g/L to trace. This response was maintained for 5m.

5/24 patients tolerated dosing as per protocol, 19/24 required dose modifications to manage AEs: 7 to 30mg, and 11 to 20mg, 1 discontinued altogether.

Conclusion

Single agent panobinostat consolidation post-ASCT improves depth of response, and in some patients, induces a further reduction in tumour burden beyond 12m post-ASCT.

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

072. NEOD001 demonstrates organ biomarker responses in patients with light chain amyloidosis and persistent organ dysfunction: results from the expansion phase of a phase 1/2 study

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Aim

Current therapies for AL amyloidosis limit light chain (LC) production but do not directly target deposits underlying multiorgan failure. NEOD001, a monoclonal antibody, targets misfolded LC and is thought to neutralize circulating LC aggregates and clear insoluble deposits. The aim of the current study is to evaluate the safety, efficacy and pharmacokinetics of NEOD001 in patients with AL amyloidosis who have achieved at least a PR to plasma cell-directed (PCD) therapy.

Method

Patients who completed ≥ 1 PCD therapy, had PR or better to any previous therapy, and had persistent organ dysfunction received NEOD001 IV every 28 days. During the dose-escalation phase, 27 patients received NEOD001 in a 3+3 study design. An additional 42 patients were enrolled and treated at the maximum planned dose (24 mg/kg) in the expansion phase. We assessed safety/tolerability, pharmacokinetics, immunogenicity, cardiac and renal responses based on consensus criteria, and neuropathy responses using the Neuropathy Impairment Score-Lower Limbs (NIS-LL).

Result

Twenty-seven patients were enrolled in the dose-escalation study. Of evaluable patients, renal response rate was 60% (n=9/15), and cardiac response rate was 57% (n=8/14). An additional 42 patients were enrolled in the expansion study, which included cohorts with renal (n=16), cardiac (n=15), and peripheral nerve (n=11) involvement. In the overall population, the median age was 60 years, and 60.9% of patients were male. Median (range) time since diagnosis was 3.02 (0.4-16.0) years for the expansion patients and 2.85 (0.4-16.0) years for the overall population. Safety/tolerability and organ response data will be presented for expansion patients.

Conclusion

Our interim results demonstrated that monthly NEOD001 infusions were safe and well tolerated, with organ response rates comparing favourably with traditional chemotherapy. These updated results may help to further elucidate our understanding of this therapeutic approach. Antibody therapy may allow for effective treatment of patients with AL amyloidosis.

This research was supported by Prothena Biosciences. The company participated in data analysis and contributed to abstract preparation.

073. Renal impairment in myeloma: a comparison of characteristics and outcomes in patients with and without renal impairment in the Australia and New Zealand myeloma and related diseases registry

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Aim

To assess the association of renal impairment with more severe disease and adverse outcomes in patients with multiple myeloma (MM) by comparing characteristics and outcomes of patients with MM and renal impairment (eGFR 15-30 mL/min/1.73m²) to those with better renal function (eGFR>30).

Methods

Data for all patients with MM registered on the Myeloma and Related Diseases Registry from 1 Feb 2013 to 7 Jun 2016 were analysed. Estimated glomerular filtration rate (eGFR) was calculated using baseline creatinine in the Modification of Diet in Renal Disease equation. Patients with an eGFR<15 at baseline were not included in the analysis as dialysis and specialised care can alter outcomes, producing misleading results. Patient characteristics, treatment, response and outcomes were compared between the 2 groups using appropriate tests.

Results

Of 704 patients with MM, 591 had eGFR available: 35 had eGFR 15 to 30, 519 eGFR >30, and 37 eGFR < 15 (not included in analysis). Patients with renal impairment (eGFR 15-30) were older than those with eGFR>30 (51% >70 years versus 32%, p=0.02), and most were classified with Stage 3 disease on the ISS (90% v 23%, p<0.001) and had higher LDH (241 v 186, p=0.004).

Bortezomib-based first-line therapy was used in 84% of patients, thalidomide in 9%, lenalidomide in 1% and other therapies for 6% of patients. Overall response rate was 67% v 84%, p=0.04. Patients with eGFR 15-30 had a shorter time to disease progression (12.8 v 29.2 months, p<0.001). Median overall survival for the cohort was not reached.

Conclusion

Renal morbidity from MM is a considerable burden. In our cohort, patients with renal impairment appeared to have more severe disease and poorer outcomes. Renal impairment (eGFR 15-30) may help to identify patients with MM requiring more vigilance.

074. Genomic characterisation of breast implant-associated anaplastic large cell lymphoma reveals a high incidence of activating JAK1/STAT3 mutations and identifies predisposing germline variants

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Aim

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a rare form of ALK-negative ALCL typically manifesting as a peri-prosthetic effusion years after implant insertion. The underlying molecular lesions associated with this unique entity are unknown. We aimed to genomically characterise cases of BIA-ALCL in regard to mutation and copy number change in order to further understand the pathogenesis and molecular drivers of this rare malignancy.

Method

Cases were referred for diagnostic testing to the Peter MacCallum Cancer Centre Molecular Haematology laboratory. Whole exome sequencing (WES) was performed using Agilent SureSelectXT Human All Exon V5 and sequenced on an Illumina HiSeq 2500. Targeted sequencing was performed using a custom Agilent SureSelect panel (PeterMac PanHaem Panel) and sequenced on an Illumina NextSeq.

Results

Tumour DNA from four cases were analysed. Two cases underwent WES and two cases underwent broad targeted sequencing (PeterMac PanHaem). In all four cases, either an activating *JAK1* or *STAT3* mutation was detected (*JAK1* G1097V, *STAT3* S614R, H410R, D661Y). Numerous copy number changes were detected including gains of chromosome 1p (*TNFRSF8* (CD30)) and a high level *MYC* amplification in one case. In addition to tumour specific variants, we identified two patients with a potential germline predisposing variant. One patient had a rare activating germline single nucleotide polymorphism in *JAK3* (V722I) previously shown to have T-cell transforming properties *in vitro* and synergistic properties with *JAK1/STAT3* mutations. Another patient had a personal and family history of malignancy, positive TP53 immunohistochemical staining in normal (non-tumour) cells and a *TP53* (R249K) mutation identified at an allele frequency consistent with germline origin.

Conclusion

We have described for the first time the genomic landscape of BIA-ALCL and have shown a high incidence of activating *JAK1/STAT3* mutations. Moreover, we have identified potential predisposing germline variants in two cases with important implications for pathogenesis.

075. The frequency of incidental malignancies detected by PET/CT scans in patients with lymphoma and the associated clinical implications

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Aim
The primary aim was to identify the frequency of incidental second malignancies detected by PET/CT scans in patients with lymphoma. Qualitative data related to histological diagnosis and staging, interruptions or obstacles to lymphoma therapy, therapy for the second malignancy and the overall impact upon prognosis were also reviewed.

Methods
A total of 550 PET/CT images were performed in 298 patients at the Prince of Wales Hospital between January 2013 – March 2016. Patients with both Hodgkin's and Non-Hodgkin's lymphoma, with PET/CT imaging performed for all medicare-approved indications were included. All PET/CT reports suggestive of an incidental second malignancy prompted further review of electronic medical records, MOSAIC cancer database and paper medical records. Where a clear diagnosis of second malignancy was confirmed, information regarding histological findings and staging as well as the implications of this diagnosis related to treatment of the underlying lymphoma and impact on overall prognosis was collected.

Results
510 PET/CT scans in 259 patients had confirmed diagnoses of lymphoma. Patients aged 17 to 96 were included in the study, with a median age of 62 years. Of the 259 patients included (M=155; F =104), 55 patients with a diagnosis of Hodgkin's lymphoma and 204 patients with Non-Hodgkin's lymphoma were included.
A total of 33 out of 259 patients with a diagnosis of malignant lymphoma had PET/CT findings suspicious for an underlying second malignancy (12.7%). Of the 33 patients, 19 underwent further invasive investigation, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (3.1%). Qualitative information was gathered regarding the patients who did not have further investigation.

Conclusions
The frequency of incidental malignancies detected by PET/CT imaging in patients with lymphoma was found to be comparable to other similar international retrospective studies. The majority of incidental second malignancies were early stage and gastrointestinal in origin. Further retrospective as well as prospective data may assist in the establishment of guidelines to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignancy.

076. Improved survival of older patients with Mantle Cell Lymphoma (MCL) with cytarabine-based immunochemotherapy

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While the role of cytarabine-based immunochemotherapy (ICT) and autologous stem cell transplantation (ASCT) in younger patients with MCL is well established, the utility of 'more intense' approaches in older patients is uncertain. With development of targeted agents, the need to define the optimal ICT strategy for comparison is pressing.

Aim

To compare the safety and efficacy of first line ICT in patients with MCL aged >60 years

Method

Patients over 60y with MCL diagnosed between 2002-2015, according to 2008 WHO criteria were identified from four Victorian hospitals. Cases with adequate information, including baseline characteristics, treatment regimens and outcome, were included. Overall survival (OS) and progression free survival (PFS) were modelled using Cox regression. For ICT comparison, patients undergoing ASCT were censored at the time of transplant.

Result

53 patients met inclusion criteria. Median age 68 years [60-91] with 77% male. All but one had advanced-stage with 88% intermediate MIPI. All received rituximab with initial therapy. Treatment regimens included: R-CHOP-like (31), alternating R-CHOP/R-DHAC (10), R-HyperCVAD/MA (7) and other (5). 11 patients underwent ASCT. The median follow up of the surviving patients was 40 months. Type of chemotherapy and ASCT were the only variables influencing PFS and OS. Cytarabine-based chemotherapy was associated with an improved median PFS (not reached vs 35 months, HR 0.27 [0.08-0.93] p=0.018) and OS (not reached vs 54 months, HR 0.28 [0.08-0.94], p=0.039) compared to R-CHOP-like regimens. Patients undergoing ASCT were younger and demonstrated improved median OS (not reached vs 46 months, HR 0.124 [0.017-0.921], p=0.041). No difference in efficacy between HyperCVAD/MA and R-CHOP/R-DHAC could be determined due to low numbers however those receiving HyperCVAD/MA therapy had higher transfusion requirements.

Conclusion

Cytarabine-based ICT is highly efficacious and well tolerated even in older patients with MCL and should serve as a benchmark with which to compare targeted therapies.

077. Frequent achievement of complete remissions in patients with relapsed / refractory mantle cell lymphoma treated with the combination of Ibrutinib and Venetoclax (ABT-199): results from the ongoing Australian Phase 2 AIM study

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Introduction

Both ibrutinib and venetoclax (ABT-199) individually have single-agent activity in relapsed/refractory Mantle Cell Lymphoma (MCL), but complete remissions (CR) occur in <25%. We sought to determine the activity of the combination in an investigator-initiated, phase 2 study.

Methods

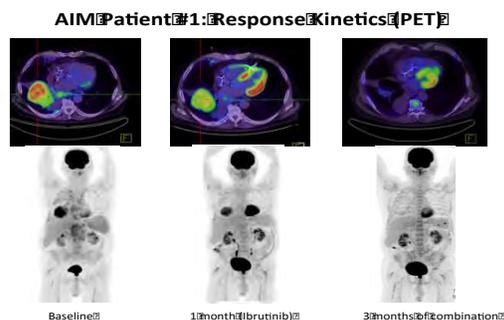
Fifteen (of target 24) patients with MCL have been recruited. All patients received 4 weeks of ibrutinib (560mg/day), followed by introduction of venetoclax (weekly ramp-up to target dose of 400mg/day). Patient #1 received venetoclax on 19-8-2015, and to our knowledge, was the first patient globally to receive this drug combination. Subsequently, 13 others have successfully completed venetoclax ramp-up. Herein, we report the results of the first 10 evaluable patients (fully restaged after 3 months of drug combination, or PD prior).

Results

Median age of patients (n=10) was 72 (range, 53-77) years. All were pretreated with a median 1.5 (1-7) prior therapies. MIPI score was high in 70%. Median time on therapy was 164 (85-339) days. Common AEs were nausea/dyspepsia (60%), diarrhoea (60%) and infections (50%). Grade 3-4 drug-related AEs included colitis (1), neutropenia (2), tumour lysis syndrome (1), atrial fibrillation (1) and BK-virus cystitis (1). Overall response rate was 80%, and the CR rate was 70%. Two patients developed PD during ibrutinib induction, and both continued to progress despite addition of venetoclax. Of the remaining 8 patients (6 SD and 2 PR after ibrutinib induction), 1 was classified as PR following combination treatment due to a small, non-PET-avid residual mass, and 7 were confirmed CR by PET, endoscopy (if baseline gut involvement) and BMAT, including MRD clearance (as assessed by flow cytometry with minimum sensitivity of 10⁻⁴) in 7/7. ASO-PCR MRD results (n=2) were 0.01% & 0.00001%. No responder had relapsed to date.

Conclusion

The combination of ibrutinib and venetoclax was deliverable, and resulted in high rates of deep remissions.



This research was supported by AbbVie, Janssen and the Victorian Cancer Agency. These entities had no role in analysing the data or preparing the abstract.

078. Primary central nervous system lymphoma: a tertiary institution experience over the last decade

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Aim/Background

Primary Central Nervous System Lymphoma (PCNSL) remains an ongoing management challenge. Whilst methotrexate-based induction is now well accepted, whole brain radiotherapy (WBRT) consolidation remains contentious due to its long-term neurocognitive impact. With growing interest in high-dose chemotherapy and autologous stem cell transplant (ASCT) as an alternative, we report on our experience in managing PCNSL over the last decade in a tertiary referral hospital.

Methods

Retrospective analysis of patients diagnosed with PCNSL from January 2006 to December 2015. A baseline prognostic score – Memorial Sloan Kettering Cancer Centre (MSKCC) score for PCNSL was determined based on performance status documented at time of diagnosis. Different parameters such as age, methotrexate dose and WBRT was examined between both non-transplanted and transplanted groups. Survival analysis was performed using the Kaplan Meier method.

Results

Overall, 26 patients were diagnosed with PCNSL with a mean age of 65 years at diagnosis. Histopathology was Diffuse Large B-cell Lymphoma in 96% and 85% were cortical lesions. High dose Methotrexate (HD-MTX) based induction was delivered in all but two patients. Eleven patients (42%) received ASCT consolidation. The ASCT patients had a younger mean age (56 vs 70 years) than non-transplanted patients. The non-transplanted group had a median overall survival of 36 months. There was a trend towards better overall survival in non-transplanted patients who were <70 years of age and those who received cytarabine on top of HD-MTX. Of note, in the ASCT group there have been no reported relapses or deaths over a median follow-up of 73 months. Furthermore, in patients receiving ASCT who functioned independently prior to diagnosis (n=10), 8 (80%) returned to independent function.

Conclusions

Local experience of ASCT consolidation in appropriately selected patients has demonstrated impressive survival outcomes without marked compromise in patient function. These findings add to increasing evidence supporting use of ASCT as consolidation after high-dose methotrexate based induction for PCNSL. Further objective assessment of neurocognitive function is required.

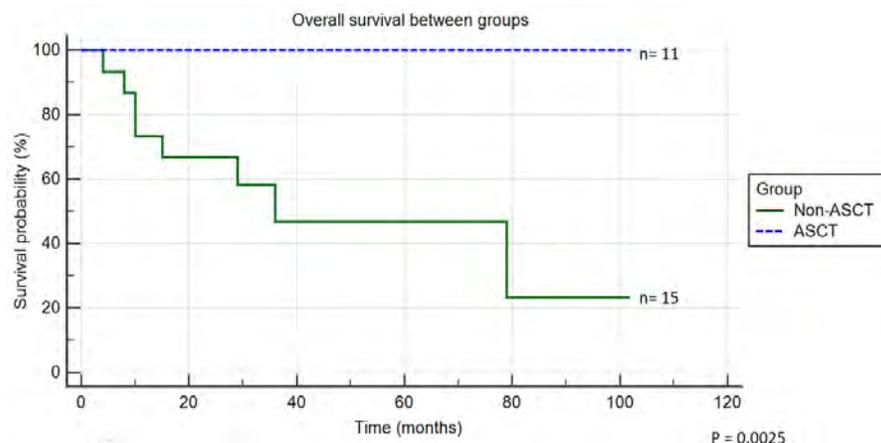


Figure 1 – Overall survival of patients receiving

079. A Retrospective Review of Treatment Outcomes in Patients Treated for Peripheral T Cell Lymphoma (PTCL) at the Peter MacCallum Cancer Centre (PMCC) between 1999 and 2015

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Aim

To assess overall survival, number and types of treatment, and time to next treatment stratified for individual treatments in a retrospective cohort of patients treated for PTCL at (PMCC) between 1999 and 2015.

Methods

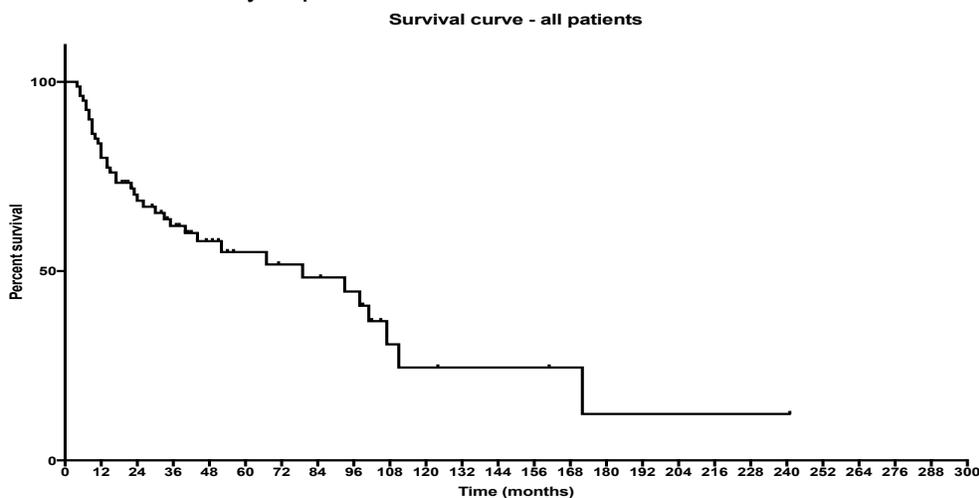
Patients were identified from the PMCC T-cell lymphoma database. Data was confirmed against electronic records. Demographics, treatments, and therapy lines were enumerated. Time to next treatment was calculated for each therapy line.

Results

83 patients were identified (53 males and 30 females). Median age at diagnosis was 56 years (range 21 to 86). Diagnoses included PTCL-not otherwise specified (n=52); anaplastic large cell lymphoma (ALCL), including ALCL-ALK positive (n=6), ALCL-ALK negative (n=12), and ALCL-ALK unspecified (n=1); angioimmunoblastic T cell lymphoma (n=6); and others (n=6).

77 individual treatments were identified, with the most common upfront regimens being CHOP or CHOP-like (n=38), HyperCVAD (n=21), and CHOEP (n=8). Median time to next treatment was six months for CHOP-treated patients (range <1 to 50 months), eight months for HyperCVAD (range 1 to 51 months), and five months for CHOEP. Autologous transplant in first remission was used in nine patients, of whom five are in ongoing complete remission (CR). Ongoing CR after first line treatment was seen in 14 patients.

Salvage chemotherapy and autologous transplant was most common after first line. Targeted molecular therapies, eg. monoclonal antibodies or small molecules were used in second line of therapy or later. Histone deacetylase inhibitors (HDACi) were used most commonly in second line or later, with sustained complete responses seen in a minority of patients.



Conclusions

Sustained remission to first line therapy for PTCL is uncommon. The use of targeted therapies, upfront autologous transplant, and treatment selection based on histology and molecular profiling are questions for future research.

080. Safety of RAPid INJECTION of Undiluted Ferric Carboxymaltose to Patients with Iron Deficiency Anaemia (RAPINJECT)

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Background

Iron deficiency is the most prevalent cause of anaemia worldwide. However oral iron supplementation may be ineffective due to gastrointestinal intolerance or delay in response. Ferric carboxymaltose (FCM) can safely be administered more rapidly than previous IV iron formulations due to its rapid uptake by cells of the reticuloendothelial system. Preliminary data suggest that FCM can also be administered undiluted as a bolus over a minute facilitating its adoption in the primary care setting.

Aim

To evaluate the safety of rapid intravenous administration of undiluted FCM (10%), up to 1000mg, over 1 minute, without premedication.

Methods

Dose escalation study with expansion cohort at maximum tolerated dose up to 1000mg. A 3 + 3 trial design with 100 subjects in the expansion cohort. Eligibility: Adult non-pregnant subjects (> 18 years) with anaemia (women Hb<120g/L; men Hb<130 g/L) and serum ferritin < 30microgram/mL. Primary Outcome: Proportion experiencing treatment emergent (TE) grade 4 or 5 serious adverse event within 1 hour of infusion.

Results

111 patients met eligibility criteria with 85% being female and median age of 44 (range 18-86) The mean pre treatment Hb was 101.6 g/L (+/- 12.8) and ferritin 10 microgram/L. No patients experienced a grade 4 or 5 event 0/111 (0% 95% Confidence Interval 0-0.5%). Grade 1, 2 and 3 events were observed in 46%, 4.5% and 1.8% of participants respectively, self-limited facial flushing being the most frequent (40.5%). Asymptomatic hypophosphatemia ($PO_4^{3-} < 0.8\text{mmol/L}$) was observed in 66% of subjects at two weeks. At four weeks the mean Hb had increased significantly to 120g/L ($p < 0.05$).

Conclusion

Up to 1000mg of undiluted FCM may be safely administered over one minute without premedication. Side effects were in general mild with self-limited facial flushing being the most common.

081. Prevalence of cardiac and hepatic siderosis in Australian patients with transfusion-dependent anaemias or non-transfusion-dependent thalassaemia, as assessed by MRI (the TIMES Study)

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Aims

Determine the prevalence and severity of iron overload assessed by MRI and its impact on clinical management in a large population of patients with transfusion-dependent anaemia or non-transfusion-dependent thalassaemia (NTDT).

Methods

The TIMES study assessed cardiac and hepatic iron load in patients with thalassaemia major (TM), NTDT, myelodysplastic syndromes (MDS) or other chronic anaemias. Prospective MRI was used to determine R2 liver iron concentration (LIC) and myocardial (m)T2*. Treatment decisions were assessed by investigator questionnaire post-MRI evaluation.

Results

243 patients (median age 49.0 years [18–92]) were enrolled. 65.8% of patients received iron chelation therapy (ICT) for ≥1 month at any time before/during the study (55.9% deferasirox, 11.9% deferoxamine, 2.5% deferiprone). The prevalence of cardiac and hepatic siderosis was 10% and 48%, respectively; TM, 22%, 33%; MDS 4%, 55%; NTDT 0%, 50%; other anaemias 5%, 57%. All TM patients had received ICT. Among patients with MDS, NTDT or other anaemias, 23–36% had hepatic siderosis but had not received ICT. Mean LIC was above target range; mean mT2* was >20 ms. While a small number of patients with high LIC had relatively low serum ferritin (SF), few (3.8%) patients with TM, MDS or other anaemia types with SF <1000 ng/mL had LIC ≥7 mg Fe/g dw; in NTDT, 2/17 patients had LIC ≥5 mg Fe/g dw despite SF <800 ng/mL. MRI assessment led to a change in management in 46% of patients (105/229).

Conclusion

Despite ICT, this study indicates a high prevalence of hepatic siderosis in transfusion-dependent MDS, anaemias and NTDT, and cardiac siderosis in TM patients. A good correlation was observed between SF and LIC in most patients; supporting SF at 1000 ng/mL as an appropriate chelation target. These data emphasize the importance of accurate monitoring of iron load to allow informed clinical decision making.

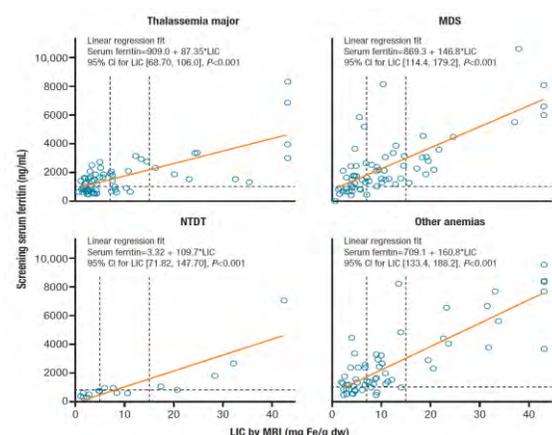
Mean LIC, mT2* and SF in the overall population and among patients who had received ICT for ≥1 month at any time before or during the study

		Mean (SD)		
Patient group	n	Serum ferritin, ng/mL	LIC, mg Fe/g dw	mT2*, ms
Overall	243	2164 (1916)	10.3 (11.2)	30.7 (9.8)
TM	81	1686 (1350)	8.1 (10.8)	27.6 (11.2)
MDS	73	2466 (1993)	11.0 (10.3)	31.6 (8.6)
NTDT	20	1230 (1614)	10.7 (12.3)	33.7 (8.5)
Other anaemias*	69	2639 (2264)	12.1 (11.9)	32.6 (8.6)

		Mean (SD)		
Chelated patients, n=160	n	Serum ferritin (ng/mL)	LIC (mg Fe/g dw)	mT2*, ms
TM	81	1686 (1350)	8.1 (10.8)	27.6 (11.2)
MDS	43	2499 (2123)	11.4 (12.1)	30.9 (9.6)
NTDT	7	2052 (2342)	19.8 (15.1)	33.4 (8.8)
Other anaemias	29	3032 (2500)	13.6 (13.4)	31.6 (10.6)

*Sickle cell disease, n=9; Diamond-Blackfan disease, n=2; aplastic anemia, n=8; myeloproliferative disease, n=16; acute myeloid leukemia, n=14; acute lymphoblastic anemia, n=9; non-Hodgkin's lymphoma, n=1; chronic myeloid leukemia, n=1; T-cell large granular lymphocytic leukemia, n=1; myeloma, n=1; pyruvate kinase deficiency, n=1; chronic cold agglutination disease, n=1; pure red cell aplasia, n=2; autoimmune hemolytic anemia, n=2; multifactorial, n=1

Regression analysis shows good correlation between LIC and SF



This research was supported by Novartis Pharma AG. The company funded statistical analyses, performed by Statistical Revelations Pty Ltd, and medical editorial assistance, provided by Mudskipper Business Ltd (Novartis Pharmaceuticals). PJH reports advisory board participation (Novartis)

082. An Observational, Non-Interventional, Multicentre, Registry Of Patients With Atypical Haemolytic Uraemic Syndrome (aHUS): A Review Of The Baseline Characteristics Of Patients Treated With Eculizumab In The Australian Cohort

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Aim

To report the characteristics of patients enrolled in the Australian Cohort of the Global aHUS Registry treated with Eculizumab.

Background

aHUS is a rare, genetic, life-threatening systemic disease. The Global aHUS Registry, a multi-centre, multi-national, non-interventional registry initiated in April 2012, prospectively collects data on aHUS patients. Eculizumab is a humanised monoclonal antibody directed against C5 and has been demonstrated to be effective as treatment of this condition.

Methods

Patients with a clinical diagnosis of aHUS are eligible for Registry enrolment. Demographics, medical and disease history, laboratory results, treatment, efficacy and safety outcome data are collected at baseline and every 6 months thereafter.

Results

As of 29th February 2016, 1054 patients were enrolled in the Global aHUS Registry, including 68 patients in Australia. In the Australian cohort, 34 patients have received Eculizumab. For this group, mean age at diagnosis of aHUS is 34 years (range 0.6 – 71.4 years), 75% female and 14% had a positive family history. Patients had significant renal dysfunction at commencement of Eculizumab: mean creatinine 162 (range 85 – 634)µmol/l, 60% of patients required dialysis and 8 had received a kidney transplant. 80% had received prior plasma exchange. Patients treated with Eculizumab were more likely to have had extra-renal complications than those never treated (cardiovascular symptoms 21 % versus 4%, CNS symptoms 36% versus 0%, GIT symptoms 40% versus 0%). The median duration of eculizumab was 0.92 years [range 0-5.8 years]. Of those patients receiving eculizumab, 9 have discontinued treatment. Ongoing follow-up to assess the efficacy of treatment and safety of drug withdrawal is ongoing.

Conclusions

Eculizumab use in Australia has increased following the PBS funding of this therapy. Patients who commence drug have a significant burden of extra-renal disease. Analyses of longitudinal data obtained through the aHUS Registry will continue to improve our understanding of aHUS and help optimise management of patients with this rare and life-threatening disease.

This research is supported by Alexion Pharmaceuticals. The company was involved in analysing the data and abstract preparation.

083. Treatment of iron deficiency anaemia of late pregnancy with a single intravenous ferric carboxymaltose or iron polymaltose infusion versus daily oral iron sulphate (TIDAL): a prospective randomised controlled trial

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Background

Iron deficiency anaemia (IDA) is the most common nutritional deficiency affecting pregnant women. There are little data available to compare the efficacy, safety and cost-effectiveness of different iron treatments for pregnancy-related IDA.

Methods and Participants

A three-arm prospective randomised controlled trial was conducted comparing intravenous 1000 mg ferric carboxymaltose (FCM) infused over 15-minutes versus oral ferrous sulphate 325mg daily or intravenous 1000 mg iron polymaltose (IPM) over two-hours for treatment of IDA in pregnancy. A total of 246 pregnant women were recruited between September 2013 and July 2014. Median age was 28 years with a median gestation of 27 weeks. Median serum ferritin was 9 µg/L. Primary outcome measure was change in ferritin. Secondary outcomes included haemoglobin improvement, safety, tolerability, resource use, total costs, quality of life (QoL) and fetal outcome.

Findings

Ferritin levels were significantly higher in the FCM group than the combined oral and IPM groups at four-weeks ($p < 0.0001$; 95%CI 39.8, 113.4) and pre-delivery. Haemoglobin levels in the FCM group had increased more than the oral iron group at 4 weeks ($p = 0.02$; 95%CI 0.4, 5.3), and at pre-delivery ($p = 0.05$; CI 5.1, 0.0). Overall QoL score improved significantly when ferritin levels were restored to normal (> 30 µg/L) in both IV iron groups versus oral iron ($p = 0.04$; 95%CI 21.3, 1.8). The average overall health service cost associated with FCM was AUD \$2,411 lower per patient than oral iron (95%CI \$1576-\$3837, $p < 0.0001$) and \$101 lower than for IPM. FCM was well tolerated, however oral iron was associated with gastrointestinal side effects in 55% of patients.

Interpretation

This study demonstrates that FCM is an effective, safe, convenient, and less costly treatment option. Rapid iron repletion with parenteral iron significantly improves iron stores, Hb and QoL in the vast majority of pregnant women.

084. Emerging Insights into Efficacy of Aplastic Anaemia Management Practices from the Australian Aplastic Anaemia Registry

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Aim

Evaluate efficacy and toxicity of therapeutic approaches for management of aplastic anaemia in an all-inclusive, prospective Australian study to obtain unbiased data for identifying factors contributing to a superior treatment outcome.

Method

Institutions treating aplastic anaemia were invited to report newly diagnosed cases, therapeutic approach and subsequent clinical outcomes via the Aplastic Anaemia Registry website. Forty three institutions enrolled patients between 2012-2016 to create a network capable of reporting >90% of newly diagnosed cases in Australia.

Results

Data from 42 of 59 currently registered patients (median age 48) are sufficient to determine efficacy of initial treatment dominated by immunosuppressive therapy (IST) with horse antithymocyte globulin (ATG) +/- cyclosporin (CSP) in 26, rabbit ATG +/- CSP in 3, and CSP in 4. Responses were achieved by 6 months in 22 (67%). Severe hepatotoxicity following horse ATG was the most serious adverse event but was uneventfully followed by effective treatment with rabbit ATG. Haematologic improvement occurred in 4 of 7 patients receiving supportive care, and in one of two receiving allogeneic haemopoietic stem cell transplantation (HSCT).

Reintroduction of IST was effective in 4 of 6 patients who relapsed after initial IST, and in one patient with minor transient responses to horse then rabbit ATG, danazol produced sustained, superior improvement. Development of myelodysplasia correlated with IST refractoriness in 2 patients and was ultimately fatal despite HSCT. Deaths from sepsis occurred in 2 elderly patients receiving supportive care or CSP. Overall survival is currently 87% at 43 months.

Conclusion

Outcomes in this patient population devoid of selection bias in treatment quality and availability were adversely influenced by advanced age and evolving myelodysplasia. Incompleteness of the initial response, and responsiveness of subsequent relapse to IST suggests incomplete suppression of the underlying immune process by current therapy, but lack of any response to IST could represent an inherited or hypocellular myelodysplastic disorder.

085. Scl and Lyl1 are critical for proplatelet formation and platelet function

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Aim

Scl and Lyl1 are the only two haematopoiesis-specific basic-helix-loop-helix transcription factors. These structurally related proteins are expressed widely throughout haematopoietic cells including megakaryocytes. We have previously shown that conditional deletion of Scl in haematopoietic cells leads to mild thrombocytopenia and defective stress thrombopoiesis (McCormack et al. Blood 2006). We postulated that this relatively mild platelet phenotype was due to compensation by Lyl1.

Method

We made platelet-specific Scl conditional knockout mice by crossing Scl-flox mice with PF4cre-recombinase mice. These mice were then crossed with Lyl1-knockout mice to generate mice with platelets deficient in both Scl & Lyl1. We then analysed the platelet phenotypes between wildtype, Scl-knockout, Lyl1-knockout and Scl/Lyl1 double knockout (dko) mice.

Result

Platelet-specific Scl/Lyl1 dko mice had marked thrombocytopenia with large platelets compared to wildtype controls (Plt count 207 vs 1147 p<0.0002, MPV 6.7 vs 5.7fl p<0.002). This is despite the elevated numbers of megakaryocytes (17 vs 7 per hpf, p<0.001). These megakaryocytes also had increased nuclear:cytoplasmic ratio which correlated with increased DNA content by flow cytometry. Electron microscopy demonstrated grossly dilated demarcated membrane system and large megakaryocyte fragments within the bone marrow sinusoidal vessels, suggesting impaired platelet production capability. Furthermore, platelet count in the Scl/Lyl1 dko mice did not increase with administration of thrombopoietin due to markedly decreased proplatelet formation. They were also unable to respond to thrombocytopenia induced by administration of anti-platelet serum or 5-FU. Finally, platelets that were formed in the Scl/Lyl1 dko mice were not functional; platelets isolated were unresponsive to several agonists across multiple assays of platelet activation and their tail bleeding times were also prolonged.

Conclusion

There is functional compensation between Scl and Lyl1 in megakaryopoiesis, with a specific requirement in platelet production. By deleting them simultaneously we have further defined the role of Scl and identified Lyl1 as a novel transcriptional regulator. Further analysis of these Scl/Lyl1 double knockout mice should reveal new insights into the molecular control of platelet formation and function.

086. Novel next generation sequencing based minimal residual disease testing utilising non-conventional molecular targets in acute myeloid leukaemia

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Background & Aims

Minimal residual disease (MRD) detection is an important predictor of outcome in acute myeloid leukaemia (AML). Standard molecular targets for MRD detection include chimeric fusion genes (*PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, *BCR-ABL*) or *NPM1* exon 12 insertion mutations. While these standard targets collectively cover between 30-60% of AML cases (depending on age) this still leaves a large proportion of patients with no standard molecular markers for MRD testing. We aimed to investigate the feasibility and utility of employing non-conventional molecular targets for MRD detection in AML.

Method

Samples with small insertion/deletion mutations in targetable genes were identified from routine diagnostic cases tested on the Peter MacCallum Cancer Centre Myeloid next generation sequencing (NGS) panel. MRD assessment was carried out on post-treatment samples using ultra-deep (500,000x) NGS. 500ng of sample DNA was PCR amplified with gene specific primers and sequenced on an Illumina MiSeq. Data analysis was carried out using an in-house developed bioinformatic pipeline and annotation tool.

Results

MRD detection was carried out for mutations identified in *RUNX1*, *WT1*, *KIT*, *TET2* and *SF3B1*. Mean sequencing depth was 325,351x giving an average estimated sensitivity of 0.0015% (1.5×10^{-5}). NGS MRD results were compared to reference methodology including flow cytometry, RT-qPCR for *WT1* expression level, *RUNX1-RUNX1T1* RT-qPCR, *CBFB-MYH11* RT-qPCR and *PML-RARA* RT-qPCR. Complete concordance between reference methodology and NGS MRD variant allele frequency (VAF) was observed. MRD-VAFs were shown to decrease with leukaemia treatment and to recur with relapse, tracking with reference methodology. In addition, testing of stored samples from diagnosis detected the presence of MRD level *RUNX1* mutations that were identified at first relapse, indicating clonal heterogeneity of the disease at diagnosis.

Conclusion

Ultra-deep NGS can be used for the sensitive and accurate MRD detection of appropriate non-conventional molecular targets in patients with AML. Mutations that are predominant at relapse may be detectable in baseline samples at MRD levels.

087. Reactive oxygen species promote leukaemogenesis via altered redox signalling pathways in FLT3-ITD+ acute myeloid leukaemia

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Aim

Acute myeloid leukaemia (AML) is a heterogeneous disease characterised by the clonal proliferation of mutant myeloid precursor cells. Despite a recent increase in our knowledge of the recurring mutations in AML, treatments have remained largely unchanged and 5-year survival remains at just 24%. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen that play important roles in normal haematopoiesis as well as leukaemia. Reversible and irreversible oxidation of critically important enzymes containing cysteine residues results in the alteration of signalling and redox pathways.

Objective

The aim of this study was to identify patient proteomes, associate them with their recurring molecular phenotypes and identify new drug targets.

Method and Results

In this study we have used quantitative proteomics to identify proteins containing reversibly oxidised cysteine residues in 13 AML patient samples, an MV4-11 cell line and a normal CD34+ control. Utilising this approach, we have identified 4,471 unique proteins showing modified cysteines. Preliminary analysis suggests patients expressing worst-case prognosis FLT3-ITD (n=6) have increased oxidation of key protein phosphatases (tumour suppressors) (PTPRC, PTPRJ, PPP2R1A, PTPRA) compared to FLT3-WT (n=6), as well as oxidation of oncogenic kinases (SRC, MAPK, PKCB). Furthermore, we have seen significant differences in antioxidant proteins between ITD and WT (GPX1, PRDX1, PRDX5, PRDX6, MPO, SOD1, VCAM1) supporting REDOX dysfunction in ITD patients. This work is supported by the detection of elevated ROS in AML cell lines using flow cytometry and reduced proliferation in FLT3-ITD cell lines upon NADPH oxidase inhibition; a driver of ROS production in FLT3-ITD+ AML.

Conclusions

Taken together these data suggest that FLT3-ITD+ AML regulate survival and resistance to therapies through the modulation of tumour suppressor networks and antioxidant defence mechanisms. Our data provides evidence that manipulation of the altered REDOX environment is a novel approach to identify new treatments in this poor prognosis subset of AML.

088. miR-10a as a Predictive Biomarker in Normal Karyotype (NK) Acute Myeloid Leukaemia (AML)

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Aim
Acute myeloid leukaemia (AML) is a fatal disease with a 5-year overall survival rate of just 25%, and a treatment regimen which has remained largely unchanged for the last four decades. There is an urgent need to identify new biomarkers and therapeutics strategies for AML. MicroRNAs (miRNAs) are known to play important roles in both haematopoiesis and cancer. We previously reported that miR-10a was expressed >26 fold higher in normal karyotype (NK) AML compared to normal bone marrow. miR-10 family members are implicated in malignant transformation, however the exact role of miR-10a in AML is unknown. In the current study we aimed to determine whether miR-10a expression was associated with outcome in NK-AML patients.

Methods
Samples from 120 NK-AML patients from the German SAL AML2003 randomized clinical trial were assayed for miR-10a expression by QPCR, and expression was correlated to clinicopathological variables, treatment response, and outcome.

Results
Analyses revealed that miR-10a may be a powerful predictive biomarker specifically in the NK-AML patients with non-mutated NPM1 who received post-induction high dose chemotherapy + allogeneic transplant (upfront allo-Tx). We compared patients who received up-front allo-Tx to a matched group who received standard induction/consolidation therapy ($n = 61$). Among patients that received upfront allo-Tx, those that expressed low miR-10a had significantly improved outcome, decreasing their risk of death by 5.6-fold compared to miR-10a-high patients (increasing overall survival from 0% to 85% after a mean followup time of 39.2 months). Conversely, miR-10a-high patients did not benefit from upfront allo-Tx, indicating that these patients should receive alternate therapy. Multivariate Cox regression analyses confirmed miR-10a added prognostic information independent of standard clinical variables for both relapse-free ($p < 0.001$) and overall survival ($p = 0.021$).

Conclusions
miR-10a may represent a valuable and novel biomarker for guiding allo-Tx in NK-AML.

089. Low-Dose Cytarabine and Thioguanine Based Metronomic Chemotherapy (STIMULus) as Salvage in Elderly Patients with Relapsed or Refractory Acute Myeloid Leukaemia (AML)

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Aim

The high toxicity of current treatments for relapsed or refractory AML necessitate the investigation of novel therapeutic approaches relevant to elderly patient cohorts. Continuous, low-dose metronomic chemotherapy approaches may have application in this poor prognosis group, through toxicity reduction, lower costs and increased rates of outpatient administration.

Methods

Data was retrospectively evaluated for 18 patients with relapsed or refractory AML treated with an outpatient-based metronomic protocol of subcutaneous cytarabine 20mg/m² (LDAC) and oral thioguanine 40mg/m² given for 14 – 21 days in monthly cycles between January 2008 and June 2015. Overall response rate, partial response rate, overall survival and relapse-free survival were compared to an age-matched historical cohort of 17 patients with relapsed AML who received alternative salvage regimens during the study period.

Results

The metronomic LDAC/thioguanine cohort was a high risk group of median age 67 years (52.8 – 83.7) consisting of 11 patients (61.1%) with secondary AML and 7 patients (38.9%) with adverse or intermediate cytogenetic risk. A CR/CRi was achieved in 5/18 patients (27.8%) in the metronomic LDAC/thioguanine cohort and 3/17 patients (17.6%) in the historical cohort, (P = 0.69). Median overall survival was 166 days (95% CI; 65 – 518 days) in the metronomic LDAC/thioguanine cohort and 102 days (95% CI; 53 – 319 days) in the historical cohort (P = 0.4995). Achievement of CR/CRi with metronomic LDAC/thioguanine was associated with prolonged overall survival (P = 0.0004). Overall survival in responders to the metronomic protocol was 770 days (95% CI; 518 days – upper limit not reached), with a median response duration of 15.16 months (7.2 – 23.5).

Conclusion

This is the first study to examine the use of metronomic chemotherapy in a cohort of patients with relapsed or refractory AML. Remission rates and overall survival were comparable to those achieved in an historical cohort of patients who received alternative salvage therapies, including intensive regimens.

090. A Single High-Dose Cytarabine (HiDAC) During Induction-Consolidation May Not be Adequate for Patients with a New Diagnosis of Acute Myeloid Leukaemia (AML) not Planned for Allogeneic Haematopoietic Stem Cell Transplantation (alloHSCT) in 1st Complete Remission (CR1)

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Introduction

It is generally accepted that treatment with HiDAC increases the cure rate of patients with newly diagnosed AML. However, it remains uncertain whether HiDAC is best given as induction or consolidation, and as to the optimal number of cycles of HiDAC.

Methods

Consecutive patients aged ≤60 yrs with a new diagnosis of AML presenting to our 5 institutions were included in the study if they were intended to receive either 7+3 induction followed by 2 HiDAC-based consolidations (HiDAC consolidation cohort) or HiDAC-based induction followed usually by 2 low dose cytarabine consolidations (HiDAC induction cohort). Patients were diagnosed from 1999 to June 2013, and were followed for at least 12 months to June 2014.

Results

486 patients were included: HiDAC consolidation cohort n=251; HiDAC induction cohort n=235. Baseline demographics were reasonably well matched. For HiDAC consolidation cohort and HiDAC induction cohort, respectively, CR1 was 80% vs 91% (p=0.001); TRM 8% vs 5% (p=ns); OS (5 yrs) 49% vs 50% (p=0.7); DFS (5 yrs) 47% vs 41% (p=0.24), and 22% vs 32% respectively of CR1 patients underwent alloHSCT in CR1. Despite the improved CR rate and higher alloHSCT in CR1 rate in the HiDAC induction cohort, DFS and OS were not improved. Subset analyses showed, that in those patients achieving CR1 and not undergoing alloHSCT in CR1 (n=301), the cumulative incidence of relapse was greater in the HiDAC induction cohort (49% vs 60%, p=0.059) leading to reduced DFS (58% vs 46%, p=0.058), and reduced OS (59% vs 49%, p=0.13).

Conclusions

Outcomes with either HiDAC given during induction or consolidation were similar. HiDAC as induction gave a better CR1 rate but did not translate into a better DFS or OS, primarily because of a greater relapse rate in this cohort. These data suggest that either a single cycle of HiDAC is not optimal in patients with newly diagnosed AML who are not planned for alloHSCT in CR1, or that HiDAC as induction therapy is getting more "bad players" into CR1 who are destined to relapse without alloHSCT in CR1.

091. Determining mechanisms of resistance to Azacitidine (Aza) in Myelodysplastic (MDS) syndromes and Acute Myeloid Leukaemia (AML) using an in-vitro model

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Aim
Aza resistance occurs in majority of Aza treated cases; however the mechanism of it is poorly understood. We investigated the mechanism of Aza resistance by using an *in-vitro* model.

Method
MOLM-13 cells were cultured with increasing concentrations of Aza. Resulting MOLM-13 cells resistant (R) to 0.1 to 10 µM Aza (R0.1, R0.4, R0.8, R1, R1.4, R2.2, R3, R5 and R10) and Aza-sensitive cells were used to explore the role of cellular transporters in mediating drug resistance, assessed by intracellular uptake and retention (IUR) of ¹⁴C-Aza and LD₅₀^{Aza}. Gene expression profiles of drug transporters were assessed using qPCR Taqman® Custom Transporter plate

Results
¹⁴C-Aza IUR was significantly (p<0.001) lower in MOLM-13-R10 compared to Aza-sensitive cells at 0.5 µM (0.5±0.1 vs. 1.4±0.1), 1 µM (1.05±0.19 vs. 3.1±0.5) and 2 µM (2.1±0.5 vs. 5.9±1.0) ¹⁴C-Aza. Similarly, ¹⁴C-Aza IUR was significantly (p<0.01) lower in all intermediate resistant MOLM-13 cells compared to Aza-sensitive cells (Fig.1A). Significantly, lower ¹⁴C-Aza IUR in resistant cells was associated with higher LD₅₀^{Aza}. Aza LD₅₀s were 18.8 and 20.5 µM in MOLM-13-R5 and MOLM-13-R10 cells respectively, compared to 4.2 µM in Aza-sensitive cells. In Aza-sensitive MOLM-13 cells, ¹⁴C-Aza IUR was significantly (p<0.01) higher at 37°C compared to 4°C. However, this temperature dependence was lost in Aza-resistant cells. Together this suggests that active cellular transporter was down regulated in Aza-resistant cells. There was no significant difference in ABCB1 or ABCG2 expression in Aza-resistant cells and Aza-sensitive cells. Gene expression profiling identified 19 drug transporter genes were differentially expressed in Aza-resistant compared to parental cells (Fig.1B).

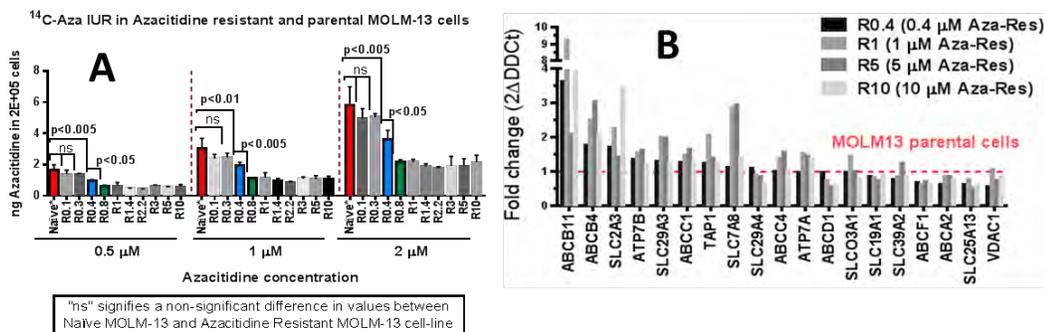


Fig 1: ¹⁴C-aza IUR is significantly lower in Aza resistant cells compared to parental cells (A), most probably due to differential expression of cellular transporters in Aza resistant cells compared to parental cells

Conclusion
In vitro Aza-resistance

is mainly due to lower intracellular concentration of Aza and is independent of ABCB1 and ABCG2. Differential expression of ATP-Binding cassette proteins and Solute Carrier Family proteins may mediate *in-vitro* Aza-resistance by reducing Aza-intracellular concentration.

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092. Stored RBC transfusions: Iron, inflammation, immunity, infection

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Whether transfusions of donor red blood cells (RBCs) after prolonged refrigerated storage induce adverse effects in recipients remains controversial. Nonetheless, refrigerated storage does induce cellular damage, known as the “RBC storage lesion.” Thus, at outdate, the 24-hour post-transfusion RBC recovery averages 75% in healthy recipients. Alternatively, at outdate, ~25% of transfused RBCs are cleared within 24 hours, most by 1-2 hours post-transfusion. In addition, post-transfusion RBC clearance is even greater in hospitalized patients. Abundant evidence suggests that oxidative damage, produced by reactive oxygen species, is, at least partly, responsible for this storage lesion.

We hypothesized that normal volunteer donors, whose RBCs, for genetic, dietary, and/or environmental reasons, are less able to withstand oxidative stress could constitute “poor storers,” whereas those whose RBCs were more able to resist oxidative stress could constitute “super storers.” We also hypothesized that post-transfusion catabolism of these rapidly cleared, storage-damaged RBCs by mononuclear phagocytes would deliver a substantial bolus of hemoglobin-derived iron to these macrophages. Finally, we hypothesized that this iron is responsible for, at least some of, the adverse effects of these transfusions.

This increased iron can induce adverse effects by several pathways. For example, increased levels of intracellular iron may play a role in producing a pro-inflammatory response to transfused RBCs, potentially through the effects of reactive oxygen species on stress pathways. Increased intracellular iron can also enhance the virulence of intracellular pathogens. In addition, some excess iron is returned to plasma through ferroportin (the cell membrane iron transporter) at a pace that can exceed the rate of iron uptake by transferrin (the physiologic iron transporter), thereby producing circulating non-transferrin-bound iron. Normally, non-transferrin-bound iron is undetectable in plasma; however, circulating non-transferrin-bound iron can cause endothelial cell toxicity and impair the host’s ability to combat infection by its innate iron-withholding pathways, thereby enhancing the virulence of extracellular pathogens.

To address the “iron hypothesis” as it relates to transfusions of RBCs after prolonged refrigerated storage, we use cell culture models *in vitro*, murine and canine models *in vivo*, and transfusion studies *in vivo* in healthy human volunteers and select patient populations.

Data will be presented demonstrating that rapid clearance of storage-damaged RBCs leads to post-transfusion release of iron, thereby inducing inflammatory and infectious sequelae. In addition, unresolved questions arising from these studies will be discussed.

093. KLF1 mutations and thalassaemia

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Heterozygous mutations in human KLF1 are common in people from Asia and the Mediterranean regions^{1,2}. Thus, they are also commonly encountered in Australia. Mutations sometimes co-occur with mutations in the α - and β -globin gene loci, which results in a phenotypic heterogeneity. KLF1 mutation carriers often go undetected throughout life but can be detected via investigation of thalassaemia families. Carriers have high but variable HbF levels and borderline HbA2 levels (between normal and α -thalassaemia carriers), which is consistent with the known critical role for KLF1 in haemoglobin switching. It is important to confirm likely cases of KLF1 heterozygosity because homozygosity for loss-of-function mutations is likely to be common, and it can lead to catastrophic anaemia in the fetus or newborn³. KLF1 disease should be considered in any case of hydrops fetalis after exclusion of α -thalassaemia and hemolytic disease. There are some simple laboratory tests which might lead one to suspect a KLF1 carrier mutation. These included the In(Lu) serological blood type, and high zinc protoporphyrin (ZPP) in the context of normal iron studies³. However, the MCV and MCH are often normal, so they cannot be relied upon for screening. Re-sequencing of the coding region of KLF1 is relatively simple as it is a small gene with just three exons. Since there are more than 60 described mutations throughout the gene, the entire coding region needs to be sequenced to be sure of detection³.

Mutations in the zinc finger domain KLF1 are usually hypo-morphic. So, when co-inherited with loss-of-function mutations, these lead to congenital non-spherocytic anaemia (NSHA) which can masquerade as enzyme deficiencies, membrane disorders or other red blood cell disorders^{2,3}. A rare dominant mutation in a DNA-contacting residue in finger 2 (E325K) leads to congenital dyserythropoietic anaemia (CDA) type IV and HPFH⁴. We undertook genetic studies to try to understand how the CDA and E339D mutations disrupt erythroid differentiation⁵ and also investigated the mechanism by which mutations in linker 2 cause disease^{6,7}. We transduced the Klf1-null cell line, K1⁸, with retroviruses expressing C-terminal ERTM fusions of wild type Klf1 and mutations E339D and E339K, and undertook ChIP-seq after tamoxifen induction. Klf1 binds the canonical KLF-binding motif, CCM-CRC-CCN, in K1-ER cells as it does in fetal liver⁹. On the other hand, Klf1-E339K (~E325K in humans) binds to a different motif, CCM-CTC-CCN, and Klf1-E339D unexpectedly binds to CCM-NGC-CCN. We confirmed aberrant binding specificities *in vitro* by EMSA and biophysical assays. We show ectopic binding leads to aberrant transcription and this leads to cell death. We have also performed ATAC-seq in these cell lines versus parental cells to show KLF1 opens chromatin at a subset of occupied sites sometimes within super enhancers, consistent with a pioneer transcription factor role for KLF1. Klf1^{E350R/-} mice develop severe post-natal haemolytic anaemia with bizarre red blood cell morphology. The phenotype closely resembles NSHA in man due to similar compound heterozygosity for KLF1⁶. We will discuss how biochemical insights into the function of linker 2 might explain the phenotype. Together, these studies suggest mutations in the DNA-binding domain of KLF1 can be hypo- or neo-morphic, and thus lead to divergent and unexpected red blood cell diseases.

094. The air they breathe

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While all living creatures share Earth's atmosphere, they have adapted respiration and tissue oxygenation to fit unique environments. Many animals demonstrate respiratory capacity that outperforms anything humanly possible.

This lecture will describe tissue oxygenation strategies used by deep diving mammals and by high-flying migratory birds, using published research on the respiratory physiology. While respiratory performance varies widely, the oxygen binding characteristics of hemoglobin are relatively constant across broad species of animals on Earth, even though the available oxygen is remarkably different in different environments.

Deep-diving marine mammals can reach depths of 1 mile where pressures are 200 times greater than at sea level. They can hold their breath for up to one hour. They oxygenate muscles using willful control of heart rate and blood flow collectively. They avoid nitrogen narcosis by emptying their lungs of air prior to dives. Myoglobin plays an important role in tissue oxygenation. Deep-diving mammals are the finest anaerobic athletes on the planet.

In contrast, birds breathe in a manner entirely different from mammals and oxygenate their blood during both inspiration and expiration. This remarkable process results in a far more efficient method of blood oxygenation than can be achieved in mammals (including humans), making birds the finest aerobic athletes on Earth.

From the perspective of oxygen delivery to tissues, athletic capacity, and adaptation to environmental stress, certain non-human species demonstrate performance far superior to humans. A better understanding of the how other creatures use the same air that we breathe may lead to greater respect for species diversity and animal life on our planet.

095. Involvement of the protein C pathway in cancer, kidney disease and malaria

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The endothelial cell protein C receptor, EPCR, is best known for its ability to bind protein C and enhance activation by the thrombin-thrombomodulin complex. More recent studies have shown that EPCR has multiple other functions. It serves as a receptor to bind malaria infected red cells. The binding site on EPCR for protein C and the malaria induced plasma membrane protein, CIDR, overlap but are not identical suggesting that antibodies to EPCR that do not inhibit protein C activation could still block the binding of the infected red cells. EPCR also plays significant roles in cancer. On the endothelium, EPCR protects against metastasis. On the cancer cells, where it is sometimes induced, EPCR contributes to cancer growth. At least in the mouse, EPCR and thrombomodulin play a central role in stem cell retention in the bone marrow. Following an inflammatory or coagulation event, EPCR is cleaved from the cell surface thereby preventing the signaling required to maintain the stem cells in the bone marrow. Thrombomodulin is down regulated in diabetes and this down regulation in the kidney contributes to diabetic nephropathy. Thrombomodulin was initially discovered based on its ability to bind thrombin and increase the rate of protein C activation. Recently, however, mutations in thrombomodulin have been identified and found to be associated with an increased risk of atypical hemolytic uremic syndrome. More detailed studies have shown that thrombomodulin regulates complement activation at several different stages. Thus, EPCR and thrombomodulin serve not only as major mediators of coagulation but also in cancer, hematopoiesis and complement regulation.

095a. The role of CXCL10 in the induction of severe malaria pathogenesis and the control of parasite biomass

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Whereas cytokine responses to malaria have been extensively investigated, the role of chemokines has only recently started to receive attention. Amongst these molecules, CXCL10 or IFN- γ -inducible protein 10 has emerged as a biomarker strongly associated with increased risk of *Plasmodium falciparum*-mediated cerebral malaria. High CXCL10 levels were found in cerebrospinal fluid and its production was detected in brain endothelial cells. Consistent with this, we have previously shown that CXCL10 neutralization or genetic deletion alleviates brain intravascular inflammation and protects malaria-infected mice from experimental cerebral malaria. In addition to organ-specific effects, here we found that the absence of CXCL10 during infection reduces parasite biomass, suggesting that this chemokine compromises the induction of protective immunity. To identify the cellular sources of CXCL10 involved in this process, wild-type and CXCL10^{-/-} mice were irradiated and reconstituted with bone marrow from either wild-type or CXCL10^{-/-} mice. Chimeric mice were challenged with luciferase-expressing *P. berghei* ANKA and parasite biomass was determined. Similar to CXCL10^{-/-} mice, chimeras unable to express CXCL10 in hematopoietic-derived cells controlled infection more efficiently than wild-type control chimeras. In contrast, expression of CXCL10 in knockout mice reconstituted with wild-type bone marrow resulted in high parasite biomass levels. The increased susceptibility to infection observed in chimeric mice able to secrete CXCL10 from their hematopoietic cell compartment resulted in higher parasite sequestration rates in the brain and increased susceptibility to cerebral malaria during *P. berghei* ANKA challenge. Neutrophils and inflammatory monocytes were identified as the main cellular sources of CXCL10 responsible for the induction of these processes. The improved control of parasitemia observed in the absence of CXCL10-mediated trafficking was associated with a preferential accumulation of CXCR3⁺CD4⁺ T follicular helper cells in the spleen and enhanced antibody responses to infection. These results are consistent with the notion that some inflammatory responses elicited in response to malaria infection contribute to the development of high parasite densities involved in the induction of severe disease in target organs.

096. Cross-species incompatibility of thrombomodulin: Implications and solutions for xenotransplantation

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The use of pigs as organ donors for human recipients (xenotransplantation) offers a potential solution to the shortage of human donors. Not surprisingly, studies in preclinical primate models have shown that pig organ xenografts induce more powerful innate and adaptive immune responses than allografts. This is due not only to the greater repertoire of antigenic differences, but also to molecular incompatibilities affecting the regulation of coagulation and inflammation. Probably the most significant incompatibility is between pig thrombomodulin (TBM) and the human protein C pathway. Pig TBM binds human thrombin, but has poor cofactor activity for the activation of human protein C. The resulting failure to generate sufficient activated protein C likely exacerbates the already pro-inflammatory/pro-coagulant milieu of the xenograft endothelium, leading to intravascular thrombosis and (in extreme cases) development of a consumptive coagulopathy in recipients. Genetic modification of the donor pig to address this problem has included deletion of xenoantigens (to reduce the activating stimuli) and transgenic expression of human TBM on pig endothelium. New genome editing techniques such as CRISPR will permit a more efficient and streamlined modification process.

097. Valves thrombosis

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There is a black box warning against the use of direct oral anticoagulants in patients with mechanical heart valves (MHV). This warning is based on the results of the RE-ALIGN study, which showed a trend for more ischemic strokes with dabigatran than with warfarin in MHV patients. Why did dabigatran fail in this setting? We have shown that MHV and other blood-contacting medical devices trigger clotting via the contact pathway and generate thrombin in concentrations that exceed those achieved with therapeutic doses of dabigatran. In contrast, by reducing the functional level of factors IX, X and prothrombin, warfarin more effectively attenuates MHV-induced thrombin generation. These findings identify factors XII and XI as potential targets for novel anticoagulants to prevent clotting on MHV and other blood-contacting devices.

098. Being in the right place at the right time: Targeted anti-thrombotic approaches

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Thrombosis occurring at sites of atherosclerotic plaque rupture leading to vascular occlusion or embolisation results in myocardial infarction and ischaemic stroke. Furthermore, the formation of pathological thrombi underpins the pathogenesis of ischaemic stroke in atrial fibrillation and venous thromboembolic disease. Given that thrombus formation is critically dependent upon platelet activation and thrombin generation, all currently used anti-thrombotic drugs inhibit these processes. However, a major drawback from current approaches is the inherent risk of bleeding associated with anti-thrombotic drugs. Indeed, major bleeding remains the most common complication afflicting inpatients with acute coronary syndromes (ACS) and affects up to 5% of patients. Importantly, bleeding cannot only result in life threatening complications such as intracerebral haemorrhage, there is now a growing awareness that bleeding events are a strong risk factor portending an increased mortality and adverse cardiovascular outcomes in patients with ACS. Therefore, there remains an unmet clinical need to develop safer anti-thrombotic approaches that specifically target the thrombotic response whilst sparing haemostasis. Importantly, recent experimental data has highlighted that the molecular mechanisms regulating the thrombotic and haemostatic responses may have important differences that can be specifically targeted. These data have provided new impetus to develop novel anti-thrombotic approaches that aim to specifically target the thrombotic response without causing bleeding. One such approach involves the delivery of anti-thrombotic drugs specifically to sites of thrombosis, by targeting the major platelet adhesion receptor, integrin $\alpha_{IIb}\beta_3$. Integrin $\alpha_{IIb}\beta_3$ undergoes a conformational change upon platelet activation thus providing a unique target allowing the specific delivery of anti-thrombotic and thrombolytic drugs to sites of thrombosis. Importantly, this approach is not associated with increased bleeding in pre-clinical animal models. In addition, other novel anti-thrombotic approaches and molecular targets will be discussed. Therefore, our growing awareness of the factors that differentially regulate thrombosis and haemostasis raises the prospect of developing safer and more efficacious anti-thrombotics.

099. Thromboelastography

Roxby D

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The best approach to the bleeding patient is to clearly identify surgical or coagulopathy associated haemorrhage and to restore the haemostatic defect through appropriate surgical interventions or guided blood component use. To achieve this, the rationale for coagulation management in acute bleeding has to be based on an understanding of the pathophysiological processes and functional assessment of the entire coagulation system leading to targeted replacement of platelets, fibrinogen and other clotting factors or the use of anti-fibrinolytic agents. It would be ideal if there was a global coagulation assay that allows assessment of an individual's haemostatic state based on the cell model of coagulation with good correlation with clinical outcomes and is rapid, inexpensive and easy to use.

Standard coagulation assays such as prothrombin time and activated partial thromboplastin time are often unsuitable for emergency use due to long turnaround times, being poor predictors of transfusion requirements and only providing an indirect correlation with the clinical picture in critical bleeding. They serve mainly as measures of clot initiation (thrombin generation) and take no account of the involvement of platelets and other aspects of the entire cell based coagulation system.

Evidence is growing that viscoelastic whole blood point of care assays, such as thromboelastometry (ROTEM) or thromboelastography (TEG) are superior to routine coagulation tests for rapid assessment and guiding haemostatic therapy in intra-operative or critical bleeding coagulation management.

ROTEM and TEG measure the viscoelastic properties of blood *in vitro* with a short turnaround for quantitative assessment of the coagulation system. They provide a dynamic and global assessment of the coagulation process with multiple end points reflecting the interaction between platelets and clotting factors and are sensitive to qualitative and quantitative differences of the factors influencing clot generation, clot quality and stability, and fibrinolysis. These devices allow visual assessment of coagulation from clot formation, through propagation, stabilisation and clot lysis. Normal results in the presence of ongoing bleeding have a high negative predictive value of surgical bleeding rather than that associated with coagulopathy and the need for blood components.

ROTEM and TEG provide useful tools for global assessment of haemostasis in critical bleeding, perioperative and acute trauma induced acquired coagulation disorders and hyperfibrinolysis, and to facilitate targeted, timely and appropriate use of blood components thereby minimising empirical administration of blood products.

100. A shear based microfluidic device to monitor platelet function: Application to von Willebrand disease screening

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Recent studies identifying a key role for micro-scale shear gradients in driving the earliest stages of platelet thrombus formation have informed the development of a novel set of microfluidic devices that have potential utility as rapid and efficient screening tools of shear dependent platelet function.

The aim of this project was to undertake a small scale clinical and laboratory based characterisation study of the microfluidic platform and to assess its ability to identify differences in platelet aggregation dynamics in citrated whole blood taken from control subjects and subjects with clinically diagnosed or undiagnosed von Willebrand disease (Types 1, 2 and 3).

Patients with VWD were recruited from the haemophilia outpatient clinic, Alfred hospital. Whole blood samples (250 μ L) or samples treated *ex vivo* to block the canonical platelet amplification loop pathways were perfused at a defined flow rate of 45 μ L/mL through a set of microfluidic device iterations incorporating a high resolution micro-contraction. Microfluidic geometries were characterised by an entry shear rate of 1,800 s^{-1} , that was accelerated to a peak shear rate ranging from 45,000 s^{-1} to 150,000 s^{-1} (dependent on geometry), returning to an exit shear rate of 1,800 s^{-1} . The rate of initial shear acceleration was varied using a series of geometries with contraction entry angles varying from $\theta = 15 - 85^\circ$. The active shear geometry of the device was derivitised with purified human von Willebrand factor isolated from plasma to allow for efficient initial platelet capture, without affecting 3-dimensional aggregation.

The microfluidic platform was able to identify patients with Types 1, 2A, 2B and 3 VWD. ROC analysis of control versus VWD samples established an end-point aggregation size cutoff for VWD of 1,269 μm^2 with a sensitivity of 80.0%, with a specificity of 84.4% for VWD. A statistically significant difference ($p < 0.05$) was observed when comparing control blood samples to type 1 VWD ($p < 0.001$) and type 2A VWD samples ($p = 0.004$), with both subtypes showing minimal to no platelet aggregation in the device. Patients presenting with bleeding symptoms but found not to have VWD (normal VWF:Ag levels) showed no significant difference ($p = 0.907$) to controls. Exogenous titration of Type 3 ($n = 2$) VWD blood samples with purified VWF (10 – 100 μ g/mL) recapitulated platelet aggregation in a concentration dependent manner. Head to head comparison with standard laboratory based tests including, VWF:Ag, VWF:CB and VWF:RCO demonstrated a strong correlation with device output. Time-course (0, 2 and 4 Hrs) trials demonstrate that the device is a sensitive measure of DDAVP treatment of VWD. Finally, head-to-head comparison of the device with the Siemens PFA100 (Collagen/EPI and Collagen/ADP) tests demonstrated that the device comparably, if not more sensitively, was able to identify platelet aggregation defects associated with VWD.

This small-scale clinical validation study demonstrates that haemodynamically sensitive platelet aggregation within our prototype platform is critically dependent on blood VWF antigen levels and demonstrates good proof-of-concept that the device prototype can selectively identify VWD dependent defects in whole blood platelet aggregation. Taken together these data demonstrate that a microfluidic platform with discrete haemodynamic control can operate as a sensitive screen for VWD. Future studies will focus on defining the haemodynamic and platelet function parameters that shift both device selectivity and sensitivity and will expand device application to a wider range of shear sensitive platelet disorders.

101. Soluble platelet proteins that help evaluate thrombocytopenia and stratify bleeding risk

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The metalloproteinase-mediated ectodomain shedding of platelet-specific surface receptors such as glycoprotein (GP) Ib and VI not only regulates the haemostatic function of platelets, but could play a critical role in regulating platelet survival and clearance. Currently, the main clinical measure related to platelets in immune- or nonimmune thrombocytopenia is platelet count, which does not discriminate between platelet production versus destruction defects and does not reliably predict bleeding risk. Interestingly, whereas GPIb is constitutively shed from human platelets and is detectable as both intact and cleaved forms on circulating platelets, GPVI is essentially all intact and the remnant platelet-associated proteolytic fragment is only detectable after GPVI shedding is experimentally induced or in some disease states. Identified triggers of shedding include ligand binding, platelet activation, coagulation, and exposure to supra-physiological shear stress. In addition, an estimate based on normal range of platelet expression copy number and the plasma concentration of soluble GPIb (glycocalicin) or of soluble GPVI (sGPVI) suggests the majority of GPIb in the vasculature is in a soluble form, while approximately 80-90% of total GPVI is on the platelet surface. This implies plasma sGPVI could represent a sensitive platelet-specific marker of platelet reactivity, shear stress exposure or coagulopathy and ultimately provide an improved clinical measure of thrombotic or bleeding risk in individuals. Furthermore, analysis of the ratio of intact:total GPIb on platelets by flow cytometry or other methods could provide a quantitative marker of platelet age, and help with clinical discrimination of production or (immune)destruction defects. While still relatively early days, there is increasing evidence supporting a clinical link between dysfunctional proteolysis of GPIb/GPVI and bleeding or thrombotic risk, and evaluation of platelet age and quality.

102. Identification of a new dysfunctional platelet P2Y₁₂ receptor variant in a family with bleeding history

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P2Y₁₂ is a member of the G protein-coupled receptor family and one of the 2 platelet ADP receptors. P2Y₁₂ defects are associated with increased bleeding risk. Only a small number of mutations have been reported so far with a majority of recessive transmission.

Aim

Inherited bleeding disorders due to platelet defects are still underdiagnosed worldwide leading to inappropriate care. To improve diagnosis, we developed a novel platelet candidate gene array for Next Generation Sequencing (NGS).

Methods

FBC, blood film examination, light transmission aggregometry, flow cytometry were performed before NGS.

Results

A family of three was referred to us for a history of easy bruising and serious bleeding following procedures and requiring transfusion. All 3 individuals had impaired ADP-induced platelet aggregation with primary wave only and disaggregation at both low and high dose ADP. Aggregation to other agonists was normal. NGS identified a single nucleotide substitution in the *P2RY12* gene in all 3 DNA samples (NM_176876 exon2 c.G794C p.R265P) subsequently confirmed by Sanger sequencing R265 is located within the extracellular loop 3 (EL3) which is important for the binding pocket conformation and rotation following ligand binding. Molecular modelling studies indicate that the hydrogen bond between amino acids 265 and 261 will be unable to form which could destabilise this end of helix H6 and thereby affect the ligand binding pocket. A milder heterozygous P2Y₁₂ R265W mutation has been previously reported in 2 siblings with reduced platelet aggregation to 4 μM ADP but normal aggregation to 10 μM or more ADP. Their father, compound heterozygous R256Z/R265W, had a lifelong history of easy bruising and bleeding.

Conclusion

A new dominant *P2RY12* mutation affecting the ligand binding domain has been identified in an Australian family with severe bleeding history.

103. Establishment of a Diagnostic Algorithm for Heparin Induced Thrombocytopenia that Includes a Rapid Chemiluminescent Immunoassay as the Initial Laboratory Test

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Heparin-induced thrombocytopenia (HIT) has characteristic clinical signs, but demand exists for rapid laboratory tests to assist in the diagnosis. The aim of this study was to assess the diagnostic agreement of a newly available Acustar chemiluminescent immunoassay (CLIA) for detection of HIT-IgG at our centre, in comparison with ELISA, platelet aggregation testing (PAT), and the serotonin release assay (SRA).

CLIA was used to test 102 stored samples and 105 presenting to the laboratory over six months. SRA was available for 60 (31 positive and 29 negative) and used as the reference method to determine a CLIA cut-off with appropriate sensitivity and specificity, using ROC analysis. For all 207 samples, CLIA was compared to ELISA (GTI Diagnostics), with PAT and 4T score also available.

In the SRA group, using the CLIA manufacturer's cut-off of 1.0 IgG U/mL as positive, the new test showed 90% sensitivity and 83% specificity. Introduction of an equivocal range for CLIA of 0.70 – 0.99 IgG U/mL improved sensitivity to 97% but reduced specificity to 76%. In the larger group with all patients, the CLIA IgG U/mL trended upwards with the ELISA optical density (OD), but with much scatter (R 0.68). Of 118 tests negative by ELISA (OD < 0.5), 117 were clearly negative by CLIA (<0.7 IgG U/mL) and one was equivocal and negative by SRA.

We conclude that CLIA is suitable as the initial screen for HIT and can replace ELISA, with PAT reserved for patients with a high suspicion of HIT and/or positive CLIA. The equivocal range can be used to flag cases for follow-up although only rarely will a case in this range have HIT. This approach gives a rapid turn-around for initial testing with improved sensitivity and workable specificity, but laboratory results must still be interpreted in the clinical context.

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

104. Improving Microangiopathic Thrombocytopenia(MAT) diagnosis in the Asia-Pacific region by standardisation of the ADAMTS-13 assay

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Background

Laboratory testing for the level of circulating ADAMTS-13 enzyme in patients suspected of microangiopathic thrombocytopenia (MAT) is crucial in distinguishing those patients with thrombotic thrombocytopenic purpura (TTP), atypical haemolytic uraemic syndrome (aHUS) and other MAT causes. The subsequent treatment, outcome and mortality are substantially different with each MAT type. A pre-plasmapheresis ADAMTS-13 level of <10% is generally accepted as confirming a TTP diagnosis when compared to aHUS. However routine laboratory testing is difficult because of variations in ADAMTS-13 assays, poor turnaround-time and little standardisation of results between individual laboratories. To overcome these concerns, the APMAT Network will examine the utilisation of identical ADAMTS-13 ELISA methods that diagnostic laboratories can adopt in centres of excellence in Asia Pacific (AP). The APMAT external quality assessment study of ADAMTS-13 assays include centres in Australia, New Zealand, China, Korea, Japan, Taiwan, Hong Kong, Malaysia, Thailand, India and Singapore.

Aims

To establish the APMAT network and standardise ADAMTS-13 testing in the AP Region.

Methods

The ADAMTS-13 activity and autoantibody were measured by chromogenic ELISA (Technoclone GmbH) separately detecting levels in patient plasma. The HRP-conjugated antibody is measured with a spectrophotometer through a 450nm absorbance filter. A wet laboratory workshop for laboratory scientists from each centre was undertaken in Hanoi 2014. Blinded lyophilised known ADAMTS-13 plasma samples (ECAT Foundation) are sent to each participant, with survey#1 closes July13th/2016 and survey#2 closes November30th/2016.

Results

23 lead laboratories across 11 sites in the AP region have agreed to participate in the ADAMTS-13 tests for the first time. De-identified analysis of data for laboratory precision and clinical impact of the result will be undertaken; outliers will be confidentially contacted for further assistance.

Conclusions

The APMAT Network, with support from ASTH, provides improved country access, a process for quality control and real world research experience of ADAMTS-13 testing in the AP Region.

This research was supported by Technoclone GmbH and Helena Laboratories Australia. The company had no role in analysing the data or preparing the abstract.

105. MYH9 disorders are not uncommon in Australia and New Zealand: results from a platelet next generation sequencing (NGS) project

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MYH9 disorders are a group of autosomal dominant platelet disorders caused by mutations in the MYH9 gene, which encodes the non-muscle myosin heavy chain IIA (NMMIIA). They are characterised by macrothrombocytopenia and the presence of leukocyte inclusion bodies (Döhle-like bodies) on blood films. They may also cause nephropathy, sensorineural hearing loss or cataract, with a strong correlation between genotype and phenotype. Immunofluorescence (IF) staining of the NMMIIA on blood film is the current gold standard but not available in Australia, possible cause of misdiagnosis and inappropriate treatment.

Aim

To identify and characterise MYH9 variants in patients with inherited macro-thrombocytopenia and to implement NMMIIA-IF in our laboratory for diagnostic use.

Methods

Blood samples were obtained following informed consent from 14 Centres.

Blood films were prepared and FBC obtained. Using a MiSeq Illumina sequencer, we performed NGS on 141 patients.

Results

NGS detected 21 MYH9 variants in 77 patients. Of these, 7 were known pathogenic variants (N=16), 5 had uncertain significance (N=7), and 9 were benign polymorphisms (N=68). The MPVs were significantly lower than expected for a disease characterised by giant platelets as larger platelets were not accounted for by our cell counter. Except for cases where only DNA samples had been received, the blood films of patients with pathogenic mutations were evaluated. All demonstrated macrothrombocytopenia; however, inclusion bodies were not easily appreciated. NMMIIA-IF was performed in 4 cases (2 pathogenic variants and 1 uncertain variant in 2 cases); all 4 cases demonstrated abnormal NMMIIA clustering confirming the pathogenicity of the new variant of uncertain significance.

Conclusion

MYH9 disorders are the commonest cause of inherited macrothrombocytopenia. However, neutrophil inclusions are often not appreciated on blood films leading to misdiagnosis, hence the need for NMMIIA-IF and/or MYH9 genotyping.

106. Point-of-care Testing of Coagulation during Extracorporeal Membrane Oxygenation in a Sheep Model

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Aim

Extracorporeal membrane oxygenation (ECMO) is a life-saving therapy for patients with cardiopulmonary failure; however, prolonged contact of the patient's blood with the artificial circuit can cause both bleeding and clotting complications. The mechanisms of haemostatic disruption caused by ECMO remain poorly defined, and difficult to delineate from that caused by the patient's underlying illness. Therefore, we characterised these effects incrementally in an *in vivo* sheep model.

Method

Eight sheep underwent smoke-induced acute lung injury (S-ALI) and veno-venous ECMO (24hr). Control sheep underwent ventilation only (i.e. without smoke or ECMO; n=4), S-ALI only (i.e. without ECMO; n=7) or ECMO only (i.e. without S-ALI; n=8). Heparin infusion targeted Activated Clotting Time (ACT) of 200-300sec (initially 4U/kg/hr). Samples were collected at baseline, pre-ECMO (immediately and 2hr post-smoke), and at 0.25, 1, 2, 6, 12, 18 and 24hr post-ECMO for full blood count, ROTEM[®] and Multiplate[®] analysis using ADP or collagen agonist.

Result

Platelet counts were unchanged. S-ALI sheep had prolonged EXTEM (tissue factor activated) clot formation time (CFT), decreased maximum clot firmness (MCF) and decreased A30 compared to healthy controls. ECMO sheep had decreased EXTEM clotting time (CT), decreased FIBTEM-MCF, and increased collagen-induced platelet aggregation. Additionally, sheep exposed to S-ALI and ECMO had increased FIBTEM-CFT corresponding with a reduced MCF and A30.

Conclusion

Exposure to either S-ALI or ECMO alone resulted in several haemostatic perturbations, while exposure to both S-ALI and ECMO resulted in additional changes. This highlights the potential contributions of the underlying illness, the ECMO therapy alone, and the interaction of the two in contributing to altered haemostasis in ECMO patients clinically. These preliminary results (unchanged platelet counts, prolonged CFT, decreased MCF and reduced A30 in EXTEM and FIBTEM) suggest that fibrinogen may play a functional role in these changes.

107. Diagnostic algorithm in Heparin Induced Thrombotic Thrombocytopenia (HITT): review of serotonin release assay results

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Aim
The diagnostic algorithm for HITT proposes a 4T score followed by screening and confirmatory testing where appropriate. The purpose of this study was to review the performance of the Serotonin Release Assay (SRA) in the laboratory confirmation of and its correlation with clinical scoring systems and screening immunoassays.

Method
The results of SRA testing performed at a reference laboratory (SEALS, Prince of Wales Hospital) from January 2010 to December 2015 were retrospectively examined. The majority of samples were accompanied with information from the referring clinician and laboratory including the 4T Score result and screening immunoassay results which were also reviewed.

Results
A total of 668 samples were tested using SRA from January 2010 to December 2015 of which 439 were SRA negative (65.7%) and 229 were SRA positive (34.3%). Screening immunoassay results were available for 591 (88.5%) of the samples and the majority of laboratories used a particle gel immunoassay (PaGIA) (46.9%) or an ELISA based immunoassay (53.1%). SRA was performed on 160 samples with a negative screening test and was negative in 156 of these (97.5%) with only 4 samples (3 from the same patient) that were PaGIA negative/SRA positive. There were 210 samples (31.4%) with a positive screening test but negative SRA. The 4T Score was available for 465 samples (69.6%). 4T scores ranged from 0-8 in both SRA negative and SRA positive groups with higher mean score in the SRA positive group (5.5 vs 4.4). There was a higher proportion of high probability 4T scores (score 6-8) in the SRA positive group (45.9% vs 25.8%) and only 10 SRA positive cases with low probability score (0-3). Clinical information was available in 517 (77.4%) cases and complication of HITT were more prevalent in SRA positive cases (47.2% vs 20.7%).

Conclusion
This review confirms the role of SRA testing in the diagnosis of HITT and supports the use of pre-test assessment in the form of clinical scoring systems and screening immunoassays.

108. Rare hereditary bleeding disorders

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Rare coagulation disorders include the inherited disorders of fibrinogen, factor (F) II, FV, FVII, FXIII as well as combined deficiency of FV and FVIII and congenital deficiency of the vitamin K dependent clotting factors. These disorders are rare but have unique phenotypic expressions and treatment requirements that require a specialised knowledge to provide an optimum standard of care.

109. Genetic testing in the investigation of patients with suspected inherited thrombocytopenia

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Inherited thrombocytopenias (ITs) comprise a heterogeneous group of disorders characterized by low numbers of platelets and a platelet functional defect (in most disorders), causing variable bleeding tendencies in affected individuals. Historically, the reliance on multiple complex phenotypic tests, coupled by the lack of consensus regarding a standardized approach to testing, as well as, a lack of established diagnostic criteria for the conditions themselves have contributed to the misdiagnosis of IT as ITP. This has resulted in inappropriate treatment with steroids, immunosuppression, and in some cases, splenectomy. The emergence of genetic sequencing platforms for the investigation of ITs has led to the discovery of new genes causing IT and increased our understanding of these conditions.

The choice of sequencing platform is determined by the goals for any given project. Moreover, considerations of cost and practical aspects of test implementation, data generation, bio-informatic analysis, and interpretation, and reporting of incidental findings are important determinants.

Our group at the Northern blood Research Centre is employing a next generation sequencing approach (Illumina MiSeq) using custom designed candidate gene arrays for the diagnosis of IT which remain uncharacterized following phenotypic testing. The gene panels designed to date comprise genes known to be associated with IT but which lack distinct clinical phenotypes where a diagnosis could be confirmed by clinical examination and appropriate ancillary investigations alone. Panels have been updated to exclude genes with low coverage and performance, and to include new genes described in the literature. In addition, where possible, in cases where a mutation has not been detected by the candidate array and where accompanying phenotypic information suggests the presence of a significant or novel gene mutation, samples have been referred to collaborators for WES. Our approach has also enabled critical appraisal of current phenotypic algorithms by comparison of molecular data with “pre-analytical” phenotypic test results.

Our research has been successful in identifying pathogenic and likely pathogenic mutations in genes encoding transcription factors, *FLI1*, *GFI1B* and *RUNX1*, genes responsible for proplatelet and/or platelet release, *MYH9*, *GPIBA*, *GP9* and *TUBB1*, as well as, *NBEAL2* which is an important gene in megakaryocyte maturation, and in which bilallelic loss-of-function mutations result in the Gray platelet syndrome. A homozygous mutation in *ITGA2B* confirming a pre-test phenotype of Glanzmann thrombasthenia was also detected. It is anticipated that through panel optimization with iterative runs that our targeted genotypic screening has the potential to be developed into a national clinical service.

This presentation will describe our experience using a candidate gene array for the diagnosis of IT and will use examples of mutations causing IT encountered in clinical practice, as well as those detected by our NGS panel (some of which are novel), to highlight current limitations of current diagnostic approaches and considerations regarding the utilisation of NGS technology.

110. Acquired haemophilia

Tran H

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Acquired haemophilia A (AHA) is a rare autoimmune disorder characterised by autoantibodies against factor VIII (FVIII) that often leads to potentially life threatening spontaneous or trauma-associated haemorrhage. A lack of familiarity of the disorder often leads to a delay in diagnosis and prompt treatment to obtain haemostasis is necessary to reduce morbidity and mortality. This presentation reviews risk factors for the occurrence of AHA, possible prognostic factors for remission and treatment, including some emerging therapeutic options.

111. Brain trauma and the role of anti-fibrinolytic agents

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The fibrinolytic system is well known for its role in the removal of fibrin deposits and has been harnessed therapeutically to remove blood clots in patients with thromboembolic conditions, including ischaemic stroke. This occurs via the generation of the potent protease, plasmin, which is formed following the activation of plasminogen by tissue-type plasminogen activator (t-PA). An attractive feature of the ability of t-PA to activate plasminogen is that this process occurs preferentially on fibrin allowing targeted generation of plasmin on the surface of blood clots with minimal systemic activation of circulating plasminogen. This targeted generation of plasmin occurs due to the presence of lysine binding sites present in both t-PA and plasminogen that recognise exposed lysine residues in fibrin. The localisation of plasmin(ogen) to the fibrin surface via these lysine residues led to the development of lysine analogues (i.e. tranexamic acid; TXA) as anti-fibrinolytic agents that compete with fibrin for the binding of t-PA and plasminogen. TXA has proven to be effective at blocking fibrinolysis (i.e. following surgery) reducing blood loss and improving outcome. It is now known that localised plasmin formation not only occurs on fibrin, but also on the surface of many misfolded proteins formed following cell injury. Plasmin formed under these conditions facilitates the breakdown and subsequent clearance of the misfolded protein. Moreover at least 12 distinct cell surface receptors for plasminogen have been described that bind to plasminogen in a manner dependent on the presence of either internal or terminal lysine residues. Receptor-mediated plasmin generation does not only facilitate cell-surface proteolysis, but can also trigger a variety of intracellular signal cascades that alters various processes, including promotion of blood brain barrier permeability, activation of the innate immune response and activation of other enzyme cascades (i.e. the MMPs). Since the binding of plasminogen to misfolded proteins and to most of the known plasminogen receptors is also blocked by TXA, it stands to reason that the primary clinical use of TXA (i.e. to stop bleeding) may also have unintended consequences outside of haemostasis by blocking the actions of plasmin on other (non-fibrin) substrates. We have been evaluating the effects of TXA in mouse models of TBI. Our preliminary results indicate a protective effect of TXA administration on blood brain barrier permeability within 3h post injury. We also have evidence to suggest that TXA modulates the immune and inflammatory response following TBI in part via alteration in immune cell migration to the regional cervical lymph nodes. Hence, anti-fibrinolytic agents have the potential to influence plasmin-dependent proteolytic events that are not necessarily related to the removal of fibrin. We are now examining the effect of TXA in various patient groups to gain further insight into the potential clinical relevance of these findings.

112. Tranexamic acid in cardiac surgery

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Background

Tranexamic acid reduces bleeding in cardiac surgery but it is unclear whether this translates into improved outcomes. Furthermore, there is some concern that tranexamic acid may be prothrombotic (increasing risk of myocardial infarction, stroke and thromboembolism) and proconvulsant.

Methods

Using a 2-by-2 factorial trial design evaluating aspirin and tranexamic acid, we randomly assigned 4,631 patients who were scheduled to undergo coronary artery surgery and were at risk for perioperative complications to receive tranexamic acid or placebo.

The primary (safety) outcome measure was a composite of death and thrombotic complications (nonfatal myocardial infarction, stroke, pulmonary embolism, renal failure or bowel infarction) within 30 days of surgery. Efficacy outcomes included blood loss and rates of blood transfusion, and need for surgical re-exploration for bleeding/tamponade.

Results of this trial will be presented at the meeting, and a consideration of how this changes current use of tranexamic acid in contemporary surgery will be discussed.

The ATACAS trial was funded by ANZCA and NHMRC

113. Thrombolysis vs thrombectomy in stroke

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Acute ischemic stroke is responsible for around 80% of all strokes and is a leading cause of disability and death globally. The only proven treatment strategy is to restore blood flow (reperfusion). The two reperfusion strategies available are intravenous thrombolysis with alteplase and endovascular clot retrieval (thrombectomy). Alteplase was first demonstrated to reduce disability with publication of the National Institute of Neurological Disorders and Stroke (NINDS) tissue plasminogen activator (tPA) trial in 1995. Since that time further trials have solidified the evidence base and demonstrated benefit when alteplase is administered within 4.5 hours of stroke onset. However, even after 20 years, rates of alteplase administration are suboptimal. Exploration of potentially more effective thrombolytics is still underway with tenecteplase but others, such as desmoteplase, have been unsuccessful in clinical trials. Endovascular clot retrieval has been practiced for several years but came of age with the publication of 5 strongly positive trials in 2015. This presentation will discuss the evidence for intravenous and intra-arterial reperfusion strategies and the advantages, disadvantages and synergies of the two approaches.

114. Residual Vein Thrombus Does Not Predict For Recurrent Vein Thrombosis in the ASPIRE study

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Background

In other studies Residual Vein Thrombosis (RVT) as assessed by ultrasound has been associated with recurrent vein thrombosis, other vascular events and mortality. We examined whether RVT in the ASPIRE was associated with subsequent clinical events.

Methods

In the ASPIRE study 822 patients who had completed 6–24 months of anticoagulant therapy after a first unprovoked VTE were randomized to aspirin, 100 mg daily, or placebo for up to 4 years. At randomisation all patients with deep vein thrombosis (DVT) undertook an ultrasound assessment for RVT. Sites recorded the presence of RVT in local CRF. Additionally RVT reports were retrieved and reviewed for the presence and extent of RVT by 2 authors (TAB, AMB). The association of RVT with recurrent vein thrombosis was evaluated using Cox regression models.

Results

In the ASPIRE study, DVT was present in 583 of 822 patients (71%) enrolled. Sites recorded RVT data in 580 patients. Ultrasounds reports were available and reviewed in 412 patients with RVT reported in 265 (64%). The presence of RVT was more prevalent in males. There are 72 first recurrent VTE events, in the 412 patients with residual thrombosis data: 32/147 (22%) without RVT and 40/265 (15%) with RVT. There was no association between presence of RVT and time to first recurrent VTE event when adjusted for study treatment (HR = 0.76, 95% CI 0.48-1.21, p=0.25). Analysis of the 580 patients with site-recorded RVT information showed similar results. There was also no association between RVT and major vascular events (HR 0.88, 95% CI 0.56-1.38) or net clinical benefit (HR 0.96, 95% CI 0.62-1.47) after adjustment for treatment.

Conclusion

In patients who had completed anticoagulation therapy after unprovoked VTE the presence and the extent of RVT was not associated with recurrent vein thrombosis or other clinically important events.

115. Venous Thromboembolism (VTE) in Patients with Primary Central Nervous System Lymphoma (PCNSL) Receiving R-MPV

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Introduction

Venous thromboembolism is common in patients with brain tumours with rates of symptomatic disease in up to 30% of patients. Important factors in pathogenesis are postulated to include release of procoagulant substances such as tissue factor and physical immobility due to lesions within the central nervous system (CNS). While combined modality therapy is highly effective, the occurrence of VTE can cause significant morbidity and impact chemotherapy delivery and rehabilitation.

Aim

To assess VTE incidence in patients with PCNSL undergoing induction chemotherapy.

Method

Retrospectively we reviewed patients diagnosed with PCNSL between 1997 and 2016 and treated at Monash Hospital with Methotrexate, Procarbazine, Vincristine +/- Rituximab (R-MPV). Patients with VTE >4 weeks prior to the lymphoma diagnosis or those with a history of recent therapeutic anticoagulation were excluded. Patient demographics, VTE incidence, adverse events of anticoagulation and survival outcomes were recorded.

Result

36 PCNSL patients were identified. Patient characteristics are shown in Table 1. 8 patients developed VTE (2 proximal and 1 distal lower limb DVTs, 1 PE, 3 with a combination and 1 catheter related axillary vein DVT) at a median of 1.1 months from diagnosis (range 0-3.7). Half had inadequate chemical prophylaxis with delayed initiation (13%), interruptions (13%) or none (25%) despite all patients being admitted prior to VTE diagnosis. Therapeutic anticoagulation was started within a median of 9hrs of diagnosis. Two patients required IVC filters, one prophylactically in the perioperative setting and another due to grade 4 intracranial haemorrhage post anticoagulation. The only other major bleed was gastrointestinal. No patients died from VTE or its treatment.

Conclusion

The VTE incidence in PCNSL was 22% and was greatest during the diagnostic and initial chemotherapy period, possibly contributed to by underlying physical disability/immobility, the prothrombotic effects of the lymphoma and chemotherapy, and difficulties with establishing adequate VTE prophylaxis.

Characteristic	Number (total = 36)	%
Male	23	64
Female	13	36
Median Age (Years) (Range)	66 (31-87)	
>60 Years Old	22	61
Median Months from Initial Diagnosis (Range)	1.15 (0-3.7)	
ECOG > 1	14	39
Elevated LDH	4	11
Elevated CSF Protein Concentration	30	83
Involvement of Deep Regions of Brain	19	53
<i>IELSG Prognostic Score</i>		
Low	6	17
Intermediate	25	69
High	5	14

116. Phase 3 Trial Results Demonstrating Efficacy and Safety of rIX-FP in Children with Haemophilia B

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Background

A fusion protein linking recombinant human coagulation factor IX with albumin (rIX-FP) was developed with favourable pharmacokinetics (PK) allowing prolonged dosing intervals.

Aims

The long-term safety and efficacy of rIX-FP was evaluated in children with moderate or severe haemophilia B (FIX activity $\leq 2\%$).

Methods

Previously treated male haemophilia B patients (<12 years) received weekly prophylaxis with 35–50 IU/kg rIX-FP; bleeding events were treated on demand. The PK of rIX-FP and previous FIX product were assessed. Primary endpoints were PK of rIX-FP and incidence of FIX inhibitors. Annualized spontaneous bleeding rates (AsBR) were calculated; haemostatic efficacy to treat bleeds was evaluated by number of injections to achieve haemostasis and investigator assessment.

Results

27 subjects enrolled and received prophylaxis with rIX-FP. Following a single intravenous dose of 50 IU/kg rIX-FP mean FIX activity levels in all patients remained above 5 IU/dl through day 10 and above 2 IU/dl through day 14, supporting a prophylaxis treatment interval of 7 to 14 days. The PK profile of rIX-FP was improved versus previous FIX treatment, with a >5-fold longer half-life (91.4 vs 18.6 hours) and 6.4-fold slower clearance (1.11 vs 6.40 ml/hr/kg).

Median (Q1, Q3) AsBR was 0.00 (0.00, 0.91), and was similar between younger and older age groups. Mean weekly consumption of rIX-FP was over 50% lower than with prior FIX treatment (47 vs 107 IU/kg). Of 106 bleeds, 97% were treated with ≤ 2 injections of rIX-FP (95% CI: 92% to 99%); 96% of treatments were rated effective (excellent or good). All 27 subjects were compliant with weekly prophylaxis. No subjects developed inhibitors to FIX or antibodies to rIX-FP or CHO proteins. There were no related adverse events.

Conclusions

rIX-FP demonstrated a favourable PK and safety profile in children and was efficacious both as weekly prophylaxis and for treatment of bleeding episodes.

This research was supported by CSL Behring. The company analysed the data and prepared the abstract.

117. Current practice regarding prophylaxis in patients with moderate and severe haemophilia A in Australia

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Aim

To characterise current practice in patients with moderate or severe Haemophilia A (HA) in Australia regarding use of prophylactic clotting factor infusion.

Method

Data was derived from the Australian Bleeding Disorder Registry (ABDR) on patients from whom consent had been obtained. Data was obtained on patient diagnosis, disease severity, age, weight, treatment regime, expected and observed clotting factor usage during the Year 2015. The percentage of patients receiving prophylaxis according to severity of Haemophilia and age was calculated, along with average prophylactic dose (IU/Kg/Year). Compliance was assessed by calculating the percentage of patients in whom $\geq 75\%$, or ≥ 50 but $< 75\%$, of expected factor consumption was documented.

Result

Data was obtained on 622 HA patients, 508 with severe HA and 114 with moderate HA. Overall 82% (416/508) of patients with severe HA and 32% (36/114) with moderate HA were on routine prophylactic therapy. The percentage of patients receiving routine prophylaxis decreased with age and in severe HA patients ranged from 100% (5-9years) to 48.15% (60 plus-years). The mean annual consumption of Factor VIII in patients with severe HA was significantly higher in patients on routine prophylaxis (4533 IU/Kg/Year, SD 3175) in comparison to patients receiving on-demand therapy (1746 IU/Kg/Year, SD 1580), $p < 0.001$. 149/416 (35.82%) patients with severe HA receiving prophylaxis received $\geq 75\%$ of expected product, and 62/416 (14.90%) patients received ≥ 50 to $< 75\%$ of expected product.

Conclusion

Consistent with current guidelines the majority of patients with severe HA are receiving routine prophylactic treatment, although the percentage decreases with age. The average dose of prophylactic therapy is at the upper end of levels reported internationally. A significant proportion of patients were not compliant with prophylactic treatment. Further information is required examining compliance and the continuation of prophylaxis into later adult life with joint health outcomes is required.

118. Choice of anticoagulation in veno-thromboembolic disease in obese patients

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Aim

Data regarding use of non-vitamin K antagonist oral anticoagulants (NOACs) in obese patients with venothromboembolism (VTE) is limited. We examined anticoagulant prescribing in this group and the role of drug specific anti-Xa levels.

Methods

We identified patients with VTE and weight >120kg attending Western Sydney Haematology Services in 2016. We collected demographic data, weight, height, indication, anticoagulant choice and rationale, laboratory drug monitoring, dose adjustments and clinical progress.

Results

We identified 12 VTE patients with weight >120kg. Median weight was 137kg (range 121-208) with 7/12 female. 2 patients were commenced on NOACs at first review. Rationale for alternative anticoagulant were: malignancy (n=2); renal impairment (n=1); cerebral sinus thrombosis (n=1) and superior mesenteric vein thrombosis (n=1). 2 proximal DVT patients received 4 weeks Clexane prior to NOAC therapy because of efficacy concerns. 3 warfarinised patients transitioned to NOACs on patient preference. Anti-Xa testing was performed in all Clexane treated patients and doses adjusted in 4/6. 6 patients received apixaban or rivaroxaban with drug levels performed in 4. Apixaban levels (5mg BD dosing) were (1) peak 99ng/ml, trough 39ng/ml (weight 137kg) and (2) peak 141ng/ml, trough 39ng/ml (weight 182kg). Rivaroxaban levels (15mg BD dosing) were peak 196ng/ml and (20mg dose) peak 90ng/ml, trough 32ng/ml (26 hours post dose) (weight 172kg). Another patient's rivaroxaban level was 65ng/ml, 17 hours post dose (weight 160kg) due to nocturnal dosing. No NOAC dose adjustments were made. There were no recurrent thromboses or bleeding complications.

Conclusion

Clinicians remain hesitant to prescribe NOACs acutely, at body weight extremes, in high risk patients, due to limited data. Our small sample shows that obese patients may have appropriate rivaroxaban and apixaban levels and adds to limited data suggesting NOACs may be efficacious and safe in obese patients. We plan further evaluation in a larger prospective cohort.

119. Clinical utility of specific DOAC plasma concentration assessments in the ASTH Anticoagulation Reversal and Events Study Collaborative (ARES1)

Baker R^{1,2}, Curnow J³, Young L⁴, McGregor S^{1,2}, Gullapalli V⁵, Brighton T⁵, Joseph J⁶, DAulerio G^{1,2}, Harper P⁷, Hugman A⁸, Gallus A⁹

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Background

The ARES Collaborative is a large ANZ prospective observational study of consecutive patients who present to hospital with haemorrhage, thromboembolism or who need urgent anticoagulant reversal and are taking either a DOAC (dabigatran, rivaroxaban or apixaban) or warfarin. The clinical context and severity of the event is recorded in addition to haemostatic strategies utilised. The pilot study (ARES1) included case records from 287 presentations, of which 83 (28.9%) involved a DOAC.

Aims

To describe the clinical utilisation of DOAC plasma concentration ascertainment in ARES1 patients on DOAC's who present with haemorrhage, thromboembolism (TE) or requiring urgent anticoagulation reversal (UAR).

Methods

Case records from 83 DOAC patients were analysed for clinical utilisation of specific DOAC plasma concentrations, defined as reports of calibrated anti-Xa activity or dilute thrombin time assay for quantitating FXa inhibitors (rivaroxaban or apixaban) or dabigatran, respectively.

Results

36 (43.4%) records of DOAC-treated patients in ARES1 included specific assessment of DOAC plasma concentrations (FXa inhibitors n=18, dabigatran n=18). The median time from patient presentation to collection of the initial DOAC plasma level was 2 hours. Major haemorrhage with dabigatran was associated with higher median drug concentrations (216ng/mL) compared to UAR (30ng/mL) or TE (10ng/mL). However, major haemorrhage with FXa inhibitors did not appear to be correlated with higher plasma concentrations. The majority of plasma DOAC levels were within therapeutic range.

Conclusion

Less than half of patients admitted to hospital with DOAC-related events had a plasma drug concentration measured. The majority of patients with major haemorrhage had a therapeutic DOAC level, although some had undetectable amounts of anticoagulant detected. It appears that obtaining a DOAC plasma level may be a critical test to inform important clinical management decisions including requirements for blood or blood products or specific reversal agents in patients on DOAC's presenting with haemorrhage or requiring urgent surgery.

Dr. Baker has received funding for clinical trials from Biogen, Sobi, Boehringer Ingelheim, Bayer, Shire, Pfizer, Daiichi Sankyo, Astellas and CSL Behring, has participated in clinical advisory boards for Amgen, Biogen, Shire, Boehringer Ingelheim, Bayer, Alexion Pharmaceuticals, and Pfizer. Research support from Shire, Bayer, Bristol Meyer Squibb and Alexion Pharmaceuticals. He has received conference travel support from Amgen, Novo Nordisk, Bayer, Bristol Meyer Squibb, Shire and Alexion pharmaceuticals.

120. Pulmonary hypertension: What Haematologists need to know

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Pulmonary hypertension (PHT) is defined as a mean pulmonary pressure above 25 mmHg at rest. The recent Nice 2013 guidelines identify 5 pathological entities (WHO group 1-5) that cause PHT. Although rare, Group 1 PHT (pulmonary arterial hypertension- previously called primary pulmonary hypertension) has seen significant improvement in outlook due to the availability of 3 new classes of selective pulmonary vasodilators exploiting endothelin, nitric oxide and prostaglandin pathways. These diseases have a likely genetic basis or association with other diseases such as connective tissue diseases, congenital heart diseases, portal hypertension or toxin exposure. PHT secondary to Cardiac (WHO group 2) and Respiratory Disease (WHO group 3) is quite common and is presently managed by optimisation of therapy for the underlying disease. Selective pulmonary vasodilators have no clear role. Chronic Thromboembolic Hypertension (WHO group 4-CTEPH) is a not infrequent complication of pulmonary embolism likely due to formation of abnormal thrombus or ineffective thrombolysis. Surgical treatment (Pulmonary EndArterectomy-PEA) in selected cases may cure PHT and is regarded as first line therapy. Medical therapies modestly improve haemodynamics and function in those unsuitable for (or with persistent PHT after) PEA. CTEPH is regarded as an indication for lifelong anticoagulation with warfarin (in the absence of data supporting the use of other oral anticoagulants). Finally a miscellaneous group (WHO group 5) includes haematologic conditions where PHT develops. These include haemolytic anaemias (sickle-cell best described) as well as myeloproliferative diseases and post splenectomy. The cause of this PHT is unclear but may reflect reduced nitric oxide (due to avidity for free haemoglobin), endothelial damage due to intravascular fragmentation, and also potentially secondary to drug therapies.

121. Pulmonary endarterectomy for chronic thromboembolic pulmonary hypertension

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Chronic thromboembolic pulmonary hypertension (CTEPH) is an uncommon disease but important for several reasons – it is substantially under recognised, may result in progressive and disabling symptoms and the natural history is progression to death. However there is a highly effective operation (pulmonary endarterectomy) that may abolish symptoms and reverse the unfavourable natural history.

96% of patients who have a pulmonary embolus have resolution. However approximately 4% of patients do not have complete lysis of the embolus and may ultimately develop CTEPH. The pathology of CTEPH is development of fibrous blind pouches, bands and webs that obstruct pulmonary blood flow, cause secondary arteriolar vascular remodelling, pulmonary hypertension and adverse right ventricular remodelling. Imaging investigations in patients in whom pulmonary endarterectomy is being considered includes a V-Q scan to detect perfusion defects which are unmatched on the ventilation scan. A normal V-Q scan excludes CTEPH. A CT scan is required to exclude large areas of pulmonary infarction which may limit the symptomatic benefit of pulmonary endarterectomy because endarterectomised vessels maybe supplying infarcted lung. A pulmonary angiogram is performed to determine the extensiveness of thromboembolic disease and for surgical decision making.

Pulmonary endarterectomy is performed using cardiopulmonary bypass, profound hypothermia and periods of circulatory arrest to prevent bronchial artery collateral blood flow impeding visualisation of the interior of the pulmonary arteries. The endarterectomy plane (which is within the wall of the pulmonary artery) is established and the cicatrised thromboembolic material is removed.

In experienced hands the mortality of the operation is between 1% and 5%. Mortality and morbidity is related to multisystem failure, (because of poor physical reserves and severe pre-operative subsystem dysfunction), reperfusion pulmonary injury, severe right ventricular failure, persistent severe pulmonary hypertension (where pulmonary vascular remodelling is excessive) and rarely pulmonary haemorrhage from perforation of the pulmonary artery wall. Long term survival of these patients is excellent and most have normalisation or near normalisation of their pulmonary artery pressures and rapid reverse remodelling of the dysfunctional right ventricle.

122. Catheter directed techniques in treatment of venous thromboembolic disease

Vrazas J

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Anticoagulation remains the mainstay of treatment of deep vein thrombosis (DVT). However, the high incidence of chronic sequelae of DVT (post thrombotic syndrome, PTS), the associated long-term health care costs and persistent disabling symptoms for many patients afflicted with this condition demand a better approach to the problem. Ilio-Femoral and caval DVT's massive clot burden risks life threatening pulmonary embolism (PE) and limb loss due to phlegmasia cerulea dolens.

Catheter directed techniques have been used to help manage the problem of large segment venous thromboembolic disease since the 1990's. Despite early problems of bleeding and poor efficacy of systemic and local pharmacological thrombolysis, mechanical and pharmacomechanical techniques have brought this treatment into a new era. Improvements in drug efficacy, improved understanding of dose-response relationships of newer thrombolytic agents and advancements in mechanical thrombus removal techniques have made these minimal invasive techniques compelling in the management of ilio-femoral and caval DVT and life-threatening PE.

This lecture will concentrate on the endovascular treatments of large segment acute DVT and massive and sub-massive PE. Some of the recent evidence will be reviewed and basic nuances of the techniques will be outlined.

123. Update on DOAC reversal: ISTH guidance document

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Antidotes for the direct oral anticoagulants (DOACs) would be useful as one component of strategies for management of serious bleeding, or for rapid reversal of the DOACs before urgent interventions. Three antidotes for the DOACs are under various stages of development. Idarucizumab (Praxbind[®]), the antidote for dabigatran, is now licensed. Andexanet alfa, the antidote for the oral factor Xa (FXa) inhibitors, is undergoing phase III investigation. Ciraparantag (PER977), an agent reported to reverse the anticoagulant effects of all of the DOACs, is at an earlier stage of development. This session provides a summary of the recent guidance document from the ISTH including: 1) the mechanism of action of the antidotes, 2) an update on the available clinical data, and 3) guidance on potential indications for their use.

124. Strategies to Minimize the Risk of Bleeding with Direct Oral Anticoagulants

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The direct oral anticoagulants (DOACs) are at least as effective as warfarin, but are associated with less intracranial bleeding and are more convenient to administer. Consequently, guidelines now recommend DOACs over warfarin for stroke prevention in most patients with non-valvular atrial fibrillation and for treatment of venous thromboembolism in patients without active cancer. Although there is less serious bleeding with the DOACs than with warfarin, strategies are needed to optimize their benefit-risk profiles. These include (a) choosing the right anticoagulant for the right patient at the right dose and for the right duration, (b) avoiding concomitant use of antiplatelet drugs whenever possible, (c) ensuring appropriate periprocedural management, and (d) ongoing follow-up and education to ensure adherence and persistence. In patients who experience major bleeding, the outcome is no worse with DOACs than with warfarin even without specific reversal agents. However, the recent licensing of idarucizumab for dabigatran reversal and the promising results with andexanet alfa for reversal of the other DOACs further enhances their safety profile.

125. ASTH Anticoagulant Reversal and Events Study Collaborative (ARES1)

Baker R, Curnow J, Young L, McGregor SR, Brighton T, Joseph J, D'Aulerio GV, Harper P, Hugman A, Gallus A, on behalf of the ASTH ARES Steering Committee

Perth Blood Institute, WACTH Murdoch University, Perth, Australia

Background

The ARES Collaborative (ARES) is a multi-centre, large observational study of consecutive patients who present with significant haemorrhage, thromboembolism or require anticoagulant reversal for urgent surgery or procedure and are taking a direct oral anticoagulant (dabigatran, rivaroxaban, apixaban) or warfarin. The clinical circumstances, event severity and management strategies were observed.

Methods

Final data from the ARES1 pilot cohort of 287 patients (DOAC, n=83; Warfarin, n= 204) from 7 centres who presented with bleeding, thromboembolism or requiring anticoagulant reversal for surgery were analysed. Outcome measures included, details of haemorrhagic events (including type of bleed, site of bleed, outcome and total deaths at day 30 and fatal bleeds), interventions utilised for haemorrhage, coagulation parameters (pre-intervention INR, aPTT, PT, DOAC plasma levels), thromboembolic events (including type of event and outcome at 30 days), length of hospital stay and OAC resumption were analysed.

Results

The mean age at presentation was 77 years (range 19-96), with approximately a 3:2 ratio (M:F). The median duration of OAC therapy prior to presentation was 31 months. Haemorrhage accounted for 70.7% of presentations (minor bleeds 20.2%; major bleeds 50.5%), whilst urgent reversal and thromboembolism accounted for 20.2% and 9.1% of presentations, respectively. DOACs are increasingly prescribed, accounting for 28.8% of ARES1 cases (dabigatran 14.6%, rivaroxaban 13.2 % and apixaban 1.0%).

Major haemorrhage represented 71.4% of bleeding events, and was associated with an extended length of hospital stay (median: 6 days) and a high day 30 all-cause mortality rate (16.5%). The most prevalent sites of major haemorrhage were the GIT (40%), CNS (24.8%) and musculoskeletal (9.6%). Moderate renal impairment (CLCr 30-59mL/min) was evident in 28% of the total haemorrhage cohort. Various haemostatic agents were utilised in an attempt to improve haemostasis in patients with DOAC-associated haemorrhage (e.g. Prothrombinex-VF® 31.5%, Tranexamic acid 26.3%, FEIBA® 7.9%).

Amongst patients presenting with major haemorrhage, elevated pre-intervention INR, aPTT and PT were prolonged in the majority of patients taking warfarin, dabigatran and rivaroxaban, respectively. Specific measurements of DOAC plasma levels were infrequently utilised in the major haemorrhage cohort (21% of FXa inhibitors and 18.4% of dabigatran presentations) and were typically within the normally accepted on-therapy ranges.

Conclusion

ARES data provides important observational information concerning current oral anticoagulation practice. Major haemorrhage in patients taking OACs is a sentinel medical event with high 30-day mortality and prolonged hospitalisation. Standard coagulation assays are prolonged in the majority of major haemorrhage cases, although specific DOAC assays are infrequently performed. ARES is continuing to recruit 2000 patients at 20 sites across Australia and New Zealand to provide further comprehensive data that will improve anticoagulation practice.

Dr. Baker has received funding for clinical trials from Biogen, Sobi, Boehringer Ingelheim, Bayer, Shire, Pfizer, Daiichi Sankyo, Astellas and CSL Behring, has participated in clinical advisory boards for Amgen, Biogen, Shire, Boehringer Ingelheim, Bayer, Alexion Pharmaceuticals, and Pfizer. Research support from Shire, Bayer, Bristol Meyer Squibb and Alexion Pharmaceuticals. He has received conference travel support from Amgen, Novo Nordisk, Bayer, Bristol Meyer Squibb, Shire and Alexion pharmaceuticals.

126. AMR (1) – Platelet production

Hoffmeister K

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Platelets participate both in hemostasis to repair vascular damage and in inflammatory events through secretion of cytokines and growth factors. Thus platelet numbers are intricately regulated to avoid spontaneous bleeding or arterial occlusion and organ damage. Chronic inflammation is often associated with reactive high platelet numbers, and responses to acute infections may be accompanied by sudden reduction or increase of platelets (thrombocytopenia or thrombocytosis, respectively), placing platelets as reporters of disease or health.

The growth factor thrombopoietin (TPO) drives platelet biogenesis by inducing megakaryocyte differentiation. Our recent studies have identified a feedback mechanism by which clearance of aged platelets exposing galactose due to in vivo desialylation engage with the hepatic galactose-binding Ashwell-Morell receptor (AMR) to stimulate TPO synthesis via a JAK2/STAT3-dependent mechanism.

New data show that platelets isolated from patients with BCR-ABL1 (Philadelphia chromosome) negative Myeloproliferative Neoplasms (MPNs) had significantly increased terminal galactose, independent of underlying JAK2, CALR or MPL mutations. The data suggest that in patients with MPNs, despite profound thrombocytosis, platelets with high exposure of terminal galactose residues could engage with the hepatic AMR to stimulate aberrant TPO synthesis, thereby contributing to the pathology of the disease.

127. Procoagulant platelets contribute to occlusive thrombus formation and are formed via the Cyclophilin D dependent necrosis pathway

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A subpopulation of platelets, known as procoagulant platelets fulfil a procoagulant role in haemostasis and thrombosis by enabling the thrombin burst required for fibrin formation and thus clot stability at the site of vascular injury. Excess procoagulant platelet activity is linked with pathological thrombosis, however, study of their functional role has been stymied by the lack of a specific and sensitive marker. Since the morphology of this platelet subset has been shown to resemble that of nucleated cells undergoing cell death, we have employed a synthetic dithiol alkylator cell death marker, GSAO, to image procoagulant platelets. GSAO specifically and rapidly enters a subpopulation of agonist-stimulated and the combination of GSAO uptake and P selectin expression identifies the platelet subpopulation that assembles coagulation factors on the membrane surface. We demonstrate that these platelets are functionally procoagulant correlating with generation of thrombin in vitro and support fibrin formation in vivo. GSAO+ platelets are generated in vivo during formation of occlusive murine thrombi in mice and are spatially associated with sites of fibrin formation, which implies that GSAO+ platelets provide a procoagulant surface for thrombus formation. Interestingly, GSAO+procoagulant platelets were detected in an occlusive model of murine thrombosis, but not in a minimal injury, non-occlusive model. GSAO/Pselectin+ procoagulant platelets are formed independently of the Bcl-xL caspase pathway, however, pharmacological and genetic studies indicate dependence on the cyclophilin D-dependent necrosis pathway involving regulation of the mitochondrial permeability pore. Targeted deletion of Cyclophilin D in murine platelets resulted in significant reduction in GSAO+ procoagulant platelets within the murine thrombus, significant reduction in platelet thrombus volume and fibrin formation and abrogated the ability to form occlusive thrombi in response to ferric chloride stimulation. These findings illustrate an unusual scenario – platelets generated via a cell death pathway have a physiological role, and may be targetable in decrease pathological thrombosis.

128. Apoptotic death prevents the functional decline of platelets *in vivo*, but apoptosis is not required for the development of the platelet storage lesion

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The intrinsic apoptosis pathway regulates the survival of platelets, where the pro-survival protein Bcl-x_L restrains the essential death mediators Bak and Bax. Hence, platelet lifespan and platelet counts in mice are increased in the absence of Bak alone or both Bak and Bax. In this study we examined the effect of apoptosis inhibition on platelet storage *ex vivo* and the functional outcome of extended platelet survival *in vivo*. For this, control and Bak/Bax deficient platelets (*Bak*^{-/-} *Bax*^{Pf4Δ/Pf4Δ}) were stored in plasma with agitation at room temperature or at 37°C. Surprisingly, inhibition of apoptosis neither improved the viability nor reduced storage-induced platelet activation of platelets stored at room temperature. While apoptosis was triggered in platelets stored at 37°C, its inhibition did not improve platelet viability. Next, we examined the *in vivo* function of platelets unable to undergo apoptosis. *Bak*^{-/-} *Bax*^{Pf4Δ/Pf4Δ} platelets showed reduced activation, aggregation and degranulation and double-deficient mice exhibited extended bleeding time and unstable thrombi upon arterial injury. Subsequently, we explored if platelet age was a factor behind these observations. To allow direct comparisons of platelet function in *Bak*^{-/-} *Bax*^{Pf4Δ/Pf4Δ} mice and wild-type controls to be made, we synchronized platelet age to 3 days in all genotypes. Platelets were depleted *in vivo* by injection of anti-platelet serum. Newly generated platelets were collected 3 days post injection, a time-point where platelet counts had returned to normal. Remarkably, synchronizing the platelet age to 3 days in *Bak*^{-/-} *Bax*^{Pf4Δ/Pf4Δ} mice normalized platelet function *in vitro* and rescued the haemostatic defect *in vivo*. Our study shows that extended platelet *in vivo* survival results in exhausted platelets, with reduced ability to mobilize granular release. We conclude that apoptotic death prevents the functional decline of platelets *in vivo*, but apoptosis is inessential for the development of the platelet storage lesion.

129. Development of Australian guidelines for the treatment of haemophilia

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Aim

To describe the development of Australian guidelines for the treatment of Haemophilia, using a structured adaptation of the World Federation of Haemophilia (WFH) Guidelines to the local setting.

Methods

The National Blood Authority (NBA) and the Australian Haemophilia Centre Directors Organisation (AHCDO) recognised the need to develop Australian guidelines regarding the management of haemophilia due to a) the lack of existing local guidelines and b) the need for standardisation of care to optimise patient outcomes and resource utilisation. It was agreed that the WFH guidelines provided a basis upon which to develop a local document. Each chapter of the WFH guidelines was reviewed by at least two AHCDO members, who were asked to appraise the chapter, assess the need for a systematic review and draft additional content. Recommended changes to the guidelines to adapt to local practice, and suggested research priorities were discussed and agreed upon by all members of AHCDO at a face-to-face meeting. Revised chapters were then reviewed for consistency, consolidated and circulated to nominated clinical experts as well as to Haemophilia Foundation Australia in a 'critical friends' consultation process.

Results

Local guidelines regarding the management of haemophilia were successfully adapted from the WFH guidelines via a consensus process. Key research questions were identified, and as a result a systematic review of the efficacy and safety of non-steroidal anti-inflammatory drugs in the management of haemophilic arthropathy was performed with the findings incorporated within the guideline. Key practice points were identified in all areas of haemophilia care.

Conclusions

The Australian Haemophilia Guidelines are an important collaborative project between the NBA and AHCDO to help with the standardisation of haemophilia care within Australia. The guidelines will provide a reference document against which haemophilia care and resource allocation can be benchmarked. It is expected that guidelines will be published in July 2016.

130. Anticoagulation for Venous Thromboembolism (VTE) and Rates of Per Vaginal Bleeding in Pre-Menopausal Women

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Background

New oral anticoagulants (NOACs) are replacing warfarin as the choice of oral anticoagulation. While grade III/IV bleeding is reported to be similar or less than vitamin K antagonists (VKA) or enoxaparin, there are anecdotal reports of increased per vaginal (PV) bleeding in those women receiving rivaroxaban.

Method

Retrospective analysis of pre-menopausal women, aged less than 50 years, who received anticoagulation for VTE between 2013-2015. All bleeding, and specifically PV bleeding incidence, was evaluated as well as previous gynaecological issues, use of oral contraceptive pill (OCP) and other baseline characteristics. Those lost to follow-up or with a prior hysterectomy were excluded.

Results

Results of 75 women, with median age of 38 years (20 – 48) are reported, with increased PV bleeding in 16 patients (21%). Of these 16, 13 (81.2%) were on rivaroxaban and 3 (18.2%) were on warfarin. Risk of increased PV bleeding for rivaroxaban and warfarin was 37% versus 9% respectively (RR: 3.35, 95% CI 1.24 – 12.64, p=0.02). Risk of PV bleeding was associated with recent use of OCP (62% vs 35%, RR: 1.75, 95% CI 1.05-2.93, p=0.03) and prior gynaecological or ovarian issues (44% vs 17%, RR: 2.58, 95% CI 1.17 – 5.70; p=0.02). With regards to complications, rates of hormone therapy required for bleeding cessation were increased in the rivaroxaban group (23% vs 1%, RR 4.57; p=0.04). No other associations with age, weight, haemoglobin at diagnosis, type of VTE or requirement of blood transfusion or iron infusions were seen. No differences were seen in non-gynaecological grade III/IV bleeding rates between the rivaroxaban and VKA groups (0.02 vs 0.06%).

Conclusions

This audit suggests there is increased risk of PV bleeding in pre-menopausal woman receiving rivaroxaban. This highlights the need for careful assessment of gynaecological issues and OCP history before commencing anticoagulation in pre-menopausal women, particularly in relation to rivaroxaban.

131. Venous thromboembolism rates and validation of the Khorana score in diffuse large B-cell lymphoma

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Aim

The Khorana score was developed to predict risk of venous thromboembolism (VTE) in patients with cancer undergoing chemotherapy. The score involves five variables; cancer type, pre-chemotherapy platelet count, haemoglobin and/or use of erythrocyte stimulating agents, pre-chemotherapy leukocyte count (WCC), and body mass index.¹ The purpose of this study was to assess the incidence of VTE in patients with diffuse large B-cell lymphoma (DLBCL) and the predictive value of the Khorana score in VTE risk stratification in this cohort.

Methods

From an established lymphoma database we identified patients with DLBCL from 2002 to 2015 at Monash Health in Melbourne Australia and sought to identify (from clinical records) those patients with objectively confirmed VTE within the month prior to or during chemotherapy for DLBCL.

Results

There were 237 DLBCL patients with data on Khorana score available. 42.3% were females and the median age was 67.4 years. The overall prevalence of VTE was 11.4%. In patients with a high risk Khorana score the rate was 20.0%, in patients with a medium risk Khorana score the rate was 10.1% ($p=0.122$). No patients had a low risk Khorana score. While advanced Ann Arbor stage ($p=0.066$), admission to hospital within 30 days of DLBCL diagnosis ($p=0.026$), WCC $>11 \times 10^9/L$ ($p=0.004$) and Platelets $>350 \times 10^9/L$ ($p=0.009$) were associated with VTE on univariate analysis, multivariate analysis showed only patients with a poor performance status (ECOG ≥ 2) were more likely to develop VTE (HR 3.008, $p=0.013$).

Conclusion

The prevalence of VTE amongst patients with DLBCL is high (11%). Whilst the Khorana score may identify patients at higher risk the absolute risk of VTE within the lower risk group is still significant and the score is minimally discriminative. This data suggests that current VTE preventative strategies need to take into account the high prevalence of VTE amongst DLBCL patients.

132. Aberrant Haemostasis from OCP, but not Metformin use in Polycystic Ovarian Syndrome (PCOS)

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Context

PCOS affects 12-18% of women with increased risks of cardiovascular disease (CVD) and venous thromboembolic disease (VTE), related to metabolic and hormonal features, obesity and potentially a hypofibrinolytic state, possibly exacerbated by current PCOS treatments.

Aim

To investigate and compare haemostatic impacts of pharmacological treatments and explore relationships with hormonal and metabolic variables in PCOS.

Method

A mechanistic sub-study using biobanked samples from a six month randomized controlled trial of pharmacological treatments on pro- and anti-thrombotic markers and overall haemostatic activity. Participants were recruited from the community and studied in an academic centre and included women 19-49 years with a BMI >26 kg/m² with PCOS (n=60). Subjects were randomised to either metformin, high-dose oral contraceptive pill (OCP) or low-dose OCP + spironolactone. Plasminogen activator inhibitor 1 (PAI-1), asymmetric dimethylarginine (ADMA), prothrombin fragments 1 and 2 (PF1 & 2), plasminogen, tissue plasminogen activator (tPA), thrombin activatable fibrinolysis inhibitor (TAFI) and thrombin generation (TG), hormonal and metabolic markers were measured.

Results

PAI-1 activity fell in all groups (p<0.010), ADMA fell in high-dose OCP (p=0.006), PF1 & 2 increased with metformin and high-dose OCP (p<0.003), TG rose (p<0.005) and tPA fell in both OCP groups (p<0.011), Plasminogen increased in all (p<0.020) and TAFI increased after high-dose OCP (p<0.001).

Conclusions

Endothelial function improved with metformin and high-dose OCP. OCP increased coagulation and induced a hypofibrinolytic state, which was not apparent with Metformin. In PCOS, a high risk group for VTE and CVD, further research on thrombotic impacts of common pharmacological treatments is needed to inform clinical practice.

133. The risk of inhibitor development among congenital haemophilia patients who receive factor concentrate replacement via continuous infusion

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Aim

To evaluate the risk of inhibitor development among haemophilia patients who receive factor concentrate replacement via continuous infusion (CI) for surgery or major bleeds.

Method

A multicentre, retrospective cohort study. Prospectively recorded databases from two large international haemophilia treatment centres that practise the administration of factor concentrate via CI for patients with haemophilia undergoing major surgeries or major bleeding episodes were examined systematically to evaluate if it is associated with an increased risk for the development of inhibitors. Patient demographics (disease severity, factor concentrate exposure days (ED), and genotype), and other important variables collected include inhibitor testing pre- and post- receipt of infusions, duration of CI and concentrate type. The rate of inhibitor development was compared against historical controls.

Result

Among 168 & 66 patients with haemophilia A (HA) and B (HB) (of all severity) respectively, who received 485 CI, 5 patients was identified with inhibitors.

Four HA patients developed inhibitors (2.4%): 1) three patients with mild HA, ED<100, the mutation [c.6506G>A (p.Arg2150His)] and prior history of inhibitor associated with bolus treatment that had resolved, including two patients with high-titre inhibitors (≥5.0 BU/mL), 2) one severe HA (ED>150) with low-titre inhibitor (<5.0 BU/mL).

Only one patient with severe HB (ED>150) developed a low-titre inhibitor (1.5%).

All developed spontaneous bleeds associated following the inhibitor.

This overall inhibitor rate of 2.14% is lower than historical reports.

Conclusion

The administration of factor concentrate via CI among patients with haemophilia for surgery or major bleeds is associated with a low rate of inhibitor development in this large cohort of patients. However, further studies are needed to explore the risk among non-severe HA patients with ED<100, and “high-risk” genotypes.

134. Preoperative assessment of bleeding risk – how are we really performing?

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Introduction

Patients undergoing surgery should have a bleeding risk assessment performed. Published guidelines advise that a bleeding history should be taken and that routine coagulation screening is inappropriate¹⁻³.

Aim

This audit was undertaken as the initial phase of a clinical practice improvement project to optimise bleeding risk assessment in patients undergoing elective surgery at a tertiary referral hospital.

Methods

Following ethics approval, 121 medical records of patients attending a surgical pre-admission clinic (PAC) in a single month were audited. Demographic and clinical data were recorded including number of days between PAC attendance and planned surgery, patient self-completion of health questionnaire, documentation of bleeding history, record of anti-platelet and anti-coagulant therapy, and coagulation screening tests requested and results.

Results

The median age of patients was 66 years (range 9 – 87) and 59% were male. The median number of days between PAC attendance and surgery was 6 days (range 0 – 126) and 66/121 patients (54%) were seen in PAC one week or less prior to surgery. 19% patients did not complete their health questionnaire and of those who did, 6/98 (6%) indicated yes to a bleeding history/tendency. Only 11% patients had documentation that a medical officer had enquired about bleeding history in any way. Coagulation testing (i.e. PT, APTT, INR) was requested in 81/121 (67% patients) and of these, coagulation tests were abnormal in 14.8%. Response to abnormal coagulation testing was varied and not always acted upon. Notably, 51/121 (42%) patients were taking anti-platelet and or anti-coagulant medication and of these, 15/51 (29%) were told to continue their medications and not to cease pre-operatively.

Conclusion

Assessment of bleeding risk is poorly documented with an over-reliance on coagulation testing. Plans are underway to implement a structured bleeding history in PAC with a long-term view to eliminate inappropriate coagulation screening.

135. Obstetric DIC

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Disseminated intravascular coagulation (DIC) is a clinipathological syndrome characterised by systemic activation of coagulation that results in the generation of fibrin clots with consumption of platelets and clotting factors. It can result in organ failure due to microvascular thrombosis and clinical bleeding can occur because of the acquired coagulation deficiencies. It occurs in a wide range of disorders including malignancy, and sepsis. It is also a major feature in severe obstetric complications such as placental abruption and amniotic fluid embolism, both of which have major haemorrhage as a key clinical feature, often at presentation. No single test can be used to diagnose DIC and the diagnosis is based on clinical symptoms and signs supported by laboratory investigations. Important routine laboratory tests that demonstrate consumption of coagulation factors i.e activated partial thromboplastin time (aPTT), prothrombin time (PT) and development of thrombocytopenia and low fibrinogen. A key feature of DIC is demonstration of activation of fibrinolysis with formation of fibrin(ogen) degradation products and D-dimers. In obstetrics development of early DIC is a frequent early finding in major obstetric haemorrhage and clinicians must maintain a high suspicion of DIC in the face of severe bleeding.

Scoring systems developed by the ISTH to aid diagnosis of DIC are not helpful in discriminating obstetric DIC. Thrombocytopenia is not an uncommon finding in an otherwise uncomplicated pregnancy –due to gestational thrombocytopenia or ITP; D-dimer levels are raised in almost all pregnant women by the 3rd trimester so using these tests would overdiagnose many women. Conversely, waiting for fibrinogen levels to drop below 1g/L before making a diagnosis could lead to a delay in diagnosis given that normal levels in pregnancy are in the range of 4-6g/L in pregnancy. Other parameters such as a fall in the fibrinogen level or use of point of care testing such as ROTEM or TEG may be of greater use in this clinical setting.

136. The protein C pathway and histone participation in DIC, thrombosis, organ failure and death

Esmon C

Oklahoma Medical Research Foundation, Oklahoma, US

The protein C pathway and histone participation in DIC, thrombosis, organ failure and death
Sepsis or trauma can induce coagulopathies that involve coagulation with simultaneous bleeding. The causes are numerous. In sepsis, the release of high levels of inflammatory cytokines like TNF alpha and IL-1 beta trigger cells to express adhesion molecules. In addition, the cells synthesize and release tissue factor. In both trauma and sepsis, neutrophils release neutrophil extracellular traps (NETS) composed of DNA and histones. NETS activate platelets and can lead to thrombocytopenia. The histones in the NETS activate toll-like receptors 2, 4 and 9, contributing to increased inflammatory cytokine elaboration. They also activate a calcium channel in the endothelium, transient receptor vanilloid 4 (TRPV4), leading to vasodilation. The released histones kill endothelium, at least in part by elevating intracellular calcium. The histones also bind to polyphosphate forming a complex that potently enhances coagulation. Finally histones bind to thrombomodulin, shutting down protein C activation and contributing to microvascular coagulation. Overall, inhibition of histones reduces thrombin generation in mice challenged with LPS more than 50% and protects against death.

137. DIC in cancer

Brighton T

SEALS, NSW, Australia

Disseminated intravascular coagulation (DIC) is a pathologic condition characterised by pathologic activation of coagulation pathways that results in widespread thrombosis, depletion of platelets and coagulation factors and excessive thrombolysis. Clinically this manifests in haemorrhage, thrombosis, and/or multiorgan failure. Malignancy can produce acute DIC (e.g. APL) but more typically is associated with chronic DIC seen in patients with advanced mucin-producing tumours (pancreatic, gastric, ovarian, and breast). While chronic DIC is stimulated by a "cancer pro-coagulant" increasing evidence suggests that damage-associated pattern molecules (DAMPs, also called "alarmins") including cell-free DNA (cfDNA) and DNA-binding proteins play a critical role in the pathogenesis of DIC. DAMPs are released from dying cells or secreted from immune cells in response to infection or tissue injury. In this presentation, cases of cancer associated DIC will be reviewed to illustrate the pathogenesis, clinical manifestations, diagnosis, management and prognosis.

138. Disaster preparedness and recovery

McMillan A

Department of Health & Human Service, VIC, Australia

Abstract to come

139. Disasters I have contributed to

Waters N

Monash University, Melbourne, Australia

“Everyone has a plan until they get punched in the mouth” (Mike Tyson)

Disasters come in many forms. Each disaster is unique with its own set of constraints and confounders, issues, problems, potential solutions and outcomes. Consequently each response must be tailored to the specific needs and the context in which it occurs.

However, various factors, including health and community infrastructure (such as facilities and transport), population and geography, and their underlying vulnerabilities or resilience, may have significant effects on disaster impacts and recovery outcomes. Disaster planning and preparedness are vital for prevention, impact minimisation and facilitation of rapid recovery. Disaster responses will ideally lead to sustainable improvement and provide a platform for increased resilience for further long term development.

This presentation will explore three different disasters, in Aceh (Indonesia), Benghazi (Libya) and Vanuatu, and explore themes including leadership, networks, flexibility and resources, focussing on transfusion issues.

140. Reflections on the Christchurch earthquake

Flanagan P

New Zealand Blood Service, Auckland, New Zealand

On 22nd February 2011 Christchurch, New Zealand's second largest city, suffered a major earthquake. This followed an earlier major earthquake in September 2010. Both earthquakes resulted in significant physical damage to buildings in the city. The February earthquake, in contrast to the earlier one, was however also associated with significant loss of life and injury with 181 deaths. More than 12000 aftershocks had occurred by February 2013 and new aftershocks continue on a regular basis even in 2016.

New Zealand Blood Service (NZBS) has a well-developed disaster management plan. This utilises the Co-ordinated Incident Management System (CIMS). This mechanism is used widely by public health agencies across New Zealand allowing a well-co-ordinated sector-wide approach to disaster management. Close integration of the Blood Service plan with the wider health sector is required for effective management of both the acute disaster and subsequent recovery plans. Effective cross sector communication strategies are also essential.

The Christchurch hospital was situated within the area suffering maximum damage. Inevitably this complicated the initial response to the disaster. Staff in the hospital Blood Bank within the hospital worked in difficult conditions. Many were concerned for the safety of their families and the impact of aftershocks was particularly unnerving for many. A group O bank was used to support patients in the early hours following the February quake. Blood component stocks were good but initial problems were encountered in easy movement of stock into the disaster area. Significant numbers of casualties were admitted to the hospital mainly with fractures and crush injuries. The demand for blood components was however low.

Considerable efforts were devoted to managing the influx of volunteers in the early days following the earthquake thus avoiding any unnecessary increase in overall collection levels. A number of approaches were utilised including social media and close involvement of the National Crisis Co-ordination Centre.

The Christchurch Blood Centre was closed for varying periods following each of the major events. This reflected concerns relating to the safety of the buildings, availability of donors and disruption to automated equipment. The NZBS infrastructure incorporates extensive risk mitigation mechanisms that enabled supply to all hospitals to be maintained during the period of closure of the Christchurch Blood Centre. Nonetheless the series of earthquakes resulted in a major review of NZBS facilities. The key outcome of this was a decision to establish a model whereby two blood centres exist each of which has the ability to process the national requirement for blood and blood products. This is being achieved by the establishment of a new blood centre in Christchurch, opened in November 2014, and a major refurbishment of the centre in Auckland.

The disaster response to the earthquakes was complicated by the fact that the staff responsible for responding to the civilian emergency were also themselves 'casualties'. The psychological impact of this was significant and far exceeded the physical impact of the event highlighting the importance of people management and support during major disasters.

141. Pathophysiology of Hemolytic Transfusion Reactions

Spitalnik S

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Antibody-mediated hemolytic transfusion reactions were first described at the dawn of transfusion medicine. Indeed, a major focus of the modern practice of transfusion medicine has been to improve our methods and understanding of immunohematology to prevent such events. However, despite our best of efforts, incompatible transfusions still occur, resulting from medical errors or the inability to obtain compatible red blood cells for patients who are alloimmunized against multiple antigens. Because incompatible blood transfusions are potentially lethal, it is not ethical to conduct most types of prospective, deliberately designed, controlled studies on human subjects. Thus, our understanding of hemolytic transfusion reactions is based on clinical case reports and case series, animal models, cell culture models, inferences from related human pathologies, and studies using small volumes of transfused incompatible red blood cells. Over the past 50 years or so, substantial new knowledge has been obtained regarding the mechanisms of hemolysis, the metabolism of hemolysis by-products, and the effects of both on transfusion recipients. This session will attempt to summarize these findings into a coherent pathophysiological framework.

142. Paediatric transfusion reactions and alloimmunisation

Luban N

Children's National Medical Center, Washington, US

This session will focus on unique aspects of transfusion reactions in the infant and child, both presentation and evaluation. A specific focus will be on understanding what triggers the development of alloimmunization in chronically and acutely transfused pediatric patients and methods to abrogate the development of alloimmunization.

143. Investigation of complex Rh antibody problems in pregnancy

Thornton N

NHS Blood and Transplant, Hertfordshire, UK

The Rh system is the most complex of the blood group systems and after the ABO system, is considered the most clinically significant with regard to transfusion and pregnancy. Investigation of antibodies to high frequency antigens of the Rh system can be especially complicated in pregnant Sickle Cell Disease patients. The use of high throughput genotyping platforms to predict Rh antigen status is now widely available, but due to the genetic complexity of the Rh system, is not without limitations, especially when dealing with rare variant alleles. It is important to understand the limitations in order to use an optimal combined serological and molecular approach to solving the most difficult Rh antibody problems.

144. Caring for pregnant women for whom transfusion is not an option

Kidson-Gerber G

Prince of Wales Hospital, Sydney, Australia

Postpartum haemorrhage is the leading cause of maternal mortality and morbidity globally. Obstetric bleeding can be catastrophic and management is challenging, involving a coordinated multidisciplinary approach, which may include blood products. In settings where blood transfusion is not an option, either because of patient refusal (most commonly in Jehovah Witnesses) or because of unavailability of blood, management becomes even more challenging. Observational studies have demonstrated an association between refusal of blood products in major obstetric haemorrhage and increased morbidity and mortality, with substandard clinical care contributing to these poorer outcomes. This oral presentation draws upon evidence in the literature, physiological principles and expert opinion for strategies and guidance to optimise the outcomes of pregnant women in whom blood transfusion is either refused or impossible. The importance of a multidisciplinary antenatal and perinatal management plan, including optimisation of haemoglobin and iron stores pre-delivery, blood loss minimisation, early haemorrhage control and postpartum anaemia treatment, will be discussed.

145. Single dose Anti-D prophylaxis in pregnancy: is it time to change?

Pennell C

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Background

Despite a national program of anti-D prophylaxis, sensitisation still occurs in ~0.33% of pregnancies due to: 1) lack of prophylaxis (43%); 2) non-obstetric sensitisation (25%); and 3) sensitisation despite adequate prophylaxis (32%). At a global level there are two approved regimens for providing antenatal prophylaxis – double-dose of 500IU or more at 28- and 34-weeks' GA; and single-dose of 1500IU at 28-weeks' GA. To date, compliance and maintenance of circulating anti-D levels at delivery between the regimens have not been well studied.

Aim

To assess compliance and maintenance of circulating anti-D levels at delivery comparing single- and double-dose regimens of prophylaxis.

Method

A RCT (n=273) was performed comparing a single 1500IU dose of anti-D at 28-week to the current Australian regimen (28- and 34-week 625IU regimen). An antibody screen at delivery assessed the circulating anti-D levels. Analyses were performed based on intention to treat (ITT) and treatment received (TR). Appropriate statistical tests were used to evaluate differences in compliance, detectability of residual circulating anti-D at delivery, and maternal and neonatal outcomes. Multivariate logistic regression assessed factors contributing to undetectable anti-D at delivery.

Results

Demographic, obstetric and neonatal outcomes were similar between groups. No women became sensitised during the study. Based on analyses by ITT and TR, the single-dose group had a higher proportion of undetectable anti-D at delivery (ITT OR 5.0; 45.4% vs. 14.2%, $p<0.001$). Compliance was improved in the single-dose group (81.2% vs. 60.7%, $p<0.001$). Time elapsed since last dose and third trimester weight were significant predictors for undetectable anti-D at delivery. Multivariate regression indicated anti-D regimen group was not a significant predictor for undetectable anti-D at delivery.

Conclusions

Despite improving compliance, the single-dose regimen resulted in a nearly half of the Rh-negative women having no circulating anti-D at delivery. Translating recent WA data to an Australian context, a single dose regimen could result in an additional 580 sensitisations per year.

146. 100 million RBC transfusions each year...but do they deliver oxygen?

Dzik W H

Massachusetts General Hospital, Massachusetts, US

There is ample evidence from laboratory studies that erythrocytes undergo structural, biochemical, and metabolic changes during refrigerated storage prior to transfusion. These changes have been collectively referred to as “the storage lesion”, and considerable controversy has surrounded whether or not the transfusion of stored erythrocytes are associated with clinical harm.

3 major prospective trials (ARIPi, ABLE, RECESS) tested whether individuals randomized to receive prolonged-storage RBCs experienced more adverse effects compared with those assigned to receive short-storage (“fresh”) RBCs. These studies gave negative outcomes and could not demonstrate a higher frequency of mortality or major morbidity among recipients of prolonged-storage RBCs. However, the demonstration of no increase in adverse events does NOT address whether or not stored erythrocytes actually deliver oxygen to tissues.

We conducted a prospective, randomized trial among children with severe anemia (hemoglobin <5 g/dL) who also had lactic acidosis (lactate > 5mM). Lactic acidosis in these patients was due to inadequate global tissue oxygenation. The patients did not have other medical conditions that can cause lactic acidosis, eg, shock, hypoxia, trauma, sepsis, hepatic failure, vascular disease. Patients were randomly assigned to receive the same dose of RBC that had been stored either for <10 days or >25 days. The primary outcome was the kinetics of clearance of lactic acidosis. In addition, we measured cerebral tissue oxygenation using near-infrared spectroscopy. We also monitored clinical and laboratory outcomes.

290 children with extreme anemia and lactic acidosis were studied. They were evenly divided between the two RBC-storage groups. The results, which will be presented at the lecture, provide direct evidence regarding whether or not erythrocytes transfused after prolonged storage function as well as shorter-storage erythrocytes for the delivery of oxygen to patients in urgent need of tissue oxygenation.

147. Paediatric PBM

Luban N

Children's National Medical Center, Washington, US

Studies have established that patient blood management programs can decrease the need for allogeneic blood transfusions, lower health care costs and ensure that there is adequate inventory for patients who require blood and blood products. Many processes used to provide blood / blood products for neonates have been utilized for years but never classified as PBM. In this session, we will review the implementation of pediatric PBM and discuss the evidence that supports or refutes these initiatives.

148. Module 6: Neonatal and Paediatric Patient Blood Management

Liley H

Mater Misericordiae Ltd, Brisbane, Australia

Module 6 Neonatal and Paediatrics (2016) is the most recently completed module of the suite of 6 evidence-based National Blood Authority Patient Blood Management (PBM) guidelines. Specific guidelines are needed for these age groups because there are considerable physiological differences between neonates and children at different developmental stages, and between children and adults. Transfusions can be lifesaving and improve health, but can also have adverse consequences. Both benefits and adverse consequences may be life-long.

A Clinical/Consumer Reference Group (CRG) was established comprising experts in; fetal-maternal medicine, neonatology, paediatric haematology, paediatric anaesthesia, intensive care, cardiac surgery, oncology, haemoglobinopathies, nursing, patient blood management in addition to a consumer and an indigenous representative. The CRG formulated clinical questions examining: the effect of red blood cell (RBC) and other blood component transfusion on patient outcomes; the effect of non-transfusion measures to increase haemoglobin on patient outcomes, and the effect of strategies to minimize blood loss and/or reduce RBC transfusion. The search strategy included electronic database search of EMBASE, Medline, the Cochrane Library Database, the Health Technology Assessment and guidelines websites, clinical trial registries and Pre-medline for the years 1995 and 2014 inclusive. The National Health and Medical Research Council (NHMRC) guideline development process was used to critically appraise the evidence found and to generate the guidelines. They underwent peer review, public consultation, and independent assessment according to the Appraisal of Guidelines for Research & Evaluation II. The guideline's recommendations were approved by the NHMRC on 21 March 2016.

Twelve evidence-based recommendations for or against the use various interventions in neonatal and/or paediatric patients were developed. These included RBC transfusion in critically ill patients and sickle cell disease (SCD), erythropoietin-stimulating agents in preterm infants, hydroxyurea in SCD, fresh frozen plasma based primes and recombinant factor VIIa in cardiac surgery, intravenous immunoglobulin for Rh haemolytic disease of the fetus and newborn, and prevention of hypothermia and anti-fibrinolytics in surgical patients. Forty practice points were developed when there was insufficient evidence to generate an evidence-based recommendation. A further 37 expert opinion points were developed on topics where a systematic review was not undertaken. All guidance was developed by CRG consensus.

Module 6 also identified areas where further evidence is needed to guide clinical practice. Where evidence was found, most studies addressed only short-term outcomes, which can be insufficient to determine the overall balance of risks and benefits. The decision to transfuse a neonate or child should consider specific patient circumstances, clinical condition, response to previous transfusion and the full range of alternatives, balancing the evidence for benefit against the potential risk.

149. International neonatal and paediatric Patient Blood Management guidelines

Crichton G

Royal Children's Hospital, Melbourne, Australia

International neonatal and paediatric Patient Blood Management guidelines

Neonatal and paediatric transfusion recipients survive longer than adults receiving transfusions and therefore over their lifetime have the highest potential for harm secondary to transfusion. Neonatal and paediatric patients have specific transfusion needs, and vulnerabilities that differ from adults. There remains a paucity of literature in this area and many transfusions given to children are not supported by evidence.

The decision to transfuse a neonate or child needs to be carefully considered and guidelines therefore help inform clinicians of best practice. Clinical practice guidelines have the potential to improve clinician's evaluations and patient healthcare and quality outcomes. However, they are reliant on a meticulous and transparent development process. Transfusion guidelines, are often based on clinician consensus and historical experiences rather than being evidence based.

Two very recent international guidelines specific to paediatric and neonatal patient blood management and transfusion practice have been published. These guidelines were produced independently, using different methodological approaches, undertaken by different authors and stakeholders. Both guidelines are however, likely to be limited by the quality of literature of evidence available.

There is a well-founded and recognised limitation that clinical research trials tend to focus on adults and that randomised controlled trials are more frequently planned in adult populations. Yet, the pathophysiology of children and especially neonates makes extrapolations from the adult literature much more challenging. Another limitation is that trials conducted in neonatal and paediatric patients frequently focus on short-term outcomes and long-term outcomes are not explored.

In this session, current evidence from neonatal and paediatric blood transfusion and patient blood management guidelines will be reviewed with exploration of any variability.

150. The immunohaematology toolbox for solving complex antibody cases

Thornton N

NHS Blood and Transplant, Hertfordshire, UK

The resolution of complex antibody cases requires a bespoke analytical process made up of many different elements, which all add an important piece to the puzzle. Serological techniques remain the essential tools required for resolving antibody problems but there are non-serological elements that are also important, especially in the initial stages of an investigation. When investigating complex cases, routine serological techniques may not be enough to reach a resolution. It is then necessary to turn to supplementary manual serological techniques and also DNA based tests, which now play an important role in helping to resolve the most complex of cases. Knowledge of blood groups and antibody characteristics is important for being able to recognise clues and ensure that decisions in the antibody identification process are made in a logical and informed way, to ensure minimal delay to patient care.

151. Dealing with challenging antibodies

Rushford K

Monash Health, Melbourne, Australia

Monash Health is a tertiary referral centre covering a large geographic area in south-eastern Melbourne. Our patient population includes trauma, haematology-oncology, obstetrics, paediatrics, neonates, and a large number of transfusion-dependent patients with myelodysplasia, thalassaemia and other haemoglobinopathies. Our transfusion service performs 52,000 antibody screens and dispenses over 20,000 units of red cells annually from a group of four centrally-managed laboratories. It deals with a complex array of patients who sometimes have antibodies that make management difficult.

This presentation will provide an overview of some of the challenging antibodies seen and complex situations we encounter. Our busy routine laboratory has to deal with both organisational (including logistical, financial and staffing) and serological challenges. Procedures need to suit the multi-skilled generalist who is providing blood for a bleeding patient. Patients with autoantibodies and underlying alloantibodies require more complex testing only performed in the central laboratory, and referral of more complex cases from branch laboratories to the central laboratory and/or the Australian Red Cross Blood Service Reference Laboratory can create challenges in the timely provision of blood products. Good communication is required between laboratory staff, clinical haematology, the Blood Service and the treating clinical team to ensure the best outcomes for our patients.

152. Antibody soup: Challenging transfusion management issues in thalassaemia major

Oh D

Monash Health, Melbourne, Australia

Beta thalassaemia major is an inherited condition associated with high morbidity due to ineffective erythropoiesis, extramedullary haematopoiesis and iron overload. Although clinical manifestation and severity of this condition is heterogeneous, most patients require chronic transfusion for life.

We present a case of an alloimmunised beta thalassaemia major patient. There is a long and extensive history including multiple alloantibodies and hyperhaemolysis. Transfusion support has been difficult over many years, both in terms of finding compatible blood, and tolerance of the infusions, and he is not on a chronic transfusion program. He has received IVIg and rituximab. Now in his late 50s, the patient is facing multiple medical problems related to his disorder and its therapy, including cardiac disease, endstage renal failure and worsening anaemia. We will outline some of the challenges facing clinicians and laboratory scientists managing patients with complex immunohaematologic problems, and also address issues relevant to ageing patients with haemoglobinopathies.

153. How to get your work published in a medical journal

Dzik W H

Massachusetts General Hospital, Massachusetts, US

While it may help to have some illicit information about the Editor in Chief of a medical journal that he or she wants desperately to keep out of the public eye, most of us need to rely on the merits of our work in order for it to be accepted for publication in a peer-reviewed medical journal.

In this Master Class session, I hope to share lessons learned over the years as an author (sometimes a successful one, sometimes not!), a peer-reviewer, and a journal Editor. Items to cover include selecting the right journal for your topic, what not to say in a cover letter, the importance of conclusions versus speculations, the virtues of brevity and clarity, surviving the peer review process, and do's and don'ts of the all-important revision manuscript. Issues of copyright laws, plagiarism, and scientific misconduct will be touched upon as well. The session is meant to be interactive so bring your problems and ideas. The goal will be to better prepare you for your next effort at academic publication.

154. Risk factors for 30-day mortality in critically bleeding patients requiring massive transfusion within 24 hours of admission to the Intensive Care Unit

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Aim

To describe risk factors associated with 30-day mortality in critically bleeding patients requiring massive transfusion (MT) within 24 hours (h) of admission to the intensive care unit (ICU) in Australia and New Zealand (ANZ).

Method

A probabilistic record linkage between the ANZ Massive Transfusion Registry and ANZ Intensive Care Society Adult Patient Database was conducted to obtain a dataset containing characteristics, laboratory, transfusion and ICU data of patients requiring MT (≥ 5 units red blood cells in 4h) in 2011-2015. Mortality information was collected from the Australian National Death Index and NZ Ministry of Health. Survival analysis was used to model the associations between patient characteristics and mortality.

Results

A linked dataset contained records of 2,317 MT patients from 18 participating hospitals. More than half (55%) of these patients had MT within 24h (prior or after) of the ICU admission. 76% of MTs occurred prior to ICU admission with a median [IQR] time between MT and ICU admission of 4.8 [2.4-7.2] h. Average age of patients admitted to ICU was 63 years, 65% of them were male, and 66% had ≥ 2 comorbidities. Cardiothoracic surgery was the most frequent bleeding context (24%), followed by trauma (17%). The 30-day mortality was 20%. The following factors were associated with increased 30-day mortality:

- older age
- more comorbidities
- vascular surgical and gastrointestinal bleeding
- elevated acute physiology and chronic health evaluation (APACHE III) score
- increased volumes of blood products transfused within 4-24h from commencement of the MT.

Conclusion

This study showed that critically ill and older patients with more comorbidities and requiring more blood products were at greater risk of death within 30 days. Studies are underway to understand how management of MT in the ICU may influence patient outcomes across different bleeding contexts.

This abstract was written "on behalf of the Australian and New Zealand Massive Transfusion Registry Steering Committee

155. Transfusion-Related Acute Lung Injury (TRALI): A Review of Reported TRALI Cases in Queensland, Australia, between 1999 and June 2016

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¹ *Australian Red Cross Blood Service*, ² *Royal Brisbane and Women's Hospital*, ³ *Mater Health Services*

Aim

Transfusion-related acute lung injury (TRALI) has become one of the leading causes of transfusion-related mortality worldwide, despite likely under-diagnosis and under-reporting. This study aimed to review patient characteristics, donor characteristics and blood product factors associated with TRALI cases.

Method

A retrospective audit evaluated all cases reported in Queensland to the Australian Red Cross Blood Service for TRALI investigation between 1999 and June 2016. Cases were categorised as "TRALI", "possible TRALI" and "not TRALI" according to the 2004 Canadian consensus criteria.

Results

The study involved 78 patients from 23 hospitals with 39 TRALI cases, 17 possible TRALI cases, 11 cases deemed not TRALI and 11 cases excluded due to insufficient clinical information. Reporting rates remained steady despite the introduction of risk reduction strategies within this timeframe. The majority of TRALI and possible TRALI cases were seen in patients with haematological malignancy (23%), surgery (14%) or liver disease (13%). Most cases (75%) occurred within 1 hour of transfusion completion. Male and female donors equally contributed associated components (49% vs. 51%). Red cells were transfused in 39 cases, platelets in 19 cases and plasma products in 24 cases; many cases involved multiple components. Of the 39 TRALI cases, 13 had laboratory findings [positive granulocyte crossmatch or identification of a donor antibody (HLA or HNA) to a cognate recipient antigen] suggestive of antibody-mediated TRALI. The 26 cases where an antibody was not identified were, on average, associated with older components.

Conclusion

Laboratory investigations are integral to donor management and can support the clinical diagnosis of TRALI. Only one-third of TRALI cases appeared to be antibody-mediated. This highlights that further investigation into component age and biological response modifiers is required to provide insight into the pathophysiology of the remaining cases and to help inform the development of further TRALI risk-reduction strategies.

156. Determining the frequency, costs and outcomes of red blood cell transfusion in hospitalised patients: a pilot study

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Aim

Pilot study to assess relationships between red blood cell (RBC) transfusion, outcomes and costs in hospitalised patients.

Method

Data on patient characteristics, ICD-10-AM diagnosis codes and ACHI procedure codes, transfusion, costs and hospital outcome for all patients admitted to the Alfred Hospital, Melbourne, between 2010 and 2014 were obtained from relevant departments and merged to create the complete dataset. Variables were created to define patients who had received a transfusion of one or more units of RBCs during their admission and patients who developed infectious complications during their admission.

Results

The final dataset contained records on 105,768 patients with 283,346 admissions. Of 79,680 overnight admissions (≥ 1 night), 11.1% received a transfusion of RBCs. During their admissions, transfused patients were more likely to be admitted to ICU (36.6% vs 7.2%), had higher rates of infectious complications (27.8% versus 4.9%), higher mortality (7.0% versus 1.9%) and longer hospital stays (median 12 versus 5 days). The median [IQR] total hospital cost of overnight admissions who received a RBC transfusion was \$29,472 [14,002-\$60,264] with a mean (sd) cost of \$49,619 (\$63,189), compared to a median of \$9,274 [\$5,531-\$16,625] and a mean of \$14,079 (\$15,850) in non-transfused patients. Infective complications, transfusion, younger age and mortality were all shown to be independently associated with higher total admission costs.

Conclusion

We have developed a methodology for comprehensive analysis of the cost of transfusion in the Australian hospital setting. Transfusion was associated with substantial hospital costs over and above the cost of the product transfused; however these associations do not imply causation. Further research will investigate costs and outcomes across different products, procedures and clinical conditions after adjusting for co-morbidities and illness severity. Increased awareness of transfusion costs and consequences will assist in implementation of patient blood management initiatives.

This research was supported by the National Blood Authority (NBA). The NBA had no role in analysing the data or preparing the abstract. All information in the abstract is solely the responsibility of the authors and do not reflect the views of the NBA. ||COI relates to the abstract Determining the frequency, costs and outcomes of red blood cell transfusion in hospitalised patients: a pilot study

157. Gastrointestinal bleeding-red blood cell transfusion and patient blood management practice in a metropolitan hospital

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Aim

Upper Gastrointestinal bleeding can be life threatening however a recent randomised trial has demonstrated a restrictive approach can increase survival and reduce re-bleeding rates (Villanueva et al 2013.) An audit was carried out over an 11 month period investigating red cell use in patients presenting to the emergency department with Upper Gastrointestinal bleeding.

Method

53 patients with 72 presentations admitted and transfused under the Gastrointestinal team were filtered from global blood use report. Admission, pre and post transfusion haemoglobin were obtained as well as number of transfusions. Haemoglobin increments were initially used and then patient medical records to ascertain need against a local guideline which recommends a single unit approach for patients not actively bleeding. Iron studies and iron infusions were also collated for these patients.

Result

The table demonstrates the pre and post average haemoglobin level and number of red cell transfusions for number of units transfused.

	Pre Transfusion Haemoglobin g/L	Post Transfusion Haemoglobin g/L	Red Cell Episodes
1 unit Transfusion	76	83	68
2 unit Transfusion	69	83	37
3 unit Transfusion	67	84	7
≥4 unit Transfusion	66	93	10

95% of the red cell transfusions were deemed necessary, with most outside the guidelines transfusing multiple units without review.

20 patients out of 28 tested had a Ferritin level <100 ug/L and received an iron infusion, there were 25 patients that did not receive anaemia screen during admission

Conclusion

This audit demonstrates restrictive transfusion practice for the majority of patients admitted with upper gastrointestinal bleeding. Red cell transfusions that may have been avoided include transfusion of two or three units without review between units. The majority of patients tested had iron deficiency but almost half of the patients did not have these tests during admission.

158. The Australian haemoglobinopathy registry: aims, plans and pilot data

Crighon G^{1,2,3}, Bowden D⁴, Cole C^{5,6}, Cole-Sinclair M⁷, Chee M¹, Finlayson J⁵, Greely C⁴, Greenway A², Hogan C³, Kaplan Z⁴, Kidson-Gerber G⁸, Pasricha S⁹, Teo J¹⁰, Vadolas J¹¹, Wood E^{1,4}, Ho J^{12,13}, on behalf of the Haemoglobinopathy steering committee

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Aim/Background

Haemoglobinopathies are inherited disorders of haemoglobin (Hb) characterised by quantitative or qualitative defects in Hb synthesis, and include β thalassaemia and sickle cell disease (SCD).

The Australian Haemoglobinopathy Registry is a national collaborative project under the auspices of the Transfusion Outcomes Research Collaborative and participating hospitals, and supported by community organisations, with the aims of: i) better defining the prevalence, natural history and clinical outcomes of patients with haemoglobinopathies in Australia, ii) exploring variation in practice, iii) benchmarking outcomes nationally and internationally, and acting as a resource for clinical studies

Method/Results

Data from the first 219 patients from 3 pilot sites in Melbourne and Sydney show that 129 (60%) have β thalassaemia major or β thalassaemia intermedia with major phenotype, 10 (4.5%) HbE/ β thalassaemia compound heterozygotes, 21 (10%) HbH disease, and 48 (22%) with HbSS, HbSC or HbS/ β thalassaemia. Median age for β thalassaemia patients is 38.9 years (range 2.7-64). All β thalassaemia patients receive top-up red cell (RBC) transfusions; median 3 units (range 1-4), every 3 weeks (range 2-5).

Patients with HbSS have median age 16.3y (range 2.1-55). 52% SCD patients receive either regular or intermittent RBC transfusion support, a median of 3 units (range 1-6) every 4 weeks, and 63% were by RBC exchange.

RBC alloantibodies were identified in 25% β thalassaemia and 45% SCD patients.

In terms of access to care, 32% patients lived within 10km of their treatment centre, 22% between 11-20km, 40.6% between 21-50km and 4.4% lived more than 50km away.

Conclusions

Pilot data from the Australian Haemoglobinopathy Registry demonstrate considerable RBC transfusion requirements and high rates of alloantibodies. Some patients travel considerable distances to receive specialist care.

The registry pilot has been well received by patients, community groups and clinicians. Participation from new sites and research proposals for the registry are very welcome.

159. Preliminary data on cost of IVIg & SCIg treatment in adult Australian patients

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Aim

To compare the immunoglobulin (Ig) product and associated disposables cost of intravenous Ig (IVIg) versus subcutaneous Ig (SCIg) treatment in adult immunodeficiency patients.

Method

We compared the cost of the final 12 months of IVIg treatment with the cost of the initial 12 months of SCIg treatment in 13 adult patients who switched from hospital based IVIg infusion to self administration of SCIg at home. Patients with primary (PID) or secondary (SID) immunodeficiency treated at the Sunshine Hospital and Health Services (SCHHS) were studied. Medical charts provided dosage data, pathology reports provided quantity of Ig product dispensed, NBA website provided product cost, and SCHHS provided cost of infusion disposables (e.g. syringes) excluding infusion pumps.

Results

Table1: Mean Ig product usage and cost per patient over 12 months. Mean cost of disposables used and serum IgG levels over 12 months of IVIg and SCIg treatment.

(n)	IVIg				SCIg			
	Product used	Product Cost	Infusion disposables	Serum IgG	Product used	Product Cost	Infusion disposables	Serum IgG
PID(8)	365g	\$22,780	\$256.80	8.9g/L [#]	387g	\$24,286	\$1,086	10.1g/L [#]
SID(5)	339g	\$21,364			388g	\$24,484		

[#] p<0.05; data missing on one PID patient

Paired t test detected no difference in product usage or cost between IVIg and SCIg. Serum IgG levels were significantly (p<0.05) higher during SCIg treatment.

Discussion and Conclusion

This preliminary data indicates that while product usage and cost of IVIg and SCIg are comparable, the latter produces higher serum IgG levels. This raises the potential for dose reduction by treating physicians which may reduce the cost of SCIg over the longer term. Continued monitoring is in place, as is investigation of labour costs, which were the major determinant of decreased SCIg healthcare cost in Canada (Gerth et al 2014 Allergy Asthma Clin Immunol).

160. Can extended genotyping, using a comprehensive and targeted blood group sequencing, assist in resolving complex serology problems in chronic transfused patients?

Hyland C, Schoeman E, Powley T, Liew Y, Lopez G, Millard G, McGowan E, O'Brien H, Flower R

Australian Red Cross Blood Service

Aim

The Blood Service uses SNP-array genotyping platforms in conjunction with serological testing to investigate unresolved blood group serology cases. However SNP-array platforms do not cover the entire range of blood group antigens.

The aim of this study was to validate a massively parallel sequencing system (MPS) and test for proof of principle that blood group sequence data would clarify cases remaining unresolved by current test algorithms.

Method

We validated a targeted exome sequencing strategy by sequencing genomic DNA from 28 well characterised donors using the TruSight One Sequencing Panel and the MiSeq platform.

CLC Genomics Workbench software v.8.5 was used for data analysis.

MPS genotyping was performed for three unresolved cases exhibiting antibody specificities against antigens in the Cromer, LAN and RH blood group systems respectively.

Result

In the validation study the average sequencing depth per target regions was 66.2+/-39.8. Each sample harboured an average of 43.9 variants of which an average of 10 +/-3 (quality score >30) was used for genotyping. For all samples there was 100% concordance between predicted phenotype based on MPS variant analysis and that based on SNP genotyping.

MPS analysis defined novel variants in the Cromer and LAN blood group genes for two cases; these were respectively a c.203G>A in *CD55* predicting a p.S68N in the first extracellular complement control repeat domain of the Cromer protein and, for LAN, a splice site disruption (c.1656-1G>A) in the *ABCB6* gene. For the third sample MPS defined a complex Rh gene complex predicting a RhD-hr^B-Hr^B phenotype.

Conclusion

The targeted sequencing approach provides accurate and extended blood group genotyping. For three cases MPS uncovered either novel or (for RH) complex gene structures that were consistent with the observed antibody specificities for these transfused recipients. Further studies are required at the protein level to confirm that the predicted phenotypes arise from observed genotypes. This study provided proof in principle that comprehensive blood group sequencing can assist in serology investigations.

161. A novel molecular mechanism in RHD caused the deletion of Exon 9 resulting in a partial DEL phenotype

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Aim

Blood donors are phenotyped for the RhD antigen to ensure that RhD+ red blood cells (RBC) are not transfused to RhD- recipients. Some RBC express low levels of D antigens only detectable by Adsorption and Elution Technique (AET), DEL (D_{elute}) phenotypes. DEL phenotypes like *RHD*(K409K) express the full D-epitope and are immunogenic. We previously reported a DEL phenotype where the molecular basis was not fully investigated for this donor. It was unknown whether *RHD* Exon 9 was deleted or replaced with *RHCE* gene.¹ We now report the molecular mechanism involved with this sample and we further investigate its serological pattern.

Method

Standard Agglutination Test (SAT) and AET were performed using a panel of anti-D monoclonal antibodies (moAb) and control RBC, untreated and papain-treated. Molecular techniques were performed to amplify *RHD* gene for DNA sequencing.

Result

The serological profile for this DEL phenotype showed no reactivity on SAT. On AET, 1/12 moAb eluate was positive with untreated RBC while 4/12 were reactive with papain-treated RBC, (Table 1). The serological pattern for this sample suggested a partial RhD phenotype. Sequencing data from this sample showed a 1013-bp *RHD* gene deletion spanning from Intron 8 to Intron 9, including the whole *RHD* Exon 9. DNA sequencing showed a repeat 25-bp sequence 5'-GGAGTTCGAGACCAGCCTGGCCAAC-3' occurred at the beginning (in Intron 8) and the end (Intron 9) of the deleted *RHD* gene.

Conclusion

We describe a partial DEL phenotype caused by Exon 9 deletion, *RHD*(delEx9). A 25-bp sequence was identified in two sites in Intron 8 and Intron 9 and may have formed a loop deleting a 1013-bp *RHD* fragment – a molecular mechanism not previously described for *RHD*. In contrast to *RHD*(K409K), *RHD*(delEx9) does not express the full D-epitope and carriers of this *RHD* variant is at risk of alloimmunisation if exposed to RhD+ RBC.

Reference

1. Scott SA, *et al.* Transfusion 2014;54: 2931-40.

	MoAb	epD	SAT	AET / UC	AET / PTC
A	LHM76/58	8.1	0	0	0
B	LHM76/59	5.1	0	0	0
C	LHM174/102	1.2	0	0	0
D	LHM50/2B	6.3	0	0	weak +
E	LHM169/81	1.1	0	0	0
F	ESD1	4.1	0	0	0
G	LHM76/55	3.1	0	0	weak +
H	LHM77/64	9.1	0	0	0
I	LHM70/45	1.2	0	0	0
J	LHM59/19	8.1	0	0	weak +
K	LHM169/80	6.3	0	+	+
L	LHM57/17	6.3	0	0	0

UC=untreated cells; PTC=papain-treated cells.

162. Managing anti-CD38 (Daratumumab) interference with pre-transfusion testing in a busy hospital transfusion laboratory

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St Vincent's, Melbourne, Australia

Daratumumab, an anti-CD38 IgG therapeutic monoclonal antibody [Ab], is a promising new therapy for plasma cell myeloma [PCM] however CD38 is expressed on erythrocytes and interferes with pre-transfusion testing. For pre-transfusion testing of patients on daratumumab we have used our currently available automated techniques including enzymes bromelin and papain and for transfusion, phenotyped matched red cells were selected. In addition, titres were performed to investigate the performance and characteristics of this Ab.

Initial testing on 7 patients pre-therapy included full red cell phenotyping, blood group, Ab screen and DAT. Once treatment commenced, an Ab screen, 11 cell IAT panel and bromelin screen or panel were performed monthly on each patient. Titres and papain panels were performed intermittently to assess Ab behaviour. The bromelin and papain techniques were validated by spiking daratumumab patient samples with known clinically significant Abs and testing by bromelin screen and pre-papainised panel.

Seven patients on daratumumab were tested over 178 episodes. A positive IAT Ab screen and panel were detected in each while on treatment, with scores +/- to 2+. For the majority of these episodes, a bromelin screen and papain panel (neither are IAT tests) were consistently negative. 19 titres were performed and remained consistent at 1:32,000 within one dilution. 46 DATs and 178 auto controls were performed which were predominantly negative, 3 patients were transiently positive at commencement of treatment.

The anti-CD38 agent, daratumumab in our hands gave apparently remarkably consistent results both over the treatment period and between patients. For most patients Hb initially decreased slightly then remained stable and no transfusions were required. Daratumumab interferes with normal pre-transfusion testing, so the automated bromelin screen and papainised panel were used to indicate the possibility of an emerging alloAb response. As these tests are not IAT, blood for transfusion was selected as phenotyped matched units. We feel that this approach is simple and automated, yet thorough, and can be performed 24/7 by the multi-disciplinary scientist to provide appropriate blood when required for the patient on daratumumab.

163. Platelets in Platelet Additive Solution (PAS) cause fewer allergic reactions compared to platelets in plasma – data from NZBS Haemovigilance and possible mechanisms

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Aim

Use New Zealand Blood Service haemovigilance data to compare reported allergic reactions with platelets in platelet additive solution (PAS platelets) or platelets in 100% plasma. We hypothesise that mechanisms other than dilution may also account for the lower rate with PAS platelets.

Results

Data for allergic reactions with a single component type (imputability ≥ probable) for 2009–2015 are shown below.

Component		Units transfused	Rate/10000 (95% CI)	
			all	≥ grade 2 severity
platelets in plasma	apheresis	29620	31.7 (25.9-38.8)	6.4 (4.0-10.1)
	pooled	13691	48.2 (37.8-61.4)	15.3 (9.9-23.6)
	total	43311	36.9 (31.6-43.1)	9.2 (6.7-12.6)
PAS platelets	apheresis	29642	12.5 (9.0-17.3)	2.4 (1.0-5.0)
	pooled	19606	25.5 (19.3-33.7)	3.1 (1.2-6.9)
	total	49248	17.7 (14.3-21.8)	2.6 (1.5-4.6)
FFP		112048	14.5 (12.4-16.9)	3.0 (2.2-4.3)
Cryoprecipitate		25640	2.7 (1.2-5.8))	1.2 (0.2-3.6)
Cryodepleted plasma		3982	47.7 (30.1-74.9)	10.0 (2.9-26.8)
RBC		793703	5.0 (4.5-5.5)	0.6 (0.5-0.8)
Total		1027942	8.1 (7.6-9.7)	6.2 (5.3-7.3)

Conclusions

Platelets are the most frequent component associated with allergic reactions. PAS platelets are less frequently involved than platelets in plasma.

Plasma proteins dilution may explain this. The lower rate of reactions with apheresis PAS platelets compared to apheresis platelets in plasma ($P < 0.001/0.009$ respectively - all severity grades/≥ grade 2 reactions) supports this.

No significant differences in rates between apheresis PAS platelets and FFP ($P = 0.2/0.3$ respectively - all severity grades/≥ grade 2 reactions), higher reaction rates with pooled platelets in plasma compared to apheresis platelets in plasma ($P = 0.005/0.009$ respectively - all severity grades/≥ grade 2 reactions) and, the fact that very little antigen can trigger other allergic reactions go against dilution being the sole explanation.

Platelets *per se* contribute to allergic transfusion reactions. Reactions are less frequent with PAS platelets. Dilution of proteins in plasma is a factor but effects of PAS, and different processing methods, directly on platelets must also be considered.

164. The challenges of providing phenotyped matched red cells in contemporary Australian practice

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Background

Sickle cell anaemia with rare Rhesus (Rh) group is extremely uncommon. We present a case of sickle cell anaemia who also is Rh rr' (dce/dCe), which imposed a significant challenge in clinical management of transfusion for clinicians.

Case report

A 16-year old girl of African heritage presented to hospital with an acute abdomen. She has sickle cell anaemia (HbSS) on daily hydroxyurea but is not on regular transfusion program. Her transfusion history is otherwise not known to any Australian blood services as she recently relocated from overseas. Blood group phenotype revealed a rare Rh group; A RhD-C+E-c+e+V-VS+, K-, Fya-Fyb- (DUFFY GATA silencing mutation), S- with anti-HrB (Rh34), anti-hrB (Rh31) antibodies. Her Hb was 88g/L on presentation.

In the event of a medical emergency Rhnull blood (from the frozen red cell bank) was deemed to be most practical should transfusion be required. Enquiries were also made to blood services overseas for fresh compatible units. In addition she was commenced on Erythropoietin therapy. Fortunately she had an uncomplicated laparoscopic appendectomy and transfusion was not required.

Rh group is the most complex and highly immunogenic blood group and is the most common cause of haemolytic transfusion reaction and haemolytic disease of the foetus and newborn. Rh complex comprises two Rh genes (RHD and RHCE), RhAG and RhAG accessory proteins. Rhnull phenotype is characterised by lack of all Rh antigens. Two genetic pathways leading to Rhnull phenotypes include Regulator Rhnull phenotype (RhAG mutation) and the rarer amorph Rhnull phenotype (RHD and RHCE mutation).

Conclusion

Rare blood group poses a significant challenge in transfusion medicine. For further management of this patient, family DNA studies, possible autologous collection and storage needs to be discussed both with clinicians and the laboratory/ARCBS. International collaboration offers significant benefit in the setting of increasing genetic diversity of our population.

165. Extending the post-thaw shelf-life of cryoprecipitate

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Introduction

Whole blood- and apheresis-derived cryoprecipitate is used for the treatment of fibrinogen deficiencies and dysfunction. However, once thawed, the shelf-life of cryoprecipitate is restricted to 6 hours. It may be possible to reduce wastage of this component by extending the post-thaw shelf-life.

Aim

The *in vitro* quality of whole blood- and apheresis-derived cryoprecipitate was measured at 24 and 48 hours post-thaw to evaluate whether expiry may be extended beyond 6 hours.

Method

Whole blood- (n=20) and apheresis-derived (n=19) cryoprecipitate units were thawed in a 37°C water bath and stored at ambient temperature (22±2°C). Units were sampled immediately and at 6, 24 and 48 hours post-thaw. Quality was assessed by measuring the concentrations of coagulation factors (FVIII, FXIII and fibrinogen), von Willebrand Factor (vWF), fibronectin, ADAMTS-13 and total protein. Data was analysed by one-way ANOVA and *post-hoc* Tukey's tests; a p-value <0.05 was considered significant.

Result

There were no significant differences in the *in vitro* quality of whole blood- or apheresis-derived cryoprecipitate over 48 hours of storage. The cryoprecipitate units met the Council of Europe specifications of ≥70 IU/unit for FVIIIc, ≥140 mg/unit for fibrinogen and >100 IU/unit for vWF throughout storage. Over 48 hours, FVIII concentrations decreased from 161±60 IU/unit upon thawing to 123±46 IU/unit in whole blood-derived cryoprecipitate, and from 286±71 to 241±73 IU/unit in apheresis-derived cryoprecipitate, but these changes were not significant (p=0.108 and 0.187, respectively). The vWF activity, FXIII, fibronectin and ADAMTS-13 concentrations remained stable throughout storage, with no significant changes after 48 hours. The mean total protein concentrations of whole blood- (64.6±5.7 g/L) and apheresis-derived (63.1±5.0 g/L) cryoprecipitate were not significantly different.

Conclusion

Whole blood- and apheresis-derived cryoprecipitate may be stored for up to 48 hours post-thaw at ambient temperature and still meet Council of Europe quality specifications. However, the risk of bacterial contamination with extended storage at this temperature remains to be assessed.

166. An update on emerging infections threats to the safety of the blood supply

Flanagan P

New Zealand Blood Service, Auckland, New Zealand

Blood Services need to be alert to the potential risks to the safety of the blood supply arising as a consequence of emerging infections. Two current viral threats are of particular interest. These are Zika virus and hepatitis E virus.

Concerns relating to Zika virus emerged early in 2016. Large epidemics of the infection in Brazil and some Pacific Islands combined with evidence that this might be associated with birth defects and neurological complications resulted in its identification by WHO as a 'Public Health Emergency of International Concern'. Zika is an arthropod borne virus and a member of the flavivirus family. At this stage there are no documented cases of Zika infection by transfusion. Zika virus however shares many common features with both Dengue and West Nile viruses which are known to be transfusion transmissible and hence blood services were quick to implement measures to reduce the risk of transfusion transmission. These largely focussed on donor deferral following travel to 'at risk' areas. Definition of these 'at risk' areas was challenging given the changing nature of the epidemic. Nucleic acid tests for the virus are now becoming available and may have a role in avoiding transmission in epidemic areas. Reports emerged of sexual transmission of the virus. Initially male to female but more recently the first report of likely female to male transmission has emerged. Risk assessments of the likelihood of transfusion transmission associated with sexually acquired Zika have been undertaken in a number of countries. These identify this as a very low risk. Nonetheless some countries have taken active steps to avoid this occurring. The future pattern of the Zika epidemic is unclear.

Concern over the risk of transfusion transmission of Hepatitis E (HEV) has emerged over a number of years. Initial evidence of transfusion transmission of the virus emerged in Japan in the early 2000s. This was shown to be linked to eating undercooked pork products. More recently cases of autochthonous HEV have been described in most Western countries and studies in blood donors have shown evidence of significant community transmission of the infection. This combined with concerns that HEV in immunocompromised recipients might lead to severe disease have prompted some countries to introduce selective or universal testing of donations for HEV RNA.

Pathogen reduction might allow a proactive approach to managing emerging threats but the absence of approved systems for use in red cell components is a significant limitation.

167. What is the role for immunoglobulin products in the era of better vaccinations and new drugs?

Buttery J

Monash University, Melbourne, Australia

From the successful inoculation of guinea pigs in 1895 with heat treated blood products by Shibasaburō and von Behring against diphtheria, the role of immunoglobulin products expanded rapidly in the latter half of the 20th century, offering pre and post exposure prophylaxis against many infectious diseases, especially in circumstances where the risk of infection is high, but there is not sufficient time to rely upon active immunity. Pooled normal and specific human immune globulin preparations remain key tools in post-exposure prophylaxis and/or therapy of many infections, including varicella, hepatitis A and B, diphtheria, tetanus, respiratory syncytial virus, measles, rabies, cytomegalovirus, Ebola and botulism. They have a proven role in the therapy of Kawasaki Disease and may have a role in the therapy of other presumed superantigen mediated illnesses, including toxic shock syndrome. In the era of improved vaccines, new drugs for viral infections and explosion of monoclonal therapies, how is the role of this traditional, often scarce, and expensive resource likely to evolve?

168. Immunohaematology case studies - Interactive!

Thornton N

NHS Blood and Transplant, Hertfordshire, UK

Several advanced immunohaematology cases will be presented in an interactive format. The majority of test results to be discussed will be serologically derived but will also include molecular testing results where appropriate to the investigation. Clinical advice and/or outcome will also be presented and discussed. The session will appeal to scientists and clinicians who have a main focus in the field of red cell immunohaematology, who are interested in learning about the investigations required for resolving complex cases and the clinical implications of laboratory findings.

169. Paediatric transfusion cases or clinical problems

Luban N

Children's National Medical Center, Washington, US

In this master class we will discuss difficult transfusion medicine scenarios and develop the rationale for specific blood product selection.

170. Comprehensive patient assessment: Rhetoric or reality?

Cohen E

Austin Health, Victoria, Australia

The clinical reality is that patients with cancer experience a high incidence of both physical, psychological and psychosocial symptoms that are sometimes not detected or alleviated. Symptoms, by definition are subjective experiences and patients play a fundamental role in the identification and management of their symptoms. While it may seem intuitive that patients are the key informants about their symptoms and symptom management, arguably, the assessment and management of symptoms most commonly rests with healthcare professionals who may or may not make comprehensive and systematic symptom assessment an explicit priority or make decisions about symptom management in collaboration with patients.

The two major conclusions of a concurrent multi-method research program that aimed to uncover the role of the patient in symptom management practices in an acute oncology setting will be presented. The first conclusion was that there are variable processes for symptom management in acute cancer care. The second was the ambivalence expressed and demonstrated by patients and nurses about patient participation in symptom management.

If optimal patient outcomes are to be achieved across the entire cancer trajectory we, as cancer clinicians, need to ensure that our processes of care support comprehensive patient assessment including staff development in this area as well as have a model of care that enables patients to participate in their care.

171. Exercise medicine

Cormie P

Australian Catholic University, Australia

Haematological cancer patients may experience serious chronic health and psychological sequelae including accelerated functional decline, fatigue, psychological distress, a higher risk of developing comorbid conditions and reduced quality of life.

This presentation will summarise the evidence of the efficacy of exercise in counteracting the detrimental side effects of haematological cancers and their treatment. Strategies to incorporate exercise into clinical practice will also be discussed.

172. Positive Change for Life

Klarica D

Alfred Health, Melbourne, Australia

A critical need to address lifestyle to minimise risk for future chronic illness has been identified in long term survivors of haematological malignancy treated with either autologous or allogeneic stem cell transplantation (SCT). Leading from this, we implemented a lifestyle modification program aiming to promote sustainable changes in nutrition and physical activity to maximise future wellness, health and quality of life (QoL).

Within the setting and work flow of the Alfred Late Effects Clinic, each project participant entered a 12 month program integrating the key components of individually tailored community-based physical activity, group physical activity, education, motivational strategies and a structured program of dietetic consultation based on the 'Coach for Heart Health' model used for cardiac rehabilitation. Patient-reported outcome measures were recorded at baseline and following 6 and 12 months of project participation and included fatigue assessments, QoL, physical activity and nutrition questionnaires. Baseline weight, waist circumference, systolic and diastolic blood pressure, fasting glucose and lipid profiles were performed at baseline and repeated at 12 months.

Marked improvements in physical activity levels and dietary habits were identified in participants resulting in statistically significant weight loss, reduction in waist circumference and improved lipid profiles at 12 months. Further, clinically meaningful improvements in fatigue levels and quality of life were demonstrated. These changes were not seen in a matched group of Late Effects Clinic attendees receiving general health advice but not participating in lifestyle modification program.

Optimising health and QoL for SCT survivors requires a continuing focus on risk factor identification. Providing practical support and education has the potential to facilitate behaviour modification to reduce lifestyle-related health risk. Our project highlights the potential of an individualised community-based physical activity program coupled with nutritional advice to improve health behaviours, enhance QoL and reduce fatigue in long term survivors of SCT.

173. Unmet needs of patients attending a Haematology Nurse-led Survivorship clinic

Gates P¹, Grigg A¹

¹ *Clinical Haematology. Olivia Newton-John Cancer & Wellness Centre. Austin Health. Heidelberg. Victoria. Australia.*, ² *Clinical Haematology. Olivia Newton-John Cancer & Wellness Centre. Austin Health. Heidelberg. Victoria. Australia.*

Aims

Patients at treatment completion for a haematological malignancy experience a range of physical and psychosocial unmet needs. The purpose of this study was to describe the needs and lifestyle behaviours of a cohort of patients attending a newly developed nurse-led haematology survivorship clinic and evaluate the strengths and weaknesses of the current model for providing survivorship care to these patients.

Methods

Patients were eligible to attend the clinic if they had received treatment for a haematological malignancy with intent for cure or long-term remission and did not have progressive disease. Participant demographic and treatment characteristics were collected. Patients completed The Distress Thermometer and problem checklist, the Cancer Survivors Unmet Needs survey and the Healthy Living self-assessment prior to attending the clinic.

Results

Fifty patients with a median age of 47 years (range 18 to 75 years) were reviewed. The majority were male (56%), and had lymphoma (58%). The median time from treatment completion to attending the survivorship clinic was four months (range 0 to 116 months). Patients reported a range of issues including fatigue (60%), worry (57%), fear (49%), memory/concentration problems (43%) and feeling depressed (42%), and demonstrated poor compliance with healthy lifestyle behaviours including diet and physical activity. A key component of the nurse-led consultation was assessment, planning and referral for ongoing support. All patients required referral including Wellness Centre (100%), community organisations (56%), dietitian (38%), psychology (36%), menopause, fertility and sexual health clinics (16%).

Conclusions

These observations highlight that patients with haematological malignancies require complex, tailored survivorship interventions and survivorship is now routinely integrated into standard follow up care early post treatment completion.

174. Listening to our colleagues – Essential Primary care feedback on LTFU care plans

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¹ Royal Melbourne Hospital & Peter MacCallum Cancer Centre, Victorian Comprehensive Cancer Centre, ² Royal Melbourne Hospital, Victorian Comprehensive Cancer Centre

Listening to our colleagues – Essential Primary care feedback on LTFU care plans

The Royal Melbourne Hospital (RMH) Allograft Long Term Follow Up (LTFU) clinic was established in 2014 in recognition that treatment of haematological malignancies including stem cell transplantation can impact an individual's long term health in many ways. The goal is that with appropriate education, monitoring and early intervention longer term, more serious health issues may be prevented. This clinic provides an additional health resource for high risk individuals and as such does not replace the valuable care that patients receive from their haematologists and primary care physicians (PCPs).

The LTFU is multi-disciplinary with attendance by haematologists, nurse coordinators and a nurse practitioner, all who have expertise in LTFU. In addition we have access to specialist providers upon referral.

All patients who are in remission and are two or more years post-transplant are eligible to attend the LTFU clinic. At initial review patients are invited to complete a series of questionnaires addressing degree of symptoms currently experienced, employment history, physical activity, nutrition and quality of life. Following initial review screening investigations are completed, results discussed and care plan developed that is sent to the patient, referring physician, PCPs and other nominated health care providers.

Currently 170 patients have attended for initial LTFU review with 230 patients having attended overall. Care plans have been sent to all PCPs and in addition PCPs have been invited to provide feedback regarding the usefulness of the care plans.

Response rate for returning surveys is 23% with overall feedback positive. Care plans have been modified according to feedback suggesting they were too lengthy.

This paper will discuss the development of the RMH Allograft LTFU clinic with a focus on feedback from PCPs. Future project will be developing resources, clinical mentorship and educational opportunities for PCPs to enable suitable allograft LTFU care to be provided in the community.

175. A novel approach in delivering vaccinations following an autologous stem cell transplant

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Introduction

Conditioning chemotherapy in the autologous transplant (ASCT) setting results in loss of pre-existing immunity. Immune recovery following ASCT can take up to 12 months. This increases patient's risk of infection from both encapsulated bacteria as well as viral pathogens. Re-vaccination of this patient group is vital. Three major international societies as well as most transplant centres recommend a program of re-vaccination starting approximately 6 months following the transplant. Despite these recommendations, uptake of vaccinations in this patient population remains poor in practice. In April 2014 at the Peter MacCallum Cancer Centre (PMCC) a comprehensive infection prevention clinic incorporating patient counselling of infection prevention strategies as well as vaccination was established.

Aim

To evaluate the benefits of a dedicated infection prevention clinic to enhance the uptake of vaccination following ASCT.

Method

PMCC transplant and clinic databases were evaluated from October 2013 to June 2016 to determine the following: 1) number of patients who received ASCT, 2) number of patients who attended for vaccination following ASCT and 3) number of visits per patient to the clinic. Clinical records for identified patients will be further reviewed for compliance and completion of recommended vaccination schedule and reasons for non-compliance.

Results

A total of 197 patients underwent ASCT. Of this, 143 patients were attended the dedicated clinic (72%). Table 1 illustrates the number of patient-visits. The median number of clinic visits was 2. Evaluation of clinical records is ongoing and results are pending.

Number of visits	Number of patients N (%)
6	1 (0.7)
5	4 (2.8)
4	24 (16.8)
3	31 (21.7)
2	28 (19.6)
1	55 (38.4)

Conclusion

We are seeing reasonable numbers of transplant patients referred to the clinic and completing at least 2 cycles of vaccinations. Further work needs to elucidate the reasons for patients not completing the recommended vaccination schedule.

176. In Sickness and in Health: Taking Care of Caregivers of Cancer Survivors

Kurtin S

The University of Arizona Cancer Center, Arizona, US

Caregivers, including partners/spouses, other family members, and friends, are expected to assume a primary role in support of and clinical management of the patient with cancer, with little or no formal training. If we accept that caregivers are most often spouses or partners (formal caregivers), we would expect the majority of these caregiver to be within a few years of age of the patient living with cancer. Given the older age of most patients at the time of diagnosis (65-75 years of age), this would place the caregivers at an age where they too may be likely to have chronic illnesses. For younger patients, the often abrupt transition into the caregiver role after a diagnosis of a cancer poses a number of challenges. The burden of caregiving is associated with physical and psychological distress in patients and caregivers. Strategies to support patients and caregivers living with cancer, including innovative strategies for improved communicative health literacy and use of health technology, may empower the patient and caregiver to engage in and master health, illness and life self-management. The focus of this lecture will be on a review of current literature relative to cancer survivorship and innovative strategies for individual and population based strategies to empower cancer survivors and their caregivers.

177. Development and implementation of a multidisciplinary (MD) support program for carers of patients undergoing allogeneic haematopoietic stem cell transplantation

Presta M

Royal Melbourne Hospital, Melbourne, Australia

Background

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is associated with complex quality of life issues and unique challenges for not only transplant recipients but also their carers. At our transplant centre, having a carer is an essential requirement to be considered for allo-HSCT. With increasing numbers of adult allo-HSCT transplants performed each year and survival rates, there is recognised need to extend our service delivery to the carers' of HSCT recipients. Studies of allo-HSCT have indicated that the presence of an active and effective carer(s) is critical to the outcomes and quality of life of transplant recipients. To date, the primary focus of HSCT service provision has been directed towards recipients with the needs of carers often inadequately addressed.

Aims

To develop and implement a targeted carer's support program designed to better identify and address the carer needs of allo-HSCT recipients.

Methods

Carers >18 years of age who were caring for a recipient, either undergoing or completed their first HSCT, for haematologic malignancies or non-malignancies, between 2013 and 2016 were invited to participate. Monthly two-hour information sessions for identified carers during pre-transplant phase and treatment phase were co-facilitated by HSCT social worker and post-HSCT nurse co-ordinator. A carer's resource package was also distributed at the pre-transplant phase. One-day self-care forums were delivered by our MD transplant team with a Carer's Victoria representative and allo-HSCT carer mentor included to maintain a carer's perspective. A qualitative methodology was used based on participant survey to elicit the satisfaction and utility of a carers support program.

Results

A total of 176 carers (66% females & 34% males) participated in the HSCT Carer's Support (self-care & information) Program since November 2014. Median age of carers was 47 years (range 23-71). Outcomes were assessed using participant surveys at the completion of each program. Over 91% participants were very satisfied with the two-hour information session with improvements in their HSCT knowledge and their caring role. Participants rated the self-care forums as highly effective in addressing their own well-being and emotional distress associated with long-term caregiving and support options available. 98% of participants would recommend the program to other carers.

Conclusion

Although the concept of carer support is not new in allo-HSCT recipient care, it continues to remain under-addressed. With carers being essential in the quality care delivered to allo-HSCT recipients, transplant centres have an obligation and opportunity in extending service provision to include carers. Preliminary results of this HSCT carer's support program demonstrate the value of transplant centres offering targeted carer's service delivery, to better address the needs of carers.

179. Addressing antibiotic allergy in haematology patients - pathways to better prescribing

Trubiano J

Austin Health, Victoria, Australia

Antibiotic “allergies” are present in up to 1 in 4 patients with a haematological malignancy. These labels are associated with inappropriate antibiotic prescriptions and inferior patient outcomes. Many allergy labels can be removed via accurate allergy recording and understanding of allergy mechanisms. This session explores pathways to antibiotic allergy ‘de-labeling’ from the bedside to the clinic.

180. Drug interactions with anti-infective agents in haematological malignancies

Rowan G

Peter MacCallum Cancer Centre, Melbourne, Australia

The treatment of haematological malignancies often places the patient at risk of infectious complications.

The use of anti-infective agents as both treatment or prophylaxis of infection can lead to the risk of drug-drug interactions and complications in a patient therapy.

These interactions can affect treatment of both the disease and the infection or potentially increase the toxicity from agents that are already high risk medications.

Many of the newer targeted therapies are metabolised via or affect the major enzyme systems including cytochrome P450, as do many anti-infective agents.

This presentation will present a number of cases where the use of anti-infectives in these patients was complicated by potential drug-drug interactions and possible solutions to these.

181. Case Study: Allografting a Jehovah's Witness patient with myelodysplasia

Roberts A

Integrated Haematology Department, Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Parkville, Victoria

Myelodysplasia in people under the age of 60 leads to premature death, and the risk so death can be reasonably estimated based on our current knowledge. The only curative form of therapy is allogeneic stem cells transplantation and this is typically offered to younger patients when their life expectancy is short and their transplant-related mortality (TRM) is low compared to their imminent risk of death from the underlying disease. Non-myeloablative transplants from sibling donors have low TRM, and often require minimal transfusion support. Nevertheless, the TRM of a patient who is unable to receive red cell or platelet transfusion support is higher, but precise estimates do not exist. The process around decision-making about care for a fit patient with poor-prognosis myelodysplasia who could not receive red cell or platelet transfusion support, but who could receive stem cells from a sibling, will be presented.

183. Ethical implications of allografting a Jehovah Witness patient with MDS

Presta M

Royal Melbourne Hospital, Melbourne, Australia

Background

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) treatment decisions in Jehovah's Witness (JW) patients with life threatening haematologic malignancies or non-malignancies involve careful assessment of potential risks and benefits. As a transplant team, it is our obligation to provide treatment and serve the needs of JW patients in a manner consistent with their beliefs. JW patient refusal to undergo potentially life-saving blood transfusions presents ethical challenges for transplant centres, in which transfusion support is standard practice within allo-HSCT. With increasing numbers of allo-HSCT transplants performed each year, how we integrate ethical decision-making into our everyday clinical practice has never been more relevant. Using a case study of a JW transplant recipient provides us the opportunity to explore some of the key ethical questions.

Aims

- Explore what are the core ethical questions in relation to allografting JW patient.
- Examine ethical decision-making process by both JW recipient and their donor when considering allo-HSCT and the autonomy that is expressed through informed consent.
- Recognise ethical issues as competing values and obligations from different perspectives (recipient, donor, family & clinicians).

Methods

We present the case of a 52-year old JW woman, diagnosed with myelodysplasia syndrome (MDS) who proceeded with a sibling allo-HSCT, to illustrate the wide spectrum of ethical considerations for both the JW patient receiving allo-HSCT and the treating transplant clinicians.

Results

This case study presented substantial psychosocial, ethical, and medico-legal challenges and highlighted how core ethical decision-making process is important in clinical management of JW recipients and their donors to ensure best interventions are carried out.

Conclusion

Although JW patients present infrequently at our transplant centre for consideration of allo-HSCT, they raise complex ethical and social considerations for our clinical practice. What we have learned from this exceptional case study presented an opportunity to examine our ethical decision-making process when allografting JW patients, without compromising the ethical principles of either the recipient and treating clinicians.

184. Fulfilling the vision of youth-friendly cancer care: How well are we doing?

McCarthy M

The Royal Children's Hospital, Melbourne, Australia

Aims

Adolescent and Young Adult (AYA) cancer has increasingly been recognised as presenting very specific challenges to patients and their families given the unique developmental tasks and trajectories of this lifespan period. Accordingly cancer health service reform for AYAs is underway in Australia and provides the opportunity to better orientate services to patients' developmental and cancer care needs. This study of AYAs and their parent carers aimed to identify significant supportive care issues for both patients and parents with the objective of assisting health services to better match health services to needs.

Methods

Following qualitative analysis of in-depth interviews (60 AYAs, 60 parents & carers), a national cross-sectional survey was undertaken that recruited 196 AYAs and 204 parents from 18 clinical sites when AYAs were 6-24 months after a cancer diagnosis at the age of 15-25. The survey used validated measures of: psychological distress; post traumatic growth; quality of life; social support; unmet needs for services and information; self-management; and experience of care.

Results

AYAs had a mean (SD) age of 21.6 (3.1) years. 86% were treated at an adult institution, 64% were from a metropolitan area and 51% were male. The most common cancers were malignant haematological cancers, Hodgkin's lymphoma and sarcoma. Many AYAs experienced significant emotional distress, had unmet needs for a wide range of information, clinical and support services, were not yet back on track with education and employment, and faced major financial challenges. Parents also had major support needs, including financial, social and psychological support needs, many of which remained unmet.

Conclusions

AYAs with cancer have many age-specific psychosocial issues and high rates of unmet needs that continue into survivorship. The extent that AYAs rely on families to meet many of their needs, together with the extent of parent needs, highlights that service frameworks for AYAs with cancer should be more patient and family centered.

185. A practical review of the haematology nurse's role in the care of adolescent and young adults

Haywood P

Royal Melbourne Hospital, Melbourne, Australia

The adolescent and young adult (AYA) haematology patient population (15-25yo) has unique needs that may not be well suited to either the paediatric or adult health care systems. There is a general recognition that this period of life is characterised by a major transition, with biological, social and community influences. A diagnosis of cancer, or a haematological disease, will potentially disrupt this transition in areas of life such as identity development, sexuality, cognitive capacity, and education/vocation. The impact that a haematological malignancy has upon the psychosocial development of AYA patients is extremely complex and AYA patients are being poorly served where their care does not take this complexity into account.

The haematology nurse, whether specialised in AYA or not, is an essential component of quality multi-disciplinary AYA care. This presentation will focus upon the haematology nurses role, with a particular focus of maximizing adherence to treatment. Using case studies and a discussion of relevant literature an emphasis will be placed on describing interventions that can be used in busy clinical environments.

186. The Continuum of Care in Multiple Myeloma Redefined

Kurtin S

The University of Arizona Cancer Center, Arizona, US

Recent advances in the diagnosis, risk stratification, treatment, symptom management, supportive care, and foundational basic sciences relative to multiple myeloma (MM) over the last decade are staggering. The improvement in survival rates in both newly diagnosed and relapsed patients treated with novel agents over the last two decades is equally impressive. Amidst all of these positive developments, delivering or receiving cancer care has become increasingly complex. Assimilating the rapidly evolving scientific advances and effectively integrating these into the care of patients and caregivers living with MM presents a challenge for oncology professionals. The focus of this lecture will be on review of the recent scientific developments in MM, the implications for oncology practitioners, and strategies to empower the patients and caregivers living with MM.

187. Hope For The Best Yet Prepare For The Rest: A Systematic Review of Advance Care Planning Prior To Stem Cell Transplantation

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¹ Royal Brisbane and Women's Hospital, ² Department of Health, Healthcare Improvement Unit, Clinical Excellence Division

Aim
To identify the current scientific evidence exploring the prevalence and effect of Advance Care Planning (ACP), prior to stem cell transplantation (SCT), for the patient and their carer.

Methods
A systematic review was performed independently by two reviewers across: PubMed; CINAHL; PsycINFO; and the Cochrane Library with inclusion criteria: 1) primary research; 2) English language 3) no restriction on date; 4) no restrictions on methodology; 5) >50% sample had haematological malignancy; 6) participants treated with allogeneic or autologous SCT; and 7) reported on ACP prevalence or associated outcomes. Keywords, synonyms and MESH were used for: stem cell transplant AND advance care planning.

Results
The search returned 84 studies of which 5 met inclusion criteria. Critical appraisal was performed using the Newcastle Ottawa Scale. Most studies were prospective (n=4), observational (n=5), utilised a quantitative methodology (n=4) and had small sample sizes. Approximately 50% of patients had a 'living will' or advance directive prior to SCT. Engaging in ACP was not associated with any increase in distress, anxiety, loss of confidence in the medical team (for the patient or carer) or risk of death (for the patient). Two studies reported patients were more likely to discuss their wishes for future end-of-life care with their family friends (63-80%) than the medical team (15-16%). Of patients who had engaged in ACP, only half of these patients had the relevant documentation held in their medical record. This suggests disconnect exists between patients' attitudes, behaviours and communication around ACP.

Conclusion
Results indicate that engaging in ACP prior to SCT is not a routine and well established practice. When performed, ACP was not associated with any adverse outcomes. As the body of literature on this topic is limited, large observational studies and interventional studies are needed.

This abstract has been presented in the nurses program at the European Society for Blood and Marrow Transplantation Annual Scientific Meeting, 2nd – 6th April 2016, Valencia, Spain.

188. Hospital and Home Based Exercise Program Following Allogeneic Haematopoietic Stem Cell Transplantation

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Aims

To investigate the feasibility of an 8-week exercise program for people following allogeneic haematopoietic stem cell transplantation (HSCT); and to measure changes in patient outcomes before and after an exercise program

Methods

Single site, prospective case series including patients undergoing allogeneic HSCT. Outcome measures included feasibility (consent, adherence and withdrawal rate), exercise compliance [Fitbit® (steps per day), exercise diary], functional exercise capacity [Incremental Shuttle Walk Test (ISWT)] muscle strength, and questionnaires [physical activity levels, exercise motivation, health related quality of life (HRQoL)]. Outcomes were measured at four time points (1) prior to HSCT; (2) 60 days post-HSCT before commencing the exercise intervention; (3) 120 days post-HSCT (after the exercise intervention); and (4) 6 months post-HSCT. The intervention was an 8-week outpatient and home-based exercise and education program commenced at 60 days post-HSCT. Preliminary results are presented on recruitment feasibility and change in patient outcomes from baseline to 60 days post-HSCT (before patients commence the exercise intervention).

Results

46 patients (60% male; mean age 45 [S.D.14] years) undergoing allogeneic HSCT (59% matched unrelated donor) for their haematological disorder (56% diagnosis of Acute Myeloid Leukaemia) were approached. The consent rate was 93%. Main reasons for non-consent were “too many appointments” and “too preoccupied with health concerns to think about exercise”. From baseline to 60 days post HSCT there was significant decline in functional exercise capacity (mean difference 208 meters, 95%CI 133 – 284, p<0.005). At time point 2, 23% (n=10) of participants did not commence the exercise program due to death, illness or cancellation of HSCT.

Conclusion

Patients experience significant decline in functional capacity from pre to 60days post HSCT. The high consent rate shows patient interest in exercise. This ongoing study will provide valuable evidence regarding the feasibility and potential benefits of exercise for people following HSCT.

189. Miss A's Treatment Journey Through a Double Cord Stem Cell Transplant ; The Importance of Palliative Care Input in Stem Cell Transplant Patients Care; A Case Study Presentation

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¹ Canterbury District Health Board, ² Canterbury District Health Board, ³ Canterbury District Health Board

Aim

The aim of this case presentation is to discuss Mrs A's journey through the South Island Bone Marrow Transplant Unit (BMTU) and the fundamental involvement of the palliative care team in her treatment.

Miss A was a 17-year-old female, diagnosed with Acute Lymphoblastic Leukemia in March 2015 after presenting to her GP with recent lethargy. She was admitted to the BMTU for treatment, where she received 2 cycles of intensive chemotherapy. Due to Miss A's high risk Leukaemia, the best treatment for her was a stem cell transplant.

Miss A received a double cord stem cell transplant in August 2016. Following transplant Miss A experienced a number of complications and on day 75 post her transplant active treatment was discontinued. Miss A passed away three days later in the comfort of her home surrounded by her large family and many friends.

In the bone marrow transplant population, both patients and families/whanau have significant palliative care needs, which are becoming increasingly recognised. Miss A and her family were seen by palliative care before the transplant and were able to put an end of life care plan in place and become familiar with the palliative care team and the care they provide.

Conclusion

This case presentation discusses Miss A's treatment journey and illustrates the benefits of implementing Palliative Care early in the transplant stage for both the patient and the family/whanau. This facilitates the development of a trusting relationship well before the patient's clinical course deteriorates.

190. Changing indications for autologous HSCT at St Vincent's Hospital and the impact of severe auto-immune conditions such as Multiple Sclerosis and Scleroderma at the general ward level.

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Background

Stem Cell Transplantation for Autoimmune Conditions is becoming an increasing workload for our unit. Randomised trials have demonstrated benefit for scleroderma (SSc) using HSCT whereas encouraging data is being demonstrated in Phase II studies for Multiple Sclerosis (MS). For the first time in 2015 Autologous Bone Marrow Transplant for Auto-Immune Conditions at St Vincent's Hospital outnumbered the autologous Malignancy Transplants. This trend that has been on a steady increase since 2011. These statistics correlate with increase nursing workload, multi disciplinary care challenges and complexities at the general ward level.

Aim

To highlight and educate nursing, medical and management about the pressures and demands on staffing, hospital beds and the general Haematology Unit, due to the changing patient population at the ward level for Bone Marrow Transplantation.

Method

Patients for Autoimmune Bone Marrow Transplantation with MS or Ssc are mobilised with Cyclophosphamide 2g/m² and GCSF 10ug/kg.

Generally collections are a one day apheresis procedure as patients have high CD34 counts compared to malignant patients.

Patient then present 2-4 weeks later for conditioning agents:

MS: BEAM/ATG

Ssc: CYCLO/ATG and Bone Marrow Transplantation.

Results

Over 80 patients with various autoimmune conditions have been treated at St. Vincents with HSCT after referral from their respective specialists usually after failing multiple therapies. Two Illustrated discussions and case studies on Multiple Sclerosis and Scleroderma will be reviewed.

Conclusion

The evolution of treatment for Auto-Immune Conditions within the Bone Marrow Transplant setting comes with many challenges for all. The awareness of caring for these types of patients and their demands on staffing, the ward and the Haematology Unit, needs to be understood fully to maintain expert staff rather than dealing with burnout in the area of Bone Marrow Transplantation.

191. Autologous stem cell transplant In patients with cardiac AL amyloidosis post an orthotopic heart transplant: a case study

Horne A, Nipperess J, Noakes C

St Vincent's Hospital and The Kinghorn Cancer Centre, Sydney

Background

Amyloidosis is a condition where abnormal amyloid protein is produced leading to organ damage and failure. AL amyloidosis is a plasma cell abnormality, where clonal bone marrow plasma cells produce a light chain immunoglobulin. Cardiac involvement occurs in approximately 50% of AL Amyloidosis patients, this portends a poor prognosis. Autologous stem cell transplant (ASCT) has become the standard of care in eligible patients with AL Amyloidosis. Patients with cardiac involvement have a substantially higher risk of transplant related complications. Orthotopic heart transplant (OHT) for AL Amyloid with cardiac disease has reported a 5 year overall survival (OS) rate of 54%. However, patients receiving OHT without subsequent amyloid therapy had an OS rate post OHT of 39% in 48 months. Tandem OHT followed by ASCT is a possible effective management approach for this patient group. An Ethics committee approved Pilot study is underway at St Vincent's Hospital Sydney, 3 patients are recruited to the study.

Case

A 64 year old man diagnosed with Cardiac Amyloid in 2012. Serum free light chains were 400mg/L at diagnosis. The patient responded very well to standard induction and maintenance treatment. In 2015 he became unwell again with rising serum free light chains and ventricular fibrillation. He underwent OHT in December 2015. In June 2016 sufficient autologous cells were mobilised using 4 days of Filgrastim 10mcg/kg. After further cardiac biopsy the patient was admitted for autologous transplant. The prescribed conditioning regimen was Melphalan 200mg/m², CD34 +ve cell dose infused 5.88 x 10⁶/kg. Post OHT immune suppression continues.

Conclusion

The application of autologous transplant to this patient group has provided opportunities and challenges to nurses unfamiliar with caring for solid organ transplant patients. Altered parameters for febrile neutropenia, increased risk of viral infection and monitoring of immune suppression are themes that will be explored in this presentation.

Acknowledgements

Staff and patients of St Vincent's Haematology/BMT unit

192. A Collaborative Approach in the Management of Late Onset Grade IV skin GVHD of an Adolescent Male Post Allogenic Bone Marrow Transplant

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Cancer Care Services Royal Brisbane & Women's Hospital

Introduction

The RBWH performs 85 allogenic transplants per year. Advanced approaches to Graft Versus Host Disease (GVHD) prophylaxis and management have resulted in decreased trends of Grade IV skin GVHD severity/incidence within the BMT Unit. This case review outlines a unique patient presentation with late onset of acute flare Grade IV skin GVHD and articulates the collaborative approach of managing the patient between the inpatient and ambulatory areas to improve outcomes.

Case Report

A 16 year old male with Haemophagocytic Lymphohistiocytosis was diagnosed 11.11.2012, medical history highly functioning Aspergers syndrome. Patient received a Flu Mel VUD Mismatch transplant 23.02.13. Post-transplant phase complicated by rash D+1, mucositis requiring PCA/TPN support, increased LFT's, febrile episodes, line removal, patient discharged D+37. D+100 patient BMAT reported full donor chimerisms, nil GVHD. Skin GVHD D+131, areas affected face, torso, proximal arms and legs, D+165 commenced on Prednisolone 1mg/kg. Patient managed with titrating immunosuppressant as an outpatient. 18/12 post transplant increasing lichenoid and sclerodermoid changes decreased ROM to neck. 2 years post-transplant admission for skin GVHD, worsening breakdown of wounds, secondary pseudomonas skin infection. APMS, Skin Integrity, Dermatology, Palliative Care input over the 6/12's for pain/wound management, patient transient between inpatient/outpatient. Patient currently 3.5 years post-transplant, last 5 months improvement in wounds and no admissions.

Conclusion

Coordination of care and management of this patient's GVHD skin was instrumental in reducing the severity/coverage of the extensive skin lesions. Leadership and clinical expertise was paramount and necessary to navigate the varied members of the health care teams within integral stages of patient journey. It was important to consider psychosocial needs of the patient/family, the benefit and cost effectiveness of discharge when it was clinically appropriate. The ambulatory procedure unit that supports the transplant program has expertise in management of BMT patients has facilitated the transition of care and continuum of management in the Ambulatory space.

193. myeNURSE: working together to improve outcomes for those with myeloma, a focus on best practice and information sharing

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Background

The HSAZ Nurses Group Myeloma Special Practice Network (M-SPN) provides nurse members with opportunities to share ideas, knowledge, information and clinical expertise; with the objective to improve the quality of nursing care and outcomes for individuals affected by Multiple Myeloma (MM).

Aim

This paper describes three projects undertaken by the M-SPN: bortezomib administration best practice document; a generic MM nurse specialist business case template; and an e-portal application (App) to store, share and deliver MM information resources.

Method

A mapping exercise was undertaken to determine existing MM nurse publications, consensus statements, guidelines, policies and resources relating to the three identified projects. Assessment of peer reviewed publications, grey literature, MM professional organisations and patient groups were undertaken. Relevant content was determined by group consensus and evidence from published literature.

Results

A toolkit for the safe administration of bortezomib was developed; including safe administration recommendations, monitoring and management of adverse effects; delivery schedules and consumer information resources. The business case was written to facilitate streamlined processes to justify and establish MM advanced practice nurse positions. It was designed as a guide to be used in conjunction with local Australian practice and policy. The myINTERACT App hosts MM information and incorporates myeNURSE - an umbrella information e-portal. myeNURSE provides access to a comprehensive range of resources including treatment and best practice guidelines, assessment tools, slide kits, third party Apps, websites and patient information resources. QR code capabilities also exist allowing for real time targeted information delivery to patients.

Conclusion

Development of the three projects will facilitate MM nurse specialists to collaborate and deliver current evidence based clinical practice, and enable provision of relevant real time consumer resources; ensuring nurse members and their patients remain updated to best practice in MM in a rapidly evolving clinical environment.

194. Trial Of The Use Of Methoxyflurane Inhalation For Analgesic Control During Bone Marrow Biopsy In A Haematology Outpatient Department

Steele A, Crawford J, Howman R, Cook D, Wood J, Charlton J, Buckland H, Williams H

Sir Charles Gairdner Hospital, Perth, WA

Aim

The aim of this study was to examine whether pain scores, interdepartmental procedure times and the patient experience could be improved by introducing the inhaled analgesia Methoxyflurane instead of IV sedation for Bone marrow biopsies. (BMB)

Methoxyflurane (Penthrox) is an inhaled analgesia which has been traditionally used by emergency services to provide safe, short acting pain relief in trauma cases and for surgical dressings. Penthrox vapour provides analgesia at low concentrations when inhaled.

Method

The study was conducted over 4 months and consisted of a pre Penthrox introduction study and a post introduction study which measured departmental visit times, patient pain and experience scores as well as nurse perception scores by use of a questionnaire. Results were collated in both studies and average time in department; pain scores and patient satisfaction were compared.

Results

In the Pre study 26 patients completed the questionnaire.

The length of stay for patients receiving I.V. sedation for their BMB, in the Haematology Day ward was 99 minutes per patient. For patients not receiving intravenous sedation, the time spent in the Haematology Day Ward was 72mins.

In the study group of 51 patients post introduction of Penthrox, the average time was down to 55.37 minutes, a mean reduction of 43.6 minutes per patient who received sedation and 16 minutes less than local only BMB.

Pain scores were largely unchanged compared to patients receiving sedation and were improved versus bone marrow biopsies performed with local anaesthetic only. The inhaler was widely commended for its ease of use and nurse and medical satisfaction with the experience was high.

Conclusion

Due to the reduced interdepartmental times, improved pain scores and high level of user and staff satisfaction, the unit has adopted the use of Penthrox as its first line analgesic control for Bone Marrow Biopsies.

195. Facilitating Effective Transition from Secondary to Primary Care: Trial of a New Nurse-Led Model for Haematology Patients Post Cancer Treatment Discharge

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Aim

The Regional Cancer Treatment Service (RCTS) based in Palmerston North Hospital provides haematology services to 500,000 people. On an average, twenty autologous stem cell transplants are performed annually within RCTS and about twenty people are diagnosed with acute leukaemia. These two named patient populations experience intensive chemotherapy and a lengthy recovery phase as part of their treatment. Traditionally, transition to primary care and follow up with this population group is largely medical focused. Our service has frequently observed patients expressing feeling of apprehension and abandonment, fear and uncertainty post-treatment. More systematic and coordinated approach utilising the CNS role would potentially have benefits for the patient, the specialists and general practice teams involved in their care. The project aims to develop a comprehensive and integrative discharge plan that is agreed upon by key stakeholders identified across the care continuum to ensure seamless care post cancer treatment.

Method

A model of change developed by Kurt Lewin in 1947 was used to develop the discharge plan. Lewin described change as a 3-stage process. Firstly, unfreezing which is getting ready for change. For this project, stakeholders had regular meetings to increase understanding of the processes involved. Secondly, is change or transition where the stakeholders worked together to develop the discharge plan. Thirdly, is refreezing, which is accepting and implementing the change, followed by evaluation.

Results

This new nurse-led model provided a clear mechanism and a rewarding experience to the key nurse leader and stakeholders in developing a discharge plan. This leadership provided an opportunity to positively influence health outcomes of patients post chemotherapy and advance nursing practice with an agreed discharge plan. A full evaluation is currently underway and results will be reported.

Conclusion

This project presented many challenges and opportunities. An effective practical discharge plan was however developed. A new collaborative practice to deliver and manage health outcomes for this group was initiated with positive outcomes. This comprehensive discharge plan will educate the patient as a cancer survivor and better equip the general practice team during the transition of care from specialist to general practice care.

196. Understanding MPNs

Vassili C

MDS/MPN Special Practice Network, Victoria, Australia

Myeloproliferative neoplasms (MPN's) are a group of relatively rare blood cancers. MPN's often manifest with a constitutional symptom burden that significantly compromises quality of life. The discovery of new genetic aberrations have enhanced our understanding of MPN's and provided a target for drug development, however patients and carers continue to struggle with chronic, often debilitating, symptoms.

Nurse-led clinics for patients with chronic health care needs are gaining a stronger foothold within Australia, mimicking the international experience. Nursing interventions based on timely assessment and management of disease and treatment related side effects can have a significant impact on patients quality of life and reduce hospital presentations.

The session aims to provide a comprehensive overview of MPN's (Myelofibrosis, Polycythaemia Vera and Essential Thrombocythaemia) to improve the understanding of the disease process, and assessment/symptom management strategies within a patient-centered, nurse-led context.

197. Understanding cytogenetics

Wall M

St Vincent's Hospital, Melbourne, Australia

Cytogenetics plays an important role in the identification and management of many malignant haematological disorders. In some disorders, cytogenetic testing aids in diagnosis. One example is chronic myeloid leukaemia (CML), which is characterised by the presence of the Philadelphia translocation. Moreover, recognition that this translocation gives rise to the *BCR-ABL1* fusion gene set the scene for the treatment of CML with tyrosine kinase inhibitors, a development that has improved the outcomes of these patients dramatically. In other disorders, such as acute leukaemia and myelodysplastic syndromes, cytogenetics is used for disease classification and for predicting prognosis. Testing may include G-banded karyotyping, which provides an overview of the whole genome, or fluorescence in situ hybridisation (FISH), which provides targeted information about specific regions of the genome, or both. FISH testing is the method of choice for identifying prognostic cytogenetic abnormalities in patients with chronic lymphocytic leukaemia and plasma cell myeloma. More recently molecular karyotyping using DNA microarrays and sequencing techniques has become available. The information provided by these newer technologies complements the G-banded karyotyping and FISH results. Comprehensive characterisation of cancer genomes provides an opportunity for the design of more effective, individualised therapies for haematological malignancies in the future.

198. Developing a robust clinical audit: How to get from your idea to presentation/publication

Botti M

Deakin University, Victoria, Australia

Clinical audit has an important role in the provision of safe, quality care. Clinical audit is a key quality improvement tool that enables point-of-care staff to conduct a systematic review of clinical care against specific evidence/standards and identify areas to improve patient care and outcomes.

There is increasing expectation that all clinicians, particularly those in specialised roles, participate in regular clinical audits to ensure that what should be done is being done.

The purpose of this presentation is to highlight what constitutes a robust clinical audit including: identifying a topic; selecting the right tool, collecting and analysing the data, implementing changes if improvements are required and when to re-audit. Key considerations associated with disseminating the results of clinical audit (in a verbal and/or written format) including the requirement for ethics will also be discussed.

199. Allograft donor selection

Hart C

Australian Red Cross Blood Service, Victoria, Australia

The Human Leucocyte Antigen (HLA) genes are known as the immune response genes. These antigens, present on nucleated cells, respond to the presence of foreign particles, including transplanted or transfused cells, to destroy them in order to protect the body.

Tests done to determine the HLA typing of patients and potential stem cell donors is performed by molecular (DNA) methods. The different HLA-A, B, C, DRB1, DQB1 and DPB1 loci are tested as required.

The search for a suitable donor for stem cell transplant starts with HLA typing the patient and family members. If there are no suitably matched family donors then a search of donors on the unrelated donor registries can be performed.

The time taken for the matching process varies depending on how easy it is to find a donor for the patient. Once a suitable donor is found the donor cells are collected and then shipped to the patient's transplant centre for infusion into the patient.

200. Apheresis case study: What to do when you're not U

Hill A

Victorian Comprehensive Cancer Centre, Victoria, Australia

The pre-surgical management of patients with sickle cell disease is an operational and logistical challenge requiring intensive forward planning. Best practice dictates that prior to any invasive surgical procedures, patients with sickle cell disease will benefit from erythrocytapheresis to minimise the potential for severe adverse sequelae related to their sickle disease.

A safe path to surgery and recovery becomes exceptionally problematic and labour intensive in the presence of anti-U on blood grouping. In this situation clear interdepartmental communication becomes paramount to achieve a desirable outcome.

We present an interesting case of a 20yo female who presented to the Royal Melbourne Hospital requiring surgical intervention for avascular necrosis of left hip due to her sickle cell/Hb-C hemoglobinopathy.

This patient's rare blood grouping dictated that without total collaboration between Australian Red Cross Blood Service, RMH Transfusion laboratory, Clinical Haematology, Apheresis service, Orthopaedic surgical team and the Orthopaedics ward, a surgical repair would be impossible and potentially fatal.

201. Establishment of an Australian cord blood-derived iPSC bank for clinical use

Elwood N

Murdoch Childrens Research Institute, Victoria, Australia

Induced pluripotent stem cells (iPSC) are created by reprogramming normal human somatic cells, such as skin cells, into stem cells. However, unlike other stem cells, iPSCs can be differentiated into all cell types within the body, potentially providing a source of cells for therapeutic function. Importantly, iPSCs are immortal and therefore can be expanded and banked, providing an inexhaustible stem cell resource for both research and clinical applications. The technology within the iPSC field has now evolved to the level where Good Manufacturing Practice (GMP) - compliant, clinical grade iPSCs can be created for therapeutic use (1). Despite this, iPSC generation is still costly and labour intensive and therefore the applicability of this technology to individual patients for autologous use will be limited.

Recent publications have explored the possibility of global iPSC banks, in which the banked lines have homozygous human leukocyte antigen (HLA) haplotypes and are derived from material selected from donors whose haplotypes are common in the target population. As such, cells derived from these banked iPSCs would be suitable for therapeutic use for many individuals within the population. There are several private and commercial banks forming in the US, Europe and Japan and an effort is being made by a group of stakeholders, the International Stem Cell Banking Initiative (ISCBI), to standardize regulatory governance of these banks, to provide safe clinically complaint GMP grade human iPSC lines.

Cord blood (CB) is an ideal source of starting cells for iPSC generation. CB cells are highly proliferative and are a more naïve source of cells; studies have shown that CB cells can be readily re-programmed, with an overall efficiency higher than in other cell types.

Within the Melbourne Childrens Campus in Parkville a unique opportunity exists to establish a clinically relevant GMP compliant bank of homozygous HLA haploidentical cord blood derived human iPSC lines for cellular therapies in Australia and globally. In collaboration between the iPSC core facility at MCRI, the BMDI Cord Blood Bank, internationally renowned experts in HLA and statistical genomics and experts in GMP and international regulatory compliance, we have embarked upon a project to establish the infrastructure and explore the feasibility of such a bank. This presentation will outline our progress to date and highlight the many challenges and considerations ahead.

202. How could cord blood help children with cerebral palsy?

Crompton K

Murdoch Childrens Research Institute, Victoria, Australia

Research into the potential of stem cells to ameliorate neurological injuries has been investigated for decades, using a range of stem cells in experimental models of neurological conditions. Of these, cord blood and bone marrow cells are attractive for a number of reasons and are currently among the few cell types with an acceptable safety profile in humans.

Cerebral palsy is the most common physical disability of childhood, arising from injury to the developing brain, affecting 1 in every 500 babies with lifelong impairments. Studies in a number of animal models of brain injuries relevant to cerebral palsy indicate functional improvement after infusion of cord blood cells. Although preclinical investigations continue, the current evidence has prompted clinical trials of stem cells as a potential treatment for cerebral palsy in humans.

Stem Cells in Umbilical Blood Infusion for Cerebral Palsy (SCUBI-CP) trial is the first human stem cell trial in cerebral palsy in Australia, commenced in March 2016. The aim of the trial is to investigate the safety of intravenously infusing children with cerebral palsy with matched sibling cord blood cells. The study includes only 12 participants, but the findings will have wide implications well beyond the cerebral palsy community. This talk will present on the current scientific evidence around the use of cord blood cells for cerebral palsy, the development of this study, and how study findings may affect the field.

203. Cord blood adjunct therapy in cardiopulmonary-bypass surgery for congenital heart diseases

Pepe S

Murdoch Childrens Research Institute, Victoria, Australia

Congenital heart disease covers a wide spectrum of complex conditions that include structural, genetic and cellular functional abnormalities of the heart, valves and vasculature which emerge during fetal development and are clinically manifest after birth. The hypoplastic left heart syndrome (HLHS) features a left ventricle that is small or unformed, a restrictive ascending aorta, poorly formed and/or positioned aortic valve and/or mitral valves. Thus the heart is unable to adequately pump blood to the entire body, rapidly leading to cardiovascular failure. HLHS surgical intervention initially provides unobstructed systemic arterial circulation from the right ventricle (RV). It is also crucial to control the pulmonary (lung) blood flow to minimize the pulmonary recirculation and to provide unobstructed pulmonary venous return. This ensures initial survival despite pulmonary-systemic blood mixing that leads to cyanosis. Ultimately in a series of staged operations, although still with only one functional ventricle, the goal is to separate pulmonary and systemic circulation to permit blood circulation in series without cyanosis. Critical to achieving this is the protection of the pulmonary circulation by alleviating excessive blood flow and pressure and the protection of the right ventricle and tricuspid valve by the reduction of RV volume overload.

Our studies show that cord blood stem cell treatment can stimulate increased muscle mass and improved pumping function in a right ventricle overload model in neonatal lambs. We have recently designed a new delivery method for human cord blood stem cells to the neonatal heart during cardiopulmonary bypass (CPB) cardiac surgery. The purpose of this treatment is to induce heart muscle 'fortification' in children with a single functional ventricle that is burdened with work overload and is vulnerable to right heart failure at birth and after surgical intervention. Our work demonstrates the acute safety and efficacy of human cord cell delivery to the neonatal lamb heart during experimental CPB surgery. Compared to other methods our approach permits high dosage of cell delivery exclusively to the coronary vasculature and greater endocardial uptake while maintaining coronary blood vessel patency. We have also recently identified that human cord blood stem cells include microRNAs that promote growth signalling and a subgroup of cardiac specific progenitors. Whether human cord blood stem cell treatment in a lamb model of CPB cardiac surgery safely provides very long term cell dose-dependent benefits to muscle mass, pumping function and remodelling (inflammation/fibrosis) is under investigation. We are also planning the first safety trial of cord blood stem cell treatment in HLHS patient CPB cardiac surgery.

204. Immunotherapy in acute lymphoblastic leukaemia

Fleming S

Alfred Health, Melbourne, Australia

Acute Lymphoblastic Leukaemia (ALL) is the third most common indication for allogeneic stem cell transplantation after Acute Myeloid Leukaemia (AML) and myelodysplasia (MDS). Despite a relatively weak graft-vs-leukaemia effects allogeneic stem cell transplantation has represented an effective therapy in salvaging patients with poor-risk disease, as either defined by conventional criteria or by presence of minimal residual disease (MRD) from the likelihood of relapse. Despite this success, other immunotherapies for ALL have lagged significantly behind those seen in other lymphoid malignancies.

Rituximab, the anti-CD20 monoclonal antibody which shows modest single agent activity in relapsed/refractory disease has been applied by the MD Anderson group in combination with Hyper-CVAD and by the GMALL group in their GMALL03/07 protocol demonstrating improvements in survival with CD20+ ALL against historical comparators. The recently presented French GRAAPH05-R study demonstrated a substantial survival effect in a randomised trial for Rituximab in combination with a paediatric inspired regimen for upfront treatment of ALL, representing the first randomised study of an immunotherapy in ALL to demonstrate a survival benefit.

The recent developments of the immunoconjugate Inotuzumab Ozogamicin (IO) and the Bi-specific T-cell engager (BiTE) Blinatumomab have both demonstrated in randomised phase III trials superiority to conventional chemotherapy based salvage in relapsed and refractory ALL. Furthermore phase II data of Blinatumomab in minimal residual disease positive ALL have demonstrated intriguing results in being able to salvage molecular failures and unexpectedly prolonged disease free survival even in patients not proceeding to allogeneic stem cell transplant. These agents are now being actively explored both as up-front therapy in combination with other agents, or as MRD targeted therapy by several groups. The early reports of the MD Anderson mini-HyperCVD + IO are encouraging in their outcome for elderly ALL.

Finally the advent of Chimeric Antigen Receptor (CAR) T-cells stands to revolutionise therapy for both children and adults with ALL, with high response rates which may be durable in some patients even in the absence of allogeneic stem cell transplant. This session will examine the current evidence in application of immunotherapies in ALL, and how they may influence our future management of this disease.

205. Initial comparison of 3 apheresis platforms for supporting the collection of CD3+ cells for CAR-T production

Watson D

Cell Therapies Pty Ltd, Melbourne, Australia

Introduction

Collection by apheresis is often overlooked in the cell therapy manufacturing process. With the decommissioning of the Cobe Spectra, there is a need to evaluate whether replacement apheresis platforms will have any negative impacts. This pilot study compared the Cobe Spectra with the Spectra Optia Mononuclear Cell (MNC) and the more recent cMNC programmes.

Method

A prospective comparison (n = 3 in each arm) of 3 Terumo apheresis platforms for the collection of CD3+ cells from steady state healthy donors was undertaken. The collection target was 2 x Total Blood Volume (TBV). Donors were consented and screened according to local protocols. To allow collection efficiency (CE) to be assessed on CD3+ cells, peripheral blood and collections were both assessed by flow cytometry for absolute lymphocyte composition.

Results

Nine unmobilised donors had mononuclear cells collected by apheresis (n=3 each platform). Median age in range 40 – 45. Run and collection data are outlined in the table below. CMNC was significantly better than MNC for collection efficiency (p= 0.035) and total CD3+ cells collected (p= 0.011). A target of < 2.5% HCT was met for all Spectra collections and 2/3 Optia cMNC collections All Optia MNC collections had HCT > 2.5% (with one >5%). cMNC granulocyte contamination (by Flow Cytometry) was < 1%.

Machine	Cobe Spectra	Optia MNC	Optia cMNC
Median donor TBV (mls)	6180 (5510-7124)	6438 (5787-7008)	6124 (5537 -6766)
Median PB CD3+ (10 ⁶ /ml) (range)	1.25 (0.8 – 1.34)	1.1 (0.7 – 1.5)	1.010 (1.0 – 1.11)
Median TNC x 10 ⁹ (range)	13.86 (12.87 – 22.62)	8.52 (6.30 – 10.14)	12.48 (11.80 – 17.2)
Median CD3+ x 10 ⁹ in product (range)	8.04 (7.59 – 8.22)	3.50 (2.74 – 5.56)	7.80 (7.44 – 10.60)
Median run time (mins)	210 (202 – 241)	259 (230 – 262)	227 (220 – 235)
Median TBV processed	12047 (11019 – 12355)	11050 (9911 – 12291)	11833 (11074 – 13533)
Median TBV/ min	54.6 (51.2 – 57.4)	46.9 (38.3 – 48.0)	53.8 (48.8 – 57.6)
Median BV ratio	2.0 (1.7 – 2.0)	1.7 (1.7 – 1.8)	2.0 (1.9 – 2.0)
Median CD3+ CE %	58 (58 – 76)	47 (27 – 59)	76 (70 – 79)
Median volume (apheresis bag - mls)	193 (181 – 205)	100 (64 – 100)	216 (181 – 228)

Discussion

The Optia cMNC was quicker, more efficient and more predictable with a higher TBV processed, whilst minimising non- target cell collection compared to Optia MNC. The cMNC benefits from the automation of the Spectra Optia interfaces, while having the advantage of continuous collection. The intermittent collection of the Optia MNC led to less certainty around both collection volumes and procedure durations. We propose that the Optia cMNC is the platform of choice to replace the Cobe Spectra for steady state MNC collections that are required for novel cellular therapy product manufacture such as chimeric antigen receptor (CAR) T cell therapies.

206. Pre-apheresis T cell counts can be used to calculate additional blood volume to be processed for donor lymphocyte cryopreservation

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Westmead and Children's Hospital Blood and Marrow Transplant Services

Introduction

Donor lymphocyte infusions (DLI) are frequently used as prophylaxis or treatment for relapse after allogeneic stem cell transplant. In high risk patients, T cells for future DLI may be requested at the time of stem cell harvest. It may therefore be appropriate to consider the ideal number of T cells and CD34⁺ cells when determining blood volume to be processed at apheresis. We looked at pre-harvest CD3 counts in mobilised stem cell donors to determine whether they correlated with harvested CD3 cell number.

Methods

CD3⁺ and CD34⁺ cell counts prior to apheresis and in final collections were determined by flow cytometry from 14 allogeneic donors.

Results

The median peripheral blood CD3⁺ count prior to collection was 2878/ μ l (826-5885/ μ l). Total harvested T cells correlated with the number of T cells processed ($R^2=0.95$, $p<0.00001$). Median T cell collection efficiency was 47% (39-55%), which was comparable to CD34⁺ collection efficiency (44.6%). An algorithm used to predict blood volume to be processed at apheresis for CD34⁺ cells was adapted for CD3⁺ T cells using the variables of target CD3⁺ dose, recipient weight, pre-collection CD3 count and T cell collection efficiency. The mean predicted volume to collect 1.2×10^8 T cells/kg was 6.2L (2.3-8.5L). This cell dose corresponds to the first three escalating doses of DLI at our centre for HLA matched transplants accounting for expected cryopreservation loss. We observed a strong correlation ($R^2=0.97$, $n=12$) between the predicted versus actual blood volumes processed to collect 1.2×10^8 T cells/kg.

Conclusion

The ability to predict T cell yield based on pre harvest CD3 count may be useful for planning apheresis when a request for cryopreservation of donor lymphocytes at the time of stem cell harvest has been received. This may also be of value for unprimed apheresis although our results need to be validated in that situation.

207. Inter-laboratory Comparison of Viable CD34+ in Aliquots of Cryopreserved HPC

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The determination of post thaw viable CD34 is an essential quality control tool for cryopreserved HPC. The accuracy and reproducibility of the results are key influences of transplant cell dosing. In 2007 the single platform flow cytometry analysis using 7AAD and beads following demonstration of improved coefficients of variations (CV) published by A Chang *et al* (Cytotherapy 2004;6:50-61) was adopted.

All laboratories are enrolled in external quality assurance programs (QAP), however, the QAP samples provided are fixed fresh cells, thus permitting evaluation of total CD34 rather than viable CD34. The value of such QAP samples is limited, given that many of the clinical products are either cryopreserved for autologous transplant or transported for more than 24 hours in the unrelated allogeneic HPC setting. Hence we initiated an inter-laboratory comparison of viable CD34 in cryopreserved HPC.

HPC(A) were obtained from four consenting donors whose HPC product contained CD34 in excess of that required for transplantation. Cells were obtained from thawed products and refrozen in multiple aliquots of approximately 1 mL then distributed. Aliquots were thawed, suspended in the routinely-used diluent then analysed for WCC, total CD34 and viable CD34 content using single platform analysis by each lab.

To date, five analyses have been conducted on three product samples. Slight modifications (e.g. fluorobeads) were made to the methods of some sites on the repeat sample testing. Additional analysis of diluent used and gating strategy (ISHAGE vs Modified ISHAGE) have not demonstrated a significant difference.

	Sample				
	A	A repeat	B	B repeat	C
WCC CV%	23.0	19.5	37.9	32.1	55.8
Total CD34 CV%	5.4	18.7	7.1	21.45	16.1
Viable CD34 CV%	25.9	26.0	23.9	22.0	19.0

Large CVs (up to 55.8%) for the WCC were reflective of the methodology used: lower WCCs were obtained when the WCC was generated by flow cytometry incorporating a viability marker. Future WCC testing will universally be performed using haematology analysers.

The viable CD34 CVs obtained in this study were less than 30% for each sample. Although these CVs are substantially lower than those reported by other groups for thawed samples (e.g. > 70% in Cytotherapy 2014;16:1558), additional focus is now on harmonising test methodology in an effort to further reduce the CV across the network.

208. Sepax II vs Spectra Optia, a comparison of two technologies for red blood cell depletion

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Background

Red blood cell (RBC) depletion is a common technique particularly in ABO major mismatched bone marrow grafts. In the last 10 years we have seen an influx of closed automated systems to assist in RBC depletion. Two of the more common technologies used in cell therapy laboratories are the Sepax II from Medtel and the Spectra Optia from Terumo BCT.

Aim

The aim of this study was to compare the two technologies. We compared results for both the total nucleated cell count (TNC) recovery and the percentage of RBC depletion. A high %TNC recovery together with high % RBC depletion is the optimum result post manipulation.

Method

For this study we performed RBC depletion on peripheral blood collected from 12 venesected healthy donors. Six donors were RBC depleted using the Sepax II Redux protocol and six donors were RBC depleted using the Spectra Optia BMP protocol. Results were analysed comparing %TNC recovery and %RBC depletion.

Result

The Sepax II running the Redux protocol produced a graft containing an average 16mL of RBC whilst the Optia produced a graft containing an average of 10mL. The %RBC depletion of the Sepax was on average 94% and 96% on the Optia. TNC recovery on the Sepax II was 71% compared to the Optia recovering 84% of TNC.

Conclusion

While both technologies produced a graft below our upper limit of 20mL incompatible RBC, there is a significant difference with regard the TNC recovery between the two technologies. Both technologies provide adequate RBC reduction, with the Optia providing a better TNC recovery. The Bone Marrow Transplant Laboratory currently utilises both systems, Sepax II as a primary RBC depletion technology with the back-up option of the Optia.

209. Thaw, Wash and Infusion of Unrelated Donor HPC, Cord Blood

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Background & Aim

This study aimed to evaluate unrelated cord blood units (CBU) received for transplant between September 2007 and December 2015.

Methods

A total of 109 CBU were received for transplant: 5 CBU were not infused; 70 were infused as single CB transplants and 34 were used in double CB transplants.

Results

The thawed CBU volume was generally 91% of the stated volume, contained 82% (range 50–131) of the stated nucleated cells (NC) and 67% (17–212) of the stated viable CD34 cells. RBC content was highest in CBU from the Stemcyte banks (approx. 50mL), intermediate in CBU from AusCord (approx. 26mL) and generally less than 20mL from other banks. Pilot thaw data (performed on a cryovial or attached segment transported with the CBU) for NC, CD34 and RBC recovery correlated well with the bag thaw data.

63% of the CBU were washed prior to infusion. The wash procedure resulted in loss of 3% of the NC and 1% of CD34 but a 65% reduction in RBC. There was no difference in engraftment rate between washed and directly infused CBU. Washing the CBU was associated in a reduction in infusion-related adverse reactions (32% vs 60% in unwashed CBU).

Engraftment rate was strongly associated with infused CD34 dose. For patients receiving a single CBU transplant, median days to ANC $>0.5 \times 10^9/L$ was 13, 15 and 23 days following infusion of CD34 doses of >2 , 1-2, or $<1 \times 10^5/kg$, respectively.

Conclusion

Clear variations exist between CBU issued by different cord blood banks. Performing a pilot thaw on an associated sample gives a good indication of the CBU quality prior to transplant. Washing of the CBU results in little loss of NC or CD34 but reduces the incidence of infusion reactions. Neutrophil engraftment was more rapid following infusion of higher viable CD34 doses.

210. BMTL Pathwest: from concept to production

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Pathwest BMTL Fiona Stanley Hospital

Introduction

The Pathwest Bone Marrow Transplant Laboratory (BMTL) is the newest addition to the recently constructed Fiona Stanley Hospital Perth, WA. The laboratory was constructed to fulfil the need for a haemopoietic processing laboratory to service the adult stem cell programme, which is the only programme in the state to perform allogeneic stem cell transplants.

Aim

To complete the construction, validation and testing of the laboratory in preparation to receive, process, store and distribute haemopoietic stem cell products to meet the demands of the hospital, the regulatory bodies and the larger international community as a distribution site.

Methods

The flow chart Pathwest followed in the conception of BMTL contains 6 key steps, Laboratory Design and Physical Space, Equipment, Supplies and Reagents, Staff and Document Control/Quality Assurance Programme. Each step of the setup required completed documentation of testing and validation to sufficiently satisfy the regulatory bodies and to ensure products comply with a high standard of quality assurance.

Results

The BMTL laboratory completed its construction at the end of May 2015 with the first haemopoietic graft processed in September 2015. A total of 4 months was spent on the installation, validation and training of staff and equipment. BMTL was audited by NATA at the end of October 2015 with accreditation being awarded in February 2016. To date BMTL have processed a total of 37 allogeneic, 29 autologous and 28 Australian Bone Marrow Donor Registry donations.

Conclusion

BMTL from conception to operation has required an implementation plan which covers the main operational areas of any laboratory. By following this flow chart, it has reduced the time from construction being completed to procedures being performed to only 4 months. With accreditation achieved 7 months post construction completion.

P001. Minimal residual disease by multiparameter flow cytometry is an independent predictor of relapse free survival and overall survival in acute myeloid leukemia

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Aim

To assess clinical impact of minimal residual disease (MRD) determined by multiparametric flow cytometry in adult patients with acute myeloid leukemia (AML) achieving complete remission after intensive chemotherapy.

Method

This was a retrospective, single institution study. Kaplan-Meier curves were used to illustrate the overall (OS) and relapse free survival (RFS) distributions from the date of the first complete remission (CR1) by various categorical variables. Log-rank tests were used to test for differences in survival distributions between groups. Cox Proportional Hazards models were used to estimate hazard ratios (HR) and their 95% confidence intervals (95% CI).

Result

Patients with negative MRD had significantly better overall survival (OS) and relapse free survival (RFS) ($P = 0.038$). Multivariate analysis, including gender, traditional prognostic groups and MRD status post-induction, confirmed that the hazard ratio of death in those with positive MRD was 4.2 times that for those with negative MRD (95%CI 1.3 to 13.7, $p=0.020$). Importantly, the MRD status post induction independently affected OS in patients across traditional prognostic groups AML ($P = 0.044$).

Conclusion

MRD status determined in CR1 is an independent predictor of OS and PFS in patients with AML regardless of their traditional prognostic group.

P002. Audit of thrombotic complications and the management of coagulopathy during chemotherapy for acute lymphoblastic leukaemia/lymphoma in adults treated at the Wellington Blood and Cancer Centre over 10 years

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Aims

Venous thromboembolism is a well-known complication of treatment for patients with acute lymphoblastic leukaemia/lymphoma. Asparaginase, an important component of the combination chemotherapy used for this condition, is a particular risk factor. Prophylaxis against thrombosis and the treatment of asparaginase induced coagulopathy remains controversial. Although antithrombin III replacement and fresh frozen plasma infusions are commonly used, there is limited evidence of clinical efficacy. This study reviews the development of coagulopathy and thrombotic complications of patients with acute lymphoblastic leukaemia treated at a single institution.

Methods

A retrospective audit of adult patients treated for acute lymphoblastic leukaemia through the Wellington Blood and Cancer Centre over a 10-year period was undertaken. The rate of thrombosis was reviewed and compared to published data. The management of coagulopathy was assessed including the frequency of antithrombin III replacement as well as the rate fresh frozen plasma and cryoprecipitate supplementation.

Results

34 adults with acute lymphoblastic leukaemia were treated with chemotherapy over a 10-year period. 25 of these patients received asparaginase containing combination chemotherapy. The rate of symptomatic thrombosis was 26% of all patients, 29% of those patients treated with asparaginase and 11% of those without. Published thrombotic rates in adult populations are variable ranging from 4.2 to 34%. Antithrombin III replacement was used in 13 (52%) of the patients treated with asparaginase. There was not a significant difference in the rate of antithrombin III replacement in patients with or without thrombosis. Evidence for the use of antithrombin III replacement remains limited.

Conclusions

This study demonstrates a relatively high rate of thrombosis in adult ALL patients treated with chemotherapy at our institution. Despite the lack of quality evidence for antithrombin III replacement, consideration should be given to its prophylactic use particularly in those patients with other risk factors.

P003. Leukaemic secretion of calcitriol: A novel mechanism of hypercalcaemia in AML

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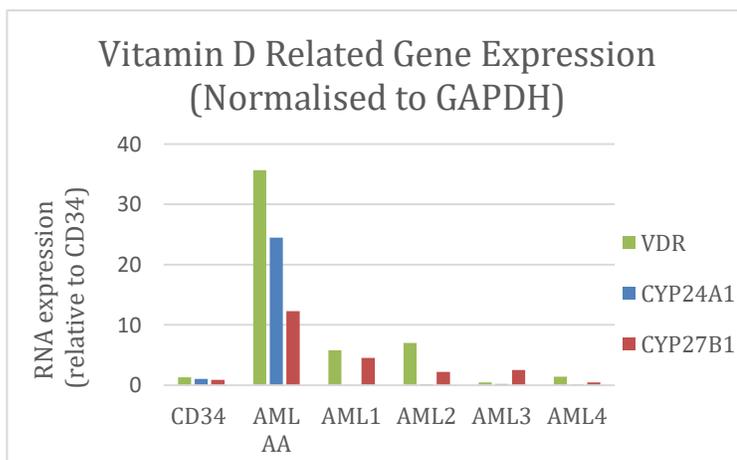
Introduction

Hypercalcaemia is a rare complication of acute myeloid leukaemia (AML). We describe a case of severe hypercalcaemia with acute kidney injury (AKI) accompanying a new diagnosis of AML, subsequently demonstrated to be secondary to leukaemic blast production of active vitamin-D (calcitriol). This is a novel pathogenic mechanism of hypercalcaemia in a myeloid malignancy.

Case Report

AA, a 68yr old male, was diagnosed with acute myelomonocytic leukaemia (78% blasts). Additionally, he had marked hypercalcaemia (calcium 3.3mmol/L) and AKI (creatinine 263umol/L). Extensive investigations failed to reveal a second malignancy. Parathyroid hormone (PTH) levels were suppressed (0.9pmol/L). Levels of PTH-related peptide and serum ACE were normal (<2pmol/L and 37units/L). Inactive vitamin-D, calcidiol [25(OH)D3] levels were also normal (88nmol/L), but the active vitamin-D, calcitriol [1,25(OH)2D3] level was grossly elevated (500pmol/L). The hypercalcaemia proved refractory to aggressive hydration and intravenous pamidronate, but responded precipitously to chemotherapy (7+3). The rapid resolution of serum calcium levels mirrored peripheral blast clearance and serum creatinine normalised. AA achieved complete remission and remains leukaemia free; his hypercalcaemia has not recurred.

To determine whether the AML blasts secreted calcitriol we performed quantitative PCR for genes essential to vitamin-D metabolism: vitamin-D receptor (VDR), CYP24A1, and CYP27B1 (1- α -hydroxylase). RNA was extracted from AML cells using the QIAGEN RNeasy kit. cDNA was synthesised from 400ng of RNA using the Roche First Strand cDNA Synthesis Kit. Gene expression was assessed by quantitative real-time PCR, relative to the housekeeping gene GAPDH. AA's leukaemia cells demonstrated markedly elevated expression of all vitamin-D related genes compared to healthy control CD34⁺ cells and four other independent primary AML (AML1-4) cells (Figure 1).



Conclusion

Hypercalcaemia secondary to secretion of calcitriol can be a manifestation of lymphoid malignancies, however our case is the first documented occurrence of this phenomenon in a myeloid cancer and represents a novel mechanism for a rare complication in AML.

P004. Identification of a novel translocation and subsequent clonal evolution in acute basophilic leukaemia

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Background and aim

Acute basophilic leukaemia is a rare acute myeloid leukaemia with poor prognosis and unknown pathogenesis [1]. Similarly, acute leukaemias with translocations involving chromosomes X and 10 are uncommon and the genes involved have not been well described. We have employed genomic and transcriptional profiling to study disease progression in an aggressive acute basophilic leukaemia.

Methods

A woman was diagnosed with acute basophilic leukaemia with t(X;10)(p10;p10). She relapsed early after standard of care induction chemotherapy, and died despite allogeneic haematopoietic stem cell transplantation. Whole exome (WES) and RNA sequencing were performed on the leukaemia samples at diagnosis and relapse. Translocation breakpoints were identified from RNA sequencing using JAFFA [2]. The partner genes involved were confirmed by RT-PCR and Sanger sequencing, as well as FISH. Clonal evolution was studied from WES by tracking mutant allele frequencies with superFreq [3]. Somatic mutations were confirmed by PCR and Sanger sequencing.

Results

The translocation t(X;10)(p10;p10) was shown to involve *DDX3X* and *MLLT10* on chromosomes X and 10 respectively. This translocation has been identified in T-cell acute lymphoblastic leukaemia, but had not been observed in acute myeloid leukaemias. Two *TP53* mutations in the DNA binding domain were present at diagnosis. Their allele frequencies increased at relapse, suggesting a role in refractoriness to chemotherapy. Clonal evolution of the leukaemia at relapse was also demonstrated by an additional *NRAS* activating mutation.

Conclusions

We have identified a novel translocation in a case of acute basophilic leukaemia. RNA sequencing can be used to identify previously unknown translocation partners. WES can be used to monitor clonal evolution and in this case revealed the genetic causes for its refractoriness to chemotherapy. These findings provide a rare insight into the pathogenesis and treatment resistance of acute basophilic leukaemia.

P005. Low rates of mortality from blood stream infection among high-risk haematology patients: a single centre retrospective analysis

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Aim

Blood stream infection (BSI) represents an important clinical problem in high-risk haematology patients. Here we have focused on organisms isolated, their resistance patterns, and mortality following proven BSI among high risk patients in our centre from 2010 - 2014.

Methods

235 patients receiving chemotherapy for acute leukemia or aggressive lymphoma at the Princess Alexandra Hospital in Brisbane, Australia were studied. Demographics, diagnosis, treatment, hospital admissions, blood culture (BC) results, absolute neutrophil count on presentation, and 30 day all-cause mortality following proven BSI were collected.

Results

Of the 958 admissions in the study period, 238 presentations were due to fever, with 33.2% of patients returning positive blood cultures for significant pathogens. The majority with positive BC were neutropenic (76.5%, $p = 0.0008$). Gram-negative organisms were dominant (82.3%), and only 7.5% of all organisms isolated were resistant to our empiric therapy (piperacillin/tazobactam and gentamicin). We demonstrate that for the 145 who were re-cultured during admission, a change in management was required for just 8 patients (5.5%), generally in the setting of prolonged hospitalisation and clinical deterioration.

To capture data from prolonged inpatient admissions, we analysed patients receiving induction therapy for acute myeloid leukemia (n=89). 9.2% of all BC collected were positive, and 13.3% of isolates were resistant to empiric antibiotics, in contrast to the ambulatory patients.

Finally, mortality within 30 days of blood stream infection was 3.4% over the study period, with just 8 patients in 5 years dying within 30 days of proven BSI.

Conclusion

BSI-related mortality in our centre is amongst the lowest in the literature, implicated organisms are predominantly Gram-negative and have low rates of antibiotic resistance. Our data support a conservative approach to repeat blood cultures in persistently febrile patients, as these rarely yield diagnostic information that alters patient management.

P006. A Case of Philadelphia-Positive B-Acute Lymphoblastic Leukaemia: Relapse Causing Obstructive Uropathy

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Aim
Solid organ relapse in Acute Lymphoblastic Leukaemia (ALL) is rare. Philadelphia-positive ALL (Ph+ ALL) has a highly unfavorable prognosis, largely due to haematological relapse. We contribute to the literature through reporting the first known case of relapsed Ph+ ALL involving the urogenital system in an elderly adult on tyrosine kinase inhibitor (TKI) therapy, who remained in haematological remission at time of relapse.

Method
Comprehensive literature review was performed (1-4), and revealed 16 previously reported cases of urogenital ALL, largely in the paediatric population and none on TKI therapy, validating the significance of our case.

Result
A 73-year-old female initially achieved complete haematological remission with dose-adjusted HyperCVAD chemotherapy and maintenance imatinib. Rising BCR-ABL transcript levels heralded molecular relapse 18 months later, but there was no evidence of morphological relapse on bone marrow biopsy. Treatment with high dose imatinib, followed by second generation TKI (dasatinib) was commenced, with an initial reduction in BCR-ABL transcript. However, 3 months later she presented with flank pain, haematuria, and rapidly progressive obstructive renal failure, requiring haemodialysis. Bladder biopsy revealed both morphological and cytogenetic evidence of relapsed Ph+ ALL. Due to poor prognosis, she was palliated. Peripheral blood BCR-ABL by that time was 50%, with no circulating blasts identified on peripheral blood. Subsequent BCR-ABL kinase domain mutation analysis identified the presence of V299L mutation, which confers resistance to dasatinib.

Conclusion
This case is a rarity, and highlights the utility of molecular studies in guiding leukaemia monitoring and treatment, as well as the potential role of TKI therapy in altering the disease course, prognosis and relapse pattern of Ph+ ALL (5).

P008. Recursive partitioning analysis for genetic stratification and prognostication of Acute Myeloid Leukaemia

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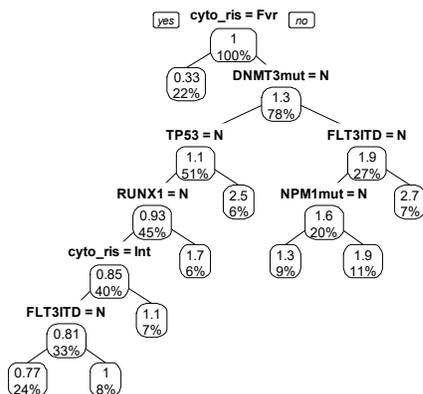
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Aim
To demonstrate the utility of an algorithmic machine-based learning approach to define independent prognostic subgroups in AML.

Method
Clinical and mutational data was collected from the CGA AML dataset with cases limited to an age of <70 years. Exome sequencing data from the CGA dataset (<http://cancergenome.nih.gov>) was analysed utilising Integrated Genomics Viewer (IGV) v2.3 with statistical analysis performed utilising R (R foundation for statistical computing) v3.2.3 and the rpart (v4.1-10), randomForest (v4.6-12) and randomForestSRC (v2.0.7) modules. The analysis was restricted to the 20 most commonly identified mutations within the CGA dataset to exclude uncommon mutations.

Results
Analysis set included 173 patients with clinical and genetic information. 143 were aged <70 years. The median overall survival (OS) was 24 months, median follow-up of 40.5 months. Recursive partitioning resulted in a decision tree highlighting the importance of favourable-risk karyotype and mutations affecting *TP53*, *RUNX1*, *DNMT3A*, *NPM1* and *FLT3-ITD*. (Figure 1). In non-CBF AML adverse prognostic impact was observed for *TP53*, *RUNX1* and *DNMT3A*, particularly with other 'driver' mutations, (e.g. *NPM1* and *FLT3-ITD*). Random-Forest Analysis confirmed the prognostic importance of these mutations predicting an error rate of 37.08%. CBF-AML and normal karyotype AML without high-risk prognostic molecular markers carried a favourable risk (median OS NR and 27 months, respectively). High-risk disease included *DNMT3A*^{mut} (median OS 11 months), *TP53*^{mut} (median OS 12 months), *RUNX1*^{mut} (median OS 11 months) and adverse-risk karyotype (median OS 12 months). Patients with *FLT3-ITD* (median OS 24 months) had an intermediate prognosis.

Conclusion
Recursive partitioning analysis allows for the formulation of a non-biased analysis tree and highlights the importance of combinations of mutations in determining prognosis for patients with AML. The impact of mutations affecting TP53, RUNX1 and the combination of mutations affecting DNMT3A with other driver mutations was relevant in patients with intermediate and poor risk karyotype.



N = absence of mutation (wild-type)
More adverse prognostic markers represented to the right
Internal numbers;
HR for death
% of total observations

P009. A linear study of clonal evolution during and after treatment in patients with acute leukaemia of ambiguous lineage

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Background

Acute leukaemia of ambiguous lineage (ALAL) includes acute undifferentiated leukaemia (AUL), mixed phenotype acute leukaemia (MPAL) with t(9;22), and MPAL, NOS (WHO 2008,2016). There is limited data on linear progression of leukaemic clones with treatment.

Aim

Assess the clonal evolution of ALAL with anti-leukaemic treatment.

Method

Nine cases of ALAL diagnosed from 2006-2015 were selected, and morphological, immunophenotypic, cytogenetic and molecular data on consecutive bone marrow biopsies were reviewed using WHO criteria. Patient demographics, anti-leukaemic therapy, response and outcome measures were collected.

Result

9 cases were included. Cases 1 and 2 diagnosed as MPAL B/myeloid on previous classifications were reclassified as acute lymphoblastic leukaemia (ALL) with aberrant markers – both achieved complete remission (CR) with ALL therapy. Case 3 diagnosed as MPAL, B/T (with myeloid markers) was treated with Acute Myeloid Leukaemia (AML) regimen with CR. Cases 4 and 5 had AUL – 1 was palliated and Case 5 (with complex cytogenetics), relapsed multiple times after AML therapy with the diagnostic clone, and died within 2 years.

Cases 6, 7 and 8 had MPAL, B/myeloid – case 6 was palliated, and case 7 achieved CR with ALL therapy+allogeneic stem cell transplant (alloSCT). Case 8 (with complex karyotype), had resolution of the ALL subclone with ALL therapy. Expansion of the myeloid clone occurred within 1 month and CR was achieved with AML therapy+ AlloSCT. Case 9, MPAL with t(9;22), achieved CR with ALL therapy + tyrosine kinase inhibitor but relapsed 2 years later with an AML subclone. 3 year mortality of the group was 44%.

Conclusion

Patients in our ALAL cohort were treated variably with ALL and AML regimens. Linear analysis showed that choice of treatment can lead to the expansion of small sub-clones noted at initial diagnosis. Further analysis with next generation sequencing is planned to further elucidate the evolution of sub-clones.

P010. The incidence of Ph-like Acute Lymphoblastic Leukaemia (ALL) increases with age and is characterised by poor outcome

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Aim

ALL remains a challenging haematological malignancy with a 5 year survival of only ~40% in adults. Recent advances in understanding key genomic events driving ALL have led to the identification of a new sub-type termed Philadelphia chromosome (Ph)-like ALL. Notably, case reports describe the successful use of tyrosine kinase inhibitors in this setting. We sought to identify the frequency and outcome of Ph-like disease in an Australian cohort.

Method

We have screened 60 adolescent/young adults (AYA) (aged 16-39) and 100 adults (>40 years) diagnosed with pre-B-ALL from 1987-2016 for Ph-like ALL using a custom Taqman Low Density Array (TLDA). Fusions were identified by Sanger sequencing, FISH or mRNA sequencing. Available outcome data was analysed by R-Studio.

Results

The frequency of Ph-like ALL in AYA was 20.3% (11/54) and increased with age to 29.8% (20/67) in adults (excluding Ph+ cases) (Table 1). Ph-like ALL patients were significantly more likely to relapse compared to pre-B-ALL patients ($p=0.03$). However, when separated, this remained significant only for AYA cases ($p=0.049$). Upon investigation, 94% (16/17) of Ph-like and 80% (32/40) of other pre-B-ALL adult patients died within 3.5 years of diagnosis. In contrast, patients with Ph+ ALL had improved relapse rates (RFS 69% n=22 where data available) and survival (OS 73% at 3.5 years), likely due to the use of tyrosine kinase inhibitors (TKIs).

Conclusion

Significantly we demonstrate that the incidence of Ph-like ALL increases with age, with Ph+ and Ph-like ALL representing the majority of cases of pre-B ALL adults diagnosed. While these results may not be reflective of contemporary protocols, the outcomes for adults with pre-B-ALL and in particular those with Ph-like ALL are poor. Importantly, rapid identification of the fusion and TKI sensitivity at diagnosis of Ph-like ALL patients may have substantial clinical impact, as observed in Ph+ patients treated with TKIs.

Table 1. Ph-like ALL fusions and outcome identified in adults and AYA. N/A – data not available.

	Fusion	n	Relapsed	N/A
Adults	IGH-CRLF2	10	6	1
	CRLF2r	1		1
	IGH-EPOR	1	0	
	GOLGA4-JAK2	1		1
	RCSD1-ABL2	1	0	
	ETV6-ABL1	1	1	
	Unknown	5	1	1
AYA	IGH-CRLF2	3	3	
	P2YR8-CFLF2	3		2
	EBF1-PDGRFB	2		
	ATF7IP-JAK2	1	1	
	PAX5-ETV6	1		
	Unknown	1	1	

P013. Active treatment for selected elderly patients with acute myeloid leukemia improves survival – results from establishment of acute leukemia service at the Northern Hospital

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Background

The incidence of acute myeloid leukemia (AML) increases with age. However, the outcome of elderly AML patients remains poor, with 3-year and 5-year overall survival (OS) <10%. Previous studies demonstrated that elderly patients receiving active treatment such as intensive or low dose chemotherapy and azacitidine, had survival benefit over those who only received supportive care. From 2014, the Northern Hospital (TNH) commenced an acute leukemia service, with the aim of delivering quality leukemia treatment to elderly patients closer to home. We examine the impact of active treatment in elderly patients with newly diagnosed AML.

Method

A retrospective analysis was performed for 22 newly diagnosed elderly AML patients (age >60 years) between 2014-2015, with median follow up of 153 days. Active treatment included intensive/low dose chemotherapy and azacitidine.

Results

Patients had median age of 73 (61-88) years, with male predominance (64%). 13 (59%) patients had secondary AML. 12 (75%) and 4 (25%) of 16 patients with known cytogenetic results had intermediate and poor classification respectively. 14 of 22 patients had active treatment, with most having good performance status (ECOG 0-1). 6 patients received intensive induction chemotherapy, whilst 8 patients received either low dose chemotherapy or azacitidine. The remaining 8 patients only received supportive treatment mainly due to poor performance status (ECOG 3, n=3) or advanced age (age >85y.o, n=3). There was significant improvement in OS of patients receiving active treatment vs supportive care (Median OS of 235 vs 40 days, p=0.0068). The 1-year OS was 64% vs 13% for active treatment vs supportive care respectively.

Conclusion

We confirm findings from previous studies that delivering treatment to selected elderly patients with good performance status resulted in significantly improved survival, supporting ongoing development of the elderly leukemia service at TNH.

P014. STIMULUS Induces Complete And Occasional Durable Responses In Elderly Patients With Azacitidine Refractory Acute Myeloid Leukaemia

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Elderly patients with Acute Myeloid Leukaemia (AML) have a poor prognosis, particularly those who progress following Azacitidine therapy. STIMULUS is a regimen of low dose cytarabine and thioguanine given on an outpatient basis with published efficacy in elderly AML. We reviewed outcomes of AML patients treated with STIMULUS following Azacitidine treatment failure at UGH, between Feb 2014 and July 2016. Of 7 patients, 4 responded, 2 with CR and 2 with haematological improvement. Responses were rapid (within 4-6 weeks of commencing treatment) and 3 of 4 responding patients exceeded the 3.4 month median life expectancy for this patient population. 1 patient is still alive 9 months after commencing therapy with good QOL. STIMULUS was well tolerated and is a cheap and effective option for patients with Azacitidine treatment failure who do not wish to adopt a purely supportive or palliative treatment approach.

Table 1

Case	1 (JB)	2 (LM)	3 (GL)	4 (WR)	5 (TK)	6 (DP)	7 (PM)
Diagnosis	AML	AML	t-AML	AML with MDS-related changes	AML with MDS-related changes	AML post MF	AML
Age /Sex	62yo F	70yo F	64yo F	88yo M	76yo M	65yo M	72yo F
Karyotype	Normal	Complex	Complex	Normal	Normal	Normal	Normal
Molecular studies	DNMT3A and CEBPA mutations present						
Best response	No response	CR	No response	No response	CR	Clearance PB blasts*	Clearance PB blasts*
Time to best response	NA	4 weeks	NA	NA	4 weeks	6 weeks	4 weeks
Toxicity	Nil	Febrile neutropenia	Nil	Nil	Myelosuppression	Myelosuppression Local irritation	Nil
<u>Survival from D1 STIMULUS</u>	2 months	6 months	1 month	1 month	9 months	4 months	3 months
Current status	Deceased	Deceased	Deceased	Deceased	Alive	Alive	Deceased

Definitions: t-AML: therapy related AML; MDS: Myelodysplasia; MF: Myelofibrosis; AMML: Acute Myelomonocytic Leukaemia

Clearance of PB blasts –clearance of blasts from peripheral blood, counts stable/improved but not normal, BMAT not performed

P016. Synergistic Targeting of Signalling Pathways Activated by the FLT3 Kinase in Acute Myeloid Leukaemia

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Aim

Carrying a 5-year survival rate of 24%, Acute Myeloid Leukaemia (AML) is the most lethal form of leukaemia. Current treatments include high-dose chemotherapy and bone marrow transplantation; however development of chemotherapy resistance and relapse is common. Internal Tandem Duplication (ITD) mutation of the receptor tyrosine kinase FLT3 is the most recurrent driver mutation in AML, leading to constitutive activation of the receptor, poor prognosis, and increased risk of relapse. Sustained therapeutic inhibition of FLT3 has proven difficult to achieve, with FLT3 inhibitors displaying limited success as single agents. Characterisation of the oncogenic signalling pathways downstream of FLT3 mutation is therefore required to identify novel therapeutic targets.

Method

The phosphoproteomes of 6 AML patient blast samples (3 x FLT3-wildtype, 3 x FLT3-ITD) were evaluated by label-based comparative and quantitative mass spectrometry. Pathways displaying differential phosphorylation in FLT3-ITD versus FLT3-wildtype patients were identified using Ingenuity Pathway Analysis. Druggability of identified pathways was assessed using resazurin assay; in FLT3-ITD and FLT3-wildtype transduced Ba/F3 cells, and a panel of AML cell lines (MV4-11 (FLT3-ITD), and HL-60, Kasumi-1, and THP-1 (FLT3-wildtype)).

Result

Three of the top four phosphorylation networks in FLT3-ITD patients showed alteration of proteins required for DNA repair, with downregulation of Base Excision Repair (BER) and upregulation of Non-Homologous End Joining (NHEJ). Targeting of NHEJ using DNA-PK inhibitor NU7441 effected selective toxicity in FLT3-ITD cell lines. Combined administration of NU7441 with low dose cytarabine elicited synergistic cell death in FLT3-ITD but not FLT3-wildtype AML cell lines. Similarly, FLT3 inhibitor sorafenib also displayed synergy in combination with NU7441, in FLT3-ITD cell lines.

Conclusion

Results presented support that increased activation of the NHEJ pathway occurs downstream of FLT3-ITD. Targeting NHEJ in combination with standard chemotherapy agents in FLT3-ITD AML has the potential to improve outcome in this poor responding AML subtype.

P017. MicroRNA-211 - a novel oncogene in acute myeloid leukemia

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MicroRNAs are a class of small, non-coding RNA molecules that regulate gene expression by post-transcriptional silencing and regulate many critical cellular processes. In cancer, microRNAs are emerging as important biomarkers of disease with distinct microRNA signatures associated with cancers of different tissues, subtypes of the disease and as predictors of patient outcome. A subset of microRNAs also has well-established roles as drivers of cancer development and is a potential therapeutic target. In a model of induced expression of an oncogene in primary murine myeloid cells, we recently identified 70 microRNAs that were differentially expressed in the presence and absence of the oncogene. This raised the hypothesis that at least some of these microRNAs have roles in the regulation of myeloid differentiation and in the development of myeloid leukemia. MicroRNA-211 (miR-211) was the most differentially expressed microRNAs in this model, and is the focus of the work presented here.

MiR-211 is an intronic microRNA encoded on human chromosome 15 and deregulated miR-211 expression has been previously described in melanoma and other cancers. The role of miR-211 in hematological malignancies, however, is unknown. Using hematopoietic reconstitution experiments in mouse models, our data shows that enforced expression of miR-211 in hematopoietic stem cells drives a partially penetrant myeloid disease, phenotypically resembling myeloid leukemia. Mice overexpressing miR-211 in hematopoietic cells demonstrate an increased proportion of immature myeloid cells in their bone marrow and spleen, with monoblastic cellular morphology. This is accompanied by severe anaemia and thrombocytopenia, and infiltration of immature hematopoietic cells into the spleen, liver and extramedullary spaces of the bone marrow. Preliminary data from a cohort of paediatric leukemic patients showed that elevated expression of miR-211 can be detected in a subset of patients with the M5 subtype of acute myeloid leukemia, suggesting miR-211 may have a previously unidentified role in AML.

P018. Successful Transplant Outcome in a Rare Case of Mast Cell Leukaemia

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Introduction

Mast cell leukemia is a rare form of aggressive systemic mastocytosis and remains a diagnostic and therapeutic challenge. It carries a poor prognosis and management is complicated by symptoms of mast cell activation and allergic reactions. Therapeutic options are limited and the disease is often fatal within a few months. The role of allogeneic stem cell transplantation remains undefined, and studies have showed a potential role for graft versus mast cell effect in reducing the mast cell burden. We report a rare case of aggressive mast cell leukaemia that responded well to cytoreductive therapy and proceeded to matched unrelated allograft with a good outcome.

Case Discussion

44 year old male was diagnosed with mast cell leukemia after presenting with pancytopenia, hepatosplenomegaly, coagulopathy, extensive skeletal involvement and life threatening mast cell activation symptoms including anaphylactic allergic reactions. Investigations showed markedly elevated tryptase level of 410 ng/L, bone marrow trephine demonstrated diffuse infiltrate of neoplastic mast cells, and positivity for the *KITD816V* mutation. Management was complicated by a background of schizophrenia, depression, type II diabetes mellitus and severe psoriasis.

His mediator release symptoms and allergic reactions responded to histamine blockade, mast cell stabilizers and steroids. Cladribine was effective at debulking the disease. He proceeded to have a matched unrelated allograft with myeloablative conditioning regimen and was successfully weaned off immune suppression with no evidence of active graft versus host disease. He remains well day 300+ post transplant with no symptoms of mast cell activation.

Conclusion

This rare case of aggressive systemic mastocytosis highlights the strategies employed to treat this rare and aggressive condition. Management consists of addressing symptoms of mast cell activation, de-bulking neoplastic mast cells with cytoreductive therapy and allogeneic stem cell transplantation in the eligible.

P019. Therapy-related acute myeloid leukaemia in Victoria, Australia 1982-2014

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Introduction

Awareness of therapy-related AML(tAML) is important due to its iatrogenicity and potential impact on cancer survivorship. tAML evolution in Australia over time has not been characterized.

Method

Cancer Council Victoria(CCV) data extracted for all AML diagnoses age \geq 20 between 1982-2014, including history of prior cancers. tAML was identified as ICD-O-3.1 morphologic code 9920/3. Cases where first malignancy was MPN or MDS were defined as secondary AML (sAML). Incidence rates were calculated using Australia Bureau Statistics data. Statistical analyses performed using GraphPad-Prism v6.0g 2015.

Results

tAML comprised 1.35% of adult AML. The incidence appears to be rising(Figure 1), with median age 66(range 24-87) and no gender predominance(males 50.6%). Median overall survival for tAML was similar to other AML cases(6.5 vs 6 mo, p=0.1143), however 26(31% tAML) and 897(15% non-tAML) had insufficient data for survival analyses. Time from first malignancy to tAML varied widely(median 82 months, range 6-373), with peaks at 1-4 and 7-8 years after first malignancy. Many occurred >10 years after the primary malignancy. Of 59 tAML cases with assessable data, almost half had prior non-Hodgkin lymphoma(NHL)(30%) or breast cancer(BC)(16%), consistent with the international literature. To determine the potential for underestimation of the true tAML caseload, we identified AML cases with \geq 1 prior malignancy(excl non-melanoma skin cancers) not classified as tAML(Table 1) as some of these cases are likely to have received chemotherapy/radiotherapy. The potential upper boundary of tAML incidence is shown in Figure 1.

Conclusion

Within limitations of a retrospective population cancer registry analysis, we propose that tAML incidence may be increasing, is likely underestimated and most commonly has BC and NHL treatment as prior causes. Risks are greatest after 1-4 and 6-8 years after original cancer diagnosis. For cases occurring >10 years after original cancer, distinction between tAML and true *de novo* AML is likely to require molecular-level dissection.

Figure 1. Incidence rate of AML in Victoria per 100 000 persons, by year, 1982-2014

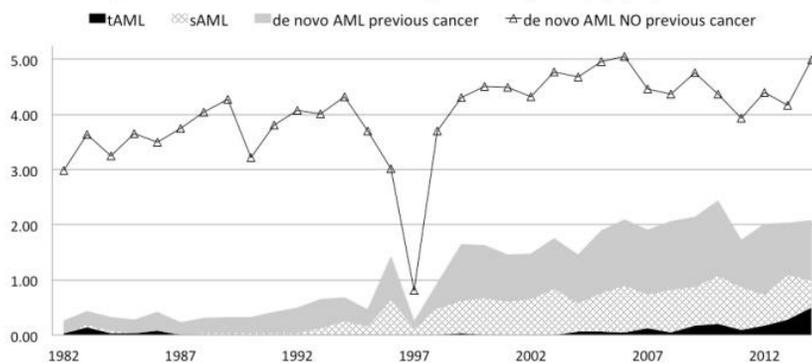


Table
patients
1.
in

Number of prior cancers in AML
Victoria, 1982-2014

	1	2	3	4
tAML	48	11	1	0
sAML	473	30	2	0
Presumed <i>de novo</i> AML with prior history of cancer	706	125	25	3

P020. Donor Derived Acute Myeloid Leukaemia 11 years Post Allogeneic Stem cell Transplantation for Multiple Myeloma

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Background

There have been reports of acute leukaemia in patients with a history of multiple myeloma. This is likely due to a complex relationship between patient factors and previous therapy including stem cell transplantation. Donor derived leukaemia is a rare entity.

Aim

To present the case of a patient with multiple myeloma with a history of autologous and allogeneic stem cell transplantation and chronic graft versus host disease on longterm Thalidomide, who was found to have acute myeloid leukaemia with no overt clinical signs.

Methods

Case report and literature review

Result

A 54 year old man with a history of non-secretory multiple myeloma diagnosed in 1999 treated with VAD chemotherapy and autologous stem cell transplant 2000 and Fludarabine/Melphalan reduced intensity conditioning sibling allogeneic transplant from his sister in 2005, was noted to have isolated neutropenia (neutrophils 1.1) on routine blood tests. Also as part of surveillance for non-secretory multiple myeloma, he underwent PET/CT which showed multiple areas of uptake mainly in the thoraco-lumbar spine. He was clinically well although had evidence of chronic graft versus host disease affecting the eyes, mouth and skin. Bone marrow biopsy showed 52% blasts and <1% plasma cells. Flow cytometry confirmed these as myeloblasts with the immunophenotype: CD45 weak, CD13+, CD33+, CD34++, HLA-DR+, MPO+ and no clonal plasma cells were detected. Cytogenetics confirmed 46, XX[40] i.e. all cells being female donor cells with no cytogenetic abnormality. A diagnosis of acute myeloid leukaemia derived from donor cells was made and the patient commenced induction therapy. The donor remains clinically well with a normal full blood count.

Conclusion

Patients may develop acute leukaemia many years after treatment for multiple myeloma. This may be secondary to previous therapy. Donor derived acute leukaemia is rare with postulated telomere shortening contributing to the pathogenesis of leukaemia with a long latency period from allogeneic transplantation. The prognosis of donor derived leukaemia generally is poor.

P021. Investigation of CRLF2-rearranged Acute Lymphoblastic Leukaemia Cases Reveals Two Groups With Distinct Secondary Lesions

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Overexpression of *Cytokine receptor-like factor 2* (*CRLF2*) occurs in 5-15% of ALLs via a cryptic deletion juxtaposing *CRLF2* to the promoter of *P2RY8* (*P2RY8-CRLF2*), or from the translocation of *CRLF2* to immunoglobulin heavy-chain locus enhancer elements (*IGH-CRLF2*). *CRLF2* overexpression requires cooperating lesions for leukaemic transformation, and 30-50% of *CRLF2*-rearranged (*CRLF2-r*) cases harbor activating *JAK2* mutations. Recent reports demonstrate that *CRLF2-r* are enriched in Ph-like ALL, a high-risk ALL sub-type, and here we show that ALL *CRLF2-r* cases associated with a Ph-like signature represent a distinct genetic sub-group likely reflecting underlying differences in secondary lesions and disease progression.

We screened 630 paediatric and adult ALL samples and identified 40 *CRLF2-r* cases (*P2RY8-CRLF2* n=20; *IGH-CRLF2* n=20) including 5 matched diagnosis and relapse samples. Activating *JAK2* mutations were found in 14/40 (35%) cases, and consistent with prior reports these samples showed response to JAK inhibitors *in vitro*. The transforming *CLRF2* F232C mutation was identified in an additional six samples (15%), and *IKZF1* deletions were detected in 24/40 (60%) of patients. Using a customized 9-gene assay we found that the majority of cases (30/40) classified as Ph-like and genome-wide expression analysis confirmed distinct clustering of these samples. Interestingly, we observed a significant association of *IKZF1* alterations and *JAK2/CRLF2* mutations in patients with a Ph-like signature, suggesting that mutation-driven *CLRF2/JAK2* activation occurs more frequently in this sub-group. While less is understood about the genetics of *CRLF2-r* cases lacking *JAK2* pathway mutations, we identified *KRAS/NRAS* mutations in 4/10 non-Ph-like cases, which correlated with increased pERK signaling, and negligible response to JAK inhibitors *in vitro*. Our findings suggest that intracellular pathway activation and drug sensitivity in *CRLF2-r* patients may be influenced by the type of secondary lesions present, and highlight the heterogeneity of ALL which can be observed within a subset of patients that harbor identical translocations.

P022. Improved Concordance of Minimal Residual Disease Measurements by Quantitative PCR and 10-colour flow cytometry in Paediatric Acute Lymphoblastic Leukaemia

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Detection of minimal residual disease (MRD) after induction and consolidation therapy is highly predictive of outcome for childhood acute lymphoblastic leukaemia (ALL) and is used to identify high risk patients in most current ALL clinical trials. Two methods broadly applicable for MRD analysis in ALL cases are real-time quantitative PCR and multi-parameter flow cytometry. We compared the two techniques using samples from patients referred for PCR-MRD analysis initially using 2-tube 4-colour flow and more recently 1-tube 10-colour flow.

Newly diagnosed consented ALL patients enrolled on ANZCHOG ALL8 (2002-2011) or AIEOP-BFM ALL 2009 (2012-2014) had duplicate bone marrow aspirates, collected at diagnosis, day 15, day 33 and day 79, and analysed by PCR-MRD and Flow-MRD techniques.

Our early comparison showed a relatively poor correlation of 4-colour flow-MRD results with PCR-MRD (Spearman rank value $\rho = 0.516$, $n = 267$) for patients enrolled at a single centre on ANZCHOG ALL8 in 2002-2009. Flow-MRD for subsequent patients on this trial (2010-11) was improved by using more antibodies and adopting a single tube approach. In our current trial, in which day 15 flow-MRD results are used for the early identification of low risk patients for a randomized treatment reduction, the correlation of the PCR-MRD and Flow-MRD methods is high ($r^2 = 0.803$). A comparison of time points found that the best correlation between the two methods was observed at day 15 when MRD is higher and the bone marrow is not regenerating. Both PCR and 10-colour flow enabled MRD to be performed for 94% of ALL patients.

We conclude that these two methods can now be used interchangeably at day 15 in BFM-style protocols for ALL patients. The concordance at later time points is weaker and warrants investigation in the whole trial cohort to enable effects of ALL subtype and patient outcomes to be evaluated.

P024. Mutant NPM1 outcomes in Acute Myeloid Leukaemia (AML) - a single institution analysis

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Background

Mutated *NPM1* plays a key prognostic role in normal karyotype (NK) AML. We aimed to examine outcomes associated with mutant *NPM1* in cytogenetic intermediate risk patients 1) with NK and non-NK; 2) with concurrent *FLT3*-ITD; and 3) following relapse.

Method

98 AML patients with intermediate-risk cytogenetics diagnosed between June 2007 and September 2015, who received induction chemotherapy and for whom *NPM1* and *FLT3*-ITD status was known were included. Statistical analyses were performed using GraphPad Prism v7.0.

Results

77% had NK AML with 58% *NPM1*^{MUT}. 23% had non-NK AML with 20% *NPM1*^{MUT} (Table 1). *NPM1*^{MUT} was associated with improved relapse-free survival (RFS) and overall survival (OS). RFS censored for stem cell transplantation stratified patients into four prognostic risk groups (*NPM1*^{MUT}/*FLT3*^{WT} > *NPM1*^{MUT}/*FLT3*-ITD > *NPM1*^{WT}/*FLT3*^{WT} > *NPM1*^{WT}/*FLT3*-ITD; data not shown). The OS, however, for *NPM1*^{MUT} was similar, independent of the presence of *FLT3*-ITD (Fig 1A). To explain this, we note that the rate of stem cell transplantation in CR1 was higher in those with concurrent *NPM1*^{MUT}/*FLT3*-ITD (43%) than *NPM1*^{MUT}/*FLT3*^{WT} (27%) (Table 1). Also, HiDAC-based induction resulted in superior RFS outcome for *NPM1*^{MUT}/*FLT3*^{WT} and *NPM1*^{MUT}/*FLT3*-ITD than other genotypes (Fig 1B). In contrast, RFS outcomes for patients receiving 7+3 were not affected by *NPM1*^{MUT} genotype (Fig 1C). The median age of the HiDAC group (41.5 years) was younger than the 7+3 group (60.7 years). After relapse, *NPM1*^{MUT} genotype had no influence on CR likelihood (Table 1) or overall survival (Fig 1D).

Conclusion

In cytogenetic intermediate risk AML, *NPM1*^{MUT} is more common in those with NK and associated with superior OS. OS was not impacted by *FLT3*-ITD in the *NPM1*^{MUT} group. Prognostic relevance for *NPM1*^{MUT} was less significant in older patients and following relapse.

Table 1: Characteristics of molecular subgroups

	<i>NPM1</i> ^{WT} <i>FLT3</i> -ITD	<i>NPM1</i> ^{MUT} <i>FLT3</i> -ITD	<i>NPM1</i> ^{WT} <i>FLT3</i> ^{WT}	<i>NPM1</i> ^{MUT} <i>FLT3</i> ^{WT}	Total
Total [n, (%)]	7 (7.1%)	24 (24.5%)	43 (43.9%)	24 (24.5%)	98
Attained CR1 [n, (%)]	6 (86%)	21 (88%)	38 (88%)	22 (92%)	87 (88%)
Median age (years)	37.1	54.0	51.9	58.0	53.7
Sex (n=87)					
Male	4 (67%)	14 (67%)	18 (47%)	9 (41%)	45 (52%)
Female	2 (33%)	7 (33%)	20 (53%)	13 (59%)	42 (48%)
Karyotype					
NK	2 (33%)	20 (95%)	26 (68%)	19 (86%)	67 (77%)
non-NK	4 (67%)	1 (5%)	12 (32%)	3 (14%)	20 (23%)
Induction chemotherapy					
HiDAC/HiDAC3	4 (67%)	7 (33%)	16 (42%)	8 (36%)	35 (40%)
7+3	0	9 (43%)	17 (45%)	12 (55%)	38 (44%)
Transplant					
Total transplants	4 (67%)	12 (57%)	18 (47%)	7 (32%)	41 (47%)
Transplanted in CR1	4 (67%)	9 (43%)	7 (18%)	6 (27%)	26 (30%)
Relapsed [n= 40]	5 (83%)	7 (33%)	20 (53%)	8 (36%)	40 (46%)
Relapsed pts who attained CR2	3 (60%)	4 (57%)	10 (50%)	3 (38%)	20 (50%)

P025. Treatment with anti-CD19 BiTE® blinatumomab in adult patients with relapsed/refractory B precursor acute lymphoblastic leukemia (r/r ALL) post-allogeneic hematopoietic stem cell transplantation (alloHSCT)

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Aim

Blinatumomab, a bispecific T-cell engager (BiTE®) antibody construct, has antileukemia activity in r/r ALL. We characterized a patient subset with r/r ALL and prior alloHSCT before blinatumomab treatment in a phase 2 study.

Method

Eligible adults had Ph- r/r ALL and one negative prognostic factor (primary refractory, 1st relapse <12 mo, post-HSCT relapse <12 mo, ≥2nd salvage). Active graft-versus-host disease (GvHD) was not allowed; immunosuppressive GvHD therapy had to be stopped ≤2 weeks before blinatumomab. Patients received blinatumomab by continuous IV infusion (4 weeks on/2 weeks off). The primary endpoint was complete remission (CR) or CR with partial hematologic recovery (CRh) within the first two cycles.

Result

64/189 (34%) patients had prior alloHSCT (matched sibling, 45%; unrelated, 48%). Median age was 32 (range, 19–74) years. At baseline, 36%, 38%, and 27% of patients had 1, 2, and ≥3 prior relapses; 69% had salvage therapy after last alloHSCT and before blinatumomab; 30% had prior GvHD; and 66% had ≥50% bone marrow blasts. 29/64 (45%; 95% CI, 33–58) patients achieved CR/CRh within the first 2 cycles. Median OS was 8.4 (4.2–9.4) months, and median RFS was 6.1 (5.0–7.7) months (median follow-up, 8.8 mo). 22/29 (76%) responders had minimal residual disease (MRD) response; 19 (66%) achieved complete MRD response. 56/64 (88%) patients had grade ≥3 treatment-emergent AEs, mostly neutropenia (22%), febrile neutropenia (20%), anemia (17%), and thrombocytopenia (14%). Six patients had GvHD (two grade ≥3) during blinatumomab; three had GvHD in skin. Eight patients had fatal AEs (gastrointestinal hemorrhage, n=1; respiratory failure, n=1; infection/infestation, n=6); one (candida infection) was possibly treatment-related per the investigator. No fatal AEs occurred during remission.

Conclusion

In heavily pretreated patients with r/r ALL and prior alloHSCT, blinatumomab induced a CR/CRh rate of 45%, with an AE profile consistent with that previously reported.

This research was supported by Amgen Inc. Amgen Inc. conducted the data analysis. The abstract was prepared by Ben Scott, PhD (Scott Medical Communications, LLC), whose work was funded by Amgen Inc.

P026. Outcomes of hematopoietic stem cell transplantation (HSCT) among adults with relapsed/refractory (r/r) acute lymphoblastic leukemia (ALL) achieving remission with blinatumomab

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Aim

We report outcomes from a multicenter, open-label phase 2 study in patients with r/r ALL who received HSCT after achieving complete remission (CR) or CR with partial hematologic recovery (CRh) with blinatumomab, a bispecific T-cell engager (BiTE[®]) antibody construct.

Method

Eligible adults had Philadelphia chromosome-negative r/r B-precursor ALL (primary refractory, relapsed <12 months after first remission or HSCT or ≥2 salvage treatments). Up to 5 cycles (4 weeks on, 2 weeks off) of blinatumomab were given by continuous IV infusion. CR/CRh in the first 2 cycles was the primary endpoint.

Result

Of 189 patients treated with blinatumomab, 83 (44%) achieved CR/CRh during the first 2 cycles. Median OS follow-up was 13.4 months post-HSCT. Overall HSCT realization rate in remission was 41% (34/83): 50% (27/54) for HSCT-naïve patients, 24% (7/29) for patients with prior HSCT. HSCT recipients received a median of 2 blinatumomab cycles (range, 1–5) before HSCT. Details of conditioning regimens were provided for 28 patients (data unavailable: 6 patients): 54% myeloablative, 43% reduced intensity, and 4% unknown. Conditioning regimens were initiated a median of 23 days (range, 8–60) after the end of blinatumomab treatment. 80% of HSCT recipients had MRD response (<10⁻⁴); 77% had complete MRD response (undetectable) before HSCT. 4 (12%) patients died within 100 days post-HSCT in remission due to infection (n=3) and GvHD (n=1). Kaplan-Meier estimates for cumulative OS and RFS at 12 months post-HSCT were 73% (95% CI, 55–85) and 53% (95% CI, 34–69), respectively.

Conclusion

In this phase 2 study, 41% of responders to blinatumomab received HSCT in remission; 80% achieved an MRD response before HSCT. Blinatumomab could be administered in close temporal proximity to myeloablative or reduced intensity conditioning regimens without evidence of increased treatment-related mortality. Blinatumomab served as an effective salvage regimen and bridge to transplant.

This research was supported by Amgen Inc. Amgen Inc. conducted the data analysis. The abstract was prepared by Ben Scott, PhD (Scott Medical Communications, LLC), whose work was funded by Amgen Inc.

P027. Allelic ratio in FLT3-ITD+ AML does not correlate with outcome

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Introduction

FMS-like tyrosine kinase 3 – internal tandem duplication (FLT3-ITD) mutations are associated with a worse prognosis in acute myeloid leukaemia (AML). Recent literature suggests that a mutant to wild type allelic ratio (AR) > 0.5 is associated with worse outcomes.

Aim

To assess the impact of allelic ratio on survival in FLT3-ITD+ AML with normal cytogenetics.

Methods

A database search was conducted to identify all cases of FLT3-ITD + AML diagnosed in the Pathology Queensland Laboratory since 2008. A retrospective review of patient charts, pathology, pharmacy and clinical databases was conducted. 71 patients were eligible, who were treated at five centres throughout Queensland. Data was collected on patient characteristics including FLT3-ITD ratio, treatment and response, transplant status, relapse and overall survival.

The primary endpoints for this study were overall survival (OS), event-free survival (EFS), and remission duration (RD).

Results

Median age at diagnosis was 51.4 years. 52 patients had a measured allelic ratio. The median allelic ratio was 0.23. 11 (21%) patients had an AR > 0.5.

The CR rate after one or two cycles of induction was 83% and 100% for patients with AR ≤0.5 and > 0.5 respectively. Baseline characteristics between patients who had AR ≤0.5 or > 0.5 were not statistically different.

20 of 34 (59%) patients with AR ≤0.5 who achieved CR relapsed compared to 5 of 11 (45%) patients with AR >0.5.

The median OS for patients with AR ≤0.5 was 11.3 months vs. 11.8 months in patients with AR>0.5. EFS was 6.4 and 7.0 months for AR ≤0.5 and >0.5 respectively. RD was 8 and 6 months for AR≤0.5 and >0.5 respectively.

There was no significant difference in median OS, EFS and RD when the population was analysed according to AR above or below the median of 0.23.

Conclusion

Our study did not demonstrate a relationship between level of FLT3-ITD allelic burden and outcome. We do not recommend using FLT3-ITD ratio to guide treatment. Further prospective studies analysing impact of allelic ratio in FLT3-ITD AML are required.

P028. Clinical Significance Of Isocitrate Dehydrogenase, Wilms' Tumor 1 and TET2 mutations in Myelodysplastic Syndromes and Acute Myeloid Leukaemia

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Wilms' Tumor 1, IDH and TET2 proteins cooperate to mediate DNA hypomethylation. Recurrent mutations of these genes are associated with AML and MDS. Therefore they may be important in the pathogenesis of AML and transformation of MDS. These mutations may be correlated with prognosis and response to chemotherapy and Azacitidine. Moreover, IDH inhibitors and WT1 vaccines are being studied by other groups as possible therapeutic agents.

Aim

1. To investigate the clinical and prognostic significance of IDH 1/2, Wilms' Tumor and TET2 mutations in MDS and AML.
2. To correlate mutation status with response to chemotherapy or Azacitidine.

Method

50 AML patients and 50 MDS patients were tested for mutations of IDH 1/2, WT1 and TET2 by PCR, fluorescent melt curve analysis, fragment analysis and Sanger Sequencing. The clinical characteristics, morphology, karyotype, genetic mutations, response to treatment and survival of patients were analysed and correlated with mutational status.

Results

MDS cohort: n=50; RCMD (46%) and MDS RAEB -2 (20%). There was no IDH1 mutation. IDH 2 mutation was detected in 1 MDS patient (2%). This patient was treated with supportive therapy and died at 17 months post diagnosis. Sanger sequencing confirmed this mutation as IDH 2 R172K. The results for WT1 and TET2 will be presented in the meeting.

AML cohort: n=50; 3 had WT1 mutations and 4 had IDH1 mutations. They were mutually exclusive. WT1 and IDH1 mutations might be associated with poor prognosis. 1 out of 4 patients with IDH1 mutation was treated with Azacitidine and had a survival of 31 months.

Conclusions

The clinical significance of IDH, WT1 and TET2 mutations in AML and MDS is still unclear. These proteins appear to mediate DNA hypomethylation. Therefore future studies and detection of these mutations is important in deciphering their role in pathogenesis, prognostic prediction and targeted therapy.

P029. miR-10a expression is regulated by methylation in NPM1-mutant Acute Myeloid Leukaemia

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Aim

Increasing evidence has demonstrated important roles for microRNAs in cancer. Acute Myeloid Leukemia (AML) with Nucleophosmin 1 (*NPM1*) gene mutation (*NPM1^{c+}*) accounts approximately 30% of AML cases. Our previous work demonstrated that miR-10a was expressed >13-fold higher in *NPM1^{c+}* AML versus *NPM1*-wildtype (*NPM1^{WT}*) patient samples. Furthermore, it may have a pro-survival role in this subtype via its direct regulation of several target genes including RB1CC1, TFAP2C, and KLF4. Here we aimed to investigate the underlying mechanism of miR-10a overexpression in *NPM1^{c+}* AML.

Results

Consistent with our previous analysis of primary patient samples, we observed a high level of miR-10a in the *NPM1^{c+}* OCI-AML3 cell line versus *NPM1^{WT}* lines. This was concordant with hypomethylation of the miR-10a promoter in *NPM1^{c+}* cell lines compared to *NPM1^{WT}* cell lines HL60 and MV4 (<10% vs >60% CpG methylation). Furthermore, exposure of the *NPM1^{WT}* AML cells to hypomethylating agents decitabine and azacitidine resulted in reduced DNA methylation (2-fold) of the miR-10a promoter and a corresponding >3-fold upregulation of miR-10a ($p < 0.5$ for all lines). A strong negative correlation between miR-10a promoter methylation and gene expression in *NPM1^{c+}* primary AML patient samples was also observed. These data suggest that hypomethylation of the miR-10a promoter leads to dysregulated expression and reveals the likely mechanism between *NPM1* mutation and high miR-10a. Examining the association of miR-10a promoter hypomethylation with mutations of epigenetic modifiers such as DNA methyltransferase 3A (DNMT3A), which is highly enriched in >60% of *NPM1^{c+}* AML, show no statistically significant association, suggesting that the overexpression of miR-10a is not due to DNMT3A mutation.

Conclusion

Overexpression of the pro-survival miRNA miR-10a in *NPM1^{c+}* AML is epigenetically regulated. This may have clinical significance and suggests a novel mechanism of action by which epigenetic modifiers such as decitabine and azacitidine may act in this subgroup.

P030. Simultaneous Acute Promyelocytic Leukaemia and Relapsed Hairy Cell Leukaemia following Cladribine therapy

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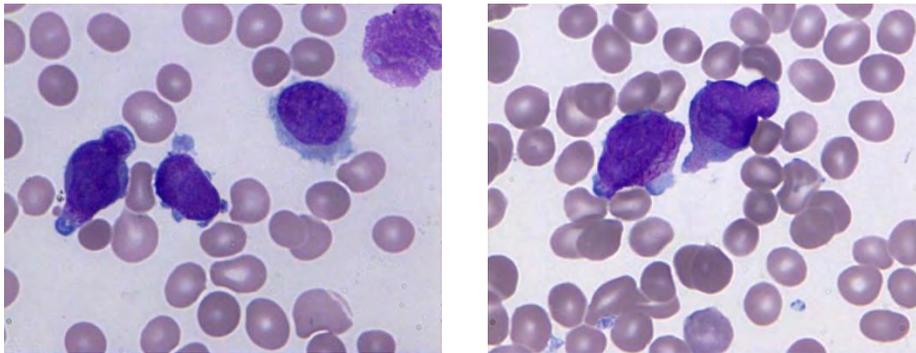
Background/Aim

Whether cladribine therapy or hairy cell leukaemia (HCL) itself leads to increased incidence of secondary malignancies is controversial and open to debate. The most frequently reported second malignancies have been solid tumours, skin cancers and other lymphoproliferative neoplasms. We describe a case of acute promyelocytic leukaemia (APL), 6 years after purine analogue therapy for HCL.

Case report

A 52-year-old woman was noted to be pancytopenic on routine follow-up testing, six years after attaining complete morphologic remission from hairy cell leukaemia (HCL) following a single course of cladribine (0.14mg/kg intravenously daily for five days). Physical examination did not reveal lymphadenopathy or hepatosplenomegaly. Full blood examination showed marked neutropenia (neutrophils $0.1 \times 10^9/L$), mild thrombocytopenia (platelets $98 \times 10^9/L$) and mild normocytic anaemia (haemoglobin 100g/L, MCV 91fL). The blood film showed occasional teardrop cells without other abnormalities.

A bone marrow biopsy revealed a marked excess of blasts (33% of nucleated cells) and abnormal promyelocytes (19% of nucleated cells). Cells containing Auer rods were readily identifiable and a few faggot cells were seen (please see figures below). Flow cytometric analysis demonstrated a large population of events within the blast gate (low CD45, high side scatter) with the immunophenotype: absent HLA-DR, positive CD13, CD33, CD34, CD117 and bright myeloperoxidase, consistent with the morphologic impression of acute promyelocytic leukaemia (APL). A small monoclonal lymphoid population was also found, with immunophenotype CD20 positive (bright), positive CD11c, CD25 and CD103, consistent with HCL. Immunohistochemical stains on the bone marrow trephine demonstrated Annexin-A1 positive large lymphoid cells with abundant cytoplasm ('fried egg cells'). Fluorescence in-situ hybridisation studies confirmed the presence of the $t(15;17)(q24;q21)$ translocation. The patient was commenced on *all-trans*-retinoic acid therapy.



Conclusion

Acute myeloid leukaemias have only been sporadically reported after purine analogue therapy for HCL. To our knowledge, ours is the first case of APL reported in this setting. Further studies are needed to fully elucidate the true risk of secondary malignancies in the setting of treated HCL.

P031. Phase 3 randomized, open-label study comparing the efficacy and safety of AG-221 vs conventional care regimens (CCR) in older patients with advanced acute myeloid leukemia (AML) with isocitrate dehydrogenase (IDH)-2 mutations in relapse or refractory to multiple prior treatments: the IDHentify trial

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Background

Mutant IDH (mIDH) enzymes produce an oncometabolite, 2-hydroxyglutarate (2HG), that can prevent DNA and histone demethylation required for lineage-specific myeloid progenitors to differentiate into mature cells. mIDH2 occurs in 8–19% of AML patients (pts) (Döhner, *NEJM* 2015) and can survive chemotherapy, leading to relapse (Chan, *Nature Med* 2015). AG-221 is a first in class, potent, selective, oral inhibitor of mIDH2. A phase I/expansion study in 159 mIDH2 R/R AML patients treated with AG-221 has reported an overall response rate (complete remission [CR], CR with incomplete platelet recovery, CR with incomplete blood count recovery, marrow CR, partial response) of 37%, including 18% CR rate (Stein, *ASH* 2015). The international IDHentify™ study (NCT02577406) in pts with mIDH2+ AML is a randomized trial to evaluate an AML Tx in older pts R/R to multiple prior Tx courses—a pt population with few Tx options. Methods: Eligible pts are age ≥ 60 years with *de novo* or secondary mIDH2 R/R AML after 2 or 3 prior Tx regimens. Physicians preselect the most appropriate of 4 conventional care regimens (CCR), given in 28-day cycles, for each pt (Table). Pts are then randomized 1:1 to AG-221 or to CCR and receive the preselected Tx. Randomization is stratified (yes/no) by prior intensive chemotherapy, primary refractory, and prior allogeneic HSCT. Assuming median OS of 5 months with CCR (Roboz, *JCO* 2014) and 8 months with AG-221 (60% improvement) and a drop-out rate of ~10%, 280 pts will allow enough events (193 deaths) to achieve ≥ 90% power to detect a statistically significant OS difference at a Type I error rate of 0.05 (two-sided). Enrollment began in Oct 2015 and continues through 2019. Clinical trial information: NCT02577406.

Table

<i>This was</i>	Tx Arm ^a	Dose	Endpoints	<i>research</i>
	CCR		Primary: Overall survival (OS)	
	Azacitidine	75 mg/m ² SC QD × 7d	Secondary: Overall response rate (IWG AML criteria), event-free survival, time to and duration of response, 1-year survival, quality of life	
	Low-dose cytarabine	20 mg SC BID × 10d		
	Intermediate dose cytarabine	0.5–1.5 g/m ² IV QD × 3–6d		
	Best supportive care (BSC) only			
	AG-221	100 mg PO QD × 28d		

^a All patients receive BSC as needed

supported by Celgene. The company assisted with preparation of the abstract.

P032. Preclinical evaluation of CD19-specific chimeric antigen receptors for use in a clinical trial of the treatment of B cell leukaemia and lymphoma

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Aim

T cells modified to express a chimeric antigen receptor with specificity for CD19 (CAR19 T cells) have shown remarkable efficacy against B cell malignancies in overseas trials. However, this CAR T cell technology is currently limited by the expense and stringent safety regulations associated with retroviral vectors used in their generation. Production of CAR T cells with the non-viral PiggyBac transposon/transposase system of gene modification avoids these issues. In order to conduct the first clinical trial of CAR19 T cells generated by this method, we needed to develop an in-house CD19-specific CAR.

Methods

We designed a panel of CD19-specific CARs incorporating single chain variable fragments (scFv) with short or long linkers between the variable heavy and light chains to direct CD19 specificity, short or long spacer domains, transmembrane and intracellular costimulatory domains derived from CD28 or 41BB, and a CD3z stimulatory domain. CARs were cloned into the PiggyBac transposon/transposase system, electroporated into T cells from healthy donors, and CAR19 T cells **selectively expanded through culture with IL-15 and CD19 stimulation**. Evaluation of CAR T cells included *in vitro* expansion, CAR expression, specificity for CD19⁺ targets, cytotoxicity, T cell memory phenotype and *in vivo* activity against ALL.

Results

CAR19 T cells with short scFv linkers and short spacers showed comparable performance to reference CAR19 T cells with a long scFv linker and long spacer. *In vivo*, CAR19 T cells with short scFv linkers, short spacers, CD28 transmembrane regions and either CD28 or 41BB costimulatory domains had activity against ALL that prolonged survival compared to untreated mice.

Conclusions

We have developed multiple in-house CD19-specific CARs suitable for use in Australian clinical trials. The results add to the growing evidence that there is complex interaction between the various CAR domains that defines activity, and that there is no single optimal CAR design.

P033. Defibrotide for the treatment of sinusoidal obstruction syndrome (SOS) after haemopoietic stem cell transplantation (HSCT): evaluation of patient outcomes

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Aim

To evaluate the effectiveness of defibrotide used within institutional guidelines for the treatment of SOS in patients undergoing HSCT.

Method

Patients who received defibrotide for the treatment of SOS after HSCT undertaken between 2006 and 2015 were retrospectively reviewed. Patients eligible for treatment with defibrotide met institutional criteria for a diagnosis of SOS, based on a composite of modified Seattle and Baltimore criteria.^{1,2} Response was evaluated at day 5, with stabilisation or improvement in symptoms and biochemical markers required for continuation of therapy.

Demographic data collected included diagnosis, timing of transplant, conditioning regimen, weight and graft versus host disease prophylaxis. Biochemical markers analysed included creatinine and bilirubin.

Symptomology that was monitored included the presence of hepatomegaly, ascites, delirium, requirement for supplemental oxygen and right upper quadrant pain. Survival at day 100 post HSCT and overall survival (OS) were calculated.

Result

Data for 23 patients was evaluated. All met institutional criteria for treatment of SOS with defibrotide. All had moderate to severe SOS based on Seattle severity criteria³. A maximum dose of 25mg/kg/day, as per protocol was used in 18 patients (range for all patients: 25mg-40mg/kg/day). With 10 year follow up available, OS for this cohort was 35%. Day 100 survival was 70%. On cessation of defibrotide, the proportion of patients exhibiting hepatomegaly (61%vs40%; p=0.03), ascites (56%vs39%, p=0.003) and requiring oxygen supplementation (78%vs43%, p=0.01) decreased, compared to on diagnosis of SOS. Bilirubin improved in 15 patients (p<0.0001) whilst on defibrotide. Weight improved and returned to baseline by cessation of defibrotide (p<0.0001). Of the 7 patients who died before day 100, only one had SOS recorded as the cause of death.

Conclusion

Defibrotide to treat SOS, initiated and response evaluated on defined criteria, demonstrated effective management of SOS with 65% survival at day 100 post HSCT. Defibrotide should be considered in any consensus protocol providing guidance on the treatment of SOS.

P034. Compliance with screening recommendations and prevalence of late effects in survivors of allogeneic haemopoietic stem cell transplantation

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Fiona Stanley Hospital

Introduction

Haematopoietic stem cell transplant (HSCT) recipients have a high risk of developing late adverse effects including osteoporosis, metabolic syndrome, liver dysfunction and secondary malignancies. We investigated whether patients at our institution receive adequate monitoring for late effects post allogeneic HSCT in accordance with hospital and international guidelines, and also investigated the prevalence of these late effects in long-term survivors.

Methods

Survivors >12 months post-allogeneic HSCT who had not relapsed and were seen in the outpatient transplant clinic between February 2015 and February 2016 were included. The frequency of bone mineral density, lipids, blood sugar, liver function and thyroid function measurements were compared to hospital and international recommendations for screening of complications post-HSCT. The prevalence of secondary malignancies, new antihypertensive and cholesterol-lowering medication as documented in the medical record was also recorded.

Results

130 HSCT survivors were included. Compliance with local and international screening recommendations is summarised in the table. Seventeen patients (15%) had a subsequent malignancy documented after transplantation, 20 (18%) had commenced a cholesterol-lowering agent and 19 (18%) had commenced a new antihypertensive. Subsequent malignancies included non-melanoma skin cancers (6), melanoma (4), and oral (2), lung (2), breast (2), and colorectal malignancies (3). 33 patients (31%) had deranged liver function during 2015. Of these, 67% were attributed to chronic GVHD by the treating physician. However, investigations to exclude other causes of deranged LFT, including liver ultrasound and lipid profile, were rarely performed.

Conclusion

Screening for late effects after allogeneic HSCT at our institution is reasonably congruent with consensus recommendations, although some areas could be improved. The moderately high prevalence of late effects, including subsequent malignancy, in our cohort is a reminder of the importance of careful monitoring and treatment of late effects after allogeneic HSCT.

P035. Non-inferior autologous hematopoietic stem cell transplantation (ASCT) outcomes in Follicular lymphoma (FL) patients receiving Cy/TBI conditioning and in older non-Hodgkin's lymphoma (NHL) patients (>60 years): A 23 year experience from Waikato hospital, New Zealand

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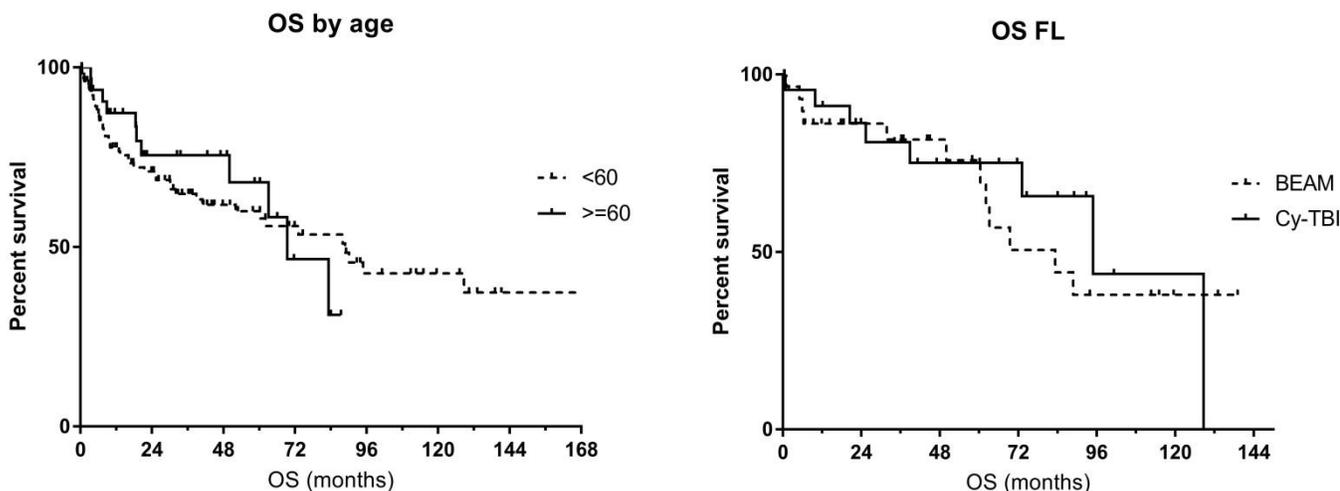
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Aim
We performed a retrospective analysis of NHL patients who received ASCT (n=140) in our centre over a period of 23 years (1992-2015). This study was done with an aim to evaluate patient outcomes and complications based on age, NHL subtypes and conditioning regimen, along with possible factors contributing to these results.

Method
Electronic records and case notes were used for data collection. Progression events were defined as refractory disease, relapse, progressive disease or death. Progression free survival (PFS) and overall survival (OS) were defined as time from ASCT till progression event or last follow-up, and time from ASCT till death or last follow-up, respectively.

Results
Median age at ASCT was 55 years (16-68). Amongst patients ≤60 years (n=109) and >60 years (n=31), there was no significant difference in PFS (p=0.756), OS (p=0.711) (Figure), neutrophil (12.5 vs 11 days) and platelet (12 vs 14 days) engraftment times. Amongst FL patients (n=54) who received BEAM (Carmustine, Etoposide, Ara-C, Melphalan) (n=30) or Cy/TBI (cyclophosphamide/total body irradiation) (n=24) conditioning, there was no significant difference between PFS (p=0.111) or OS (p=0.667) (Figure). There was no significant difference (p=0.46) in incidence of second malignancies in patient receiving BEAM (5%, 5/100) or TBI based (9%, 3/33) conditioning.

Conclusion
ASCT can be safely performed for NHL in patients > 60 years with outcomes similar to those ≤60 years. TBI based conditioning appear safe with similar outcomes to BEAM in FL patients. Prospective studies are needed to confirm these findings.



P036. Characterization of Human CD83 Expression on Immune Cells Identifies a Unique CD83+ T Cell Population Correlated with Graft versus Host Disease

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Aim

CD83 was identified on activated lymphocytes and dendritic cells (DC). We showed that polyclonal anti-CD83 antibody and a human anti-CD83 monoclonal antibody, 3C12C, depleted activated human DC, prevented graft versus host disease (GVHD) and preserved human T cell responses after transplantation in SCID mice. Further studies on CD83 are required to refine it as a therapeutic target.

Method

CD83 expression on human immune cells was analysed by flow cytometry and PCR. Soluble CD83 (sCD83) secreted by activated Mo-DC, monocytes and blood DC was measured. T cells were activated and CD83 expression examined. A dose escalation study was performed in baboons (NHP) documenting immune cell counts. CD83 expression on DC and T cells from allogeneic haematopoietic cell transplant (alloHCT) recipients was monitored.

Results

There were distinct CD83 splice variants in different immune cells. Monocytes and Mo-DC released more sCD83 than blood DC. Different T cell stimuli increased T cell CD83 expression with different kinetics. Human natural Treg did not express CD83 in contrast to mouse Treg. 3C12C had a specific immunosuppressive effect in human in vitro cultures and 3C12C reduced DC counts and increased Treg in the peripheral blood and lymph nodes of NHP compared to controls. CD83 expression was increased on both CD4⁺ and CD8⁺ T cell in association with clinical acute GVHD in alloHCT recipients.

Conclusion

The form and expression of CD83 by human immune cells is likely to contribute to several immune regulatory pathways. Our NHP studies suggest that 3C12C will target DC and revealed an unexpected effect on Treg. The 3C12C immunosuppressive effect may also involve depletion of specifically reactive T cells. Our clinical data suggests CD83 expression is a potential target induced in alloHCT recipients with acute GVHD. Targeting CD83 to induce immunosuppressive function has therapeutic applications in clinical transplantation and autoimmune diseases.

This research was supported by DendroCyte BioTech Pty Ltd. The company had no role in analysing the data or preparing the abstract.

P037. Infusion of Partially HLA-Matched Unrelated Donor Cells Appears to be Safe and Results in Detectable Microchimerism in Patients with Acute Myeloid Leukaemia

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Aim

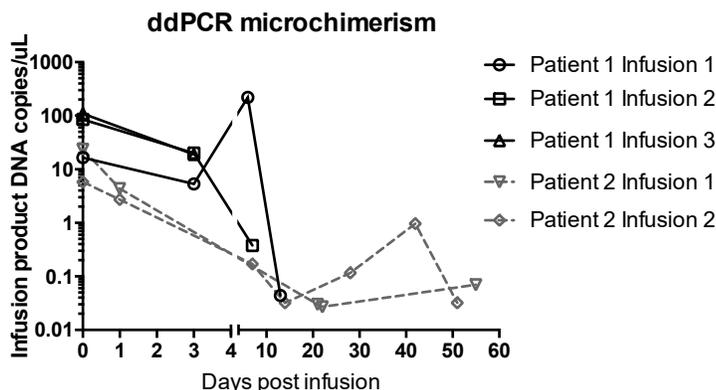
Relapse remains the leading cause of death for patients with acute myeloid leukaemia (AML). Some patients at high risk of relapse are ineligible for allogeneic stem cell transplantation due to age, comorbidities or the absence of a suitable donor. Infusion of fresh haploidentical cells from family donors has resulted in improvement in disease-free survival without the development of graft-versus-host disease. We investigated the use of cryopreserved partially HLA-matched cells from unrelated donors as an immediately available alternative in AML patients ineligible for transplant.

Method

Patients were in first complete remission of AML following induction therapy. Partially HLA-matched (2/6 antigens at HLA-A,C,DR) unrelated donor G-CSF-primed apheresis products at a dose of $0.5 \times 10^6/\text{kg}$ CD3⁺ cells were identified in surplus BMT laboratory inventory, thawed and infused following 3 days of cytarabine $2\text{g}/\text{m}^2$ BD. No GVHD prophylaxis was given. Up to three cycles were given at 2-month intervals. The presence of microchimerism was assessed via droplet digital PCR using a commercial set of primers/probes targeting 29 bi-allelic insertion/deletion loci.

Results

A total of seven cell infusions have been administered to three patients with AML. There were no immediate infusion reactions. Non-neutropenic fever occurred in 4 of 7 infusions 1 day (range 0-1) post-infusion. There were no cases of GVHD following infusion. Median time to neutrophil recovery was 21.5 days (range 13-34) and platelets 17 days (9-39). One patient relapsed 256 days after their 3rd infusion and subsequently died, the other two remain in CR on trial. Of the five infusions analysed, microchimerism has been detected in all cases up to 42 days post-infusion (Figure 1).



Conclusion

Infusion of matched cells minimal toxicity effect. Donor cells post-infusion with The trial is

cryopreserved partially HLA- from unrelated donors results in and may offer an anti-leukaemic can be detected over 30 days a sensitive chimerism assay. ongoing.

P038. Treatment of Steroid Refractory Acute Graft Versus Host Disease (SR-GVHD) with TNF inhibitors: results from a Single Centre

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Aim

The prognosis of patients with SR-GVHD is poor, and there is no consensus on the best standard of care for SR-GVHD. We report the outcomes of patients with SR-GVHD who were treated with etanercept or infliximab +/- daclizumab (anti-CD25 antibody) at our transplant unit.

Methods

Patients with SR-GVHD from 2003 – 2015 who were treated with infliximab or etanercept were included in this retrospective study. Patients who were treated with infliximab received either 10mg/kg weekly for 2 weeks, or 5mg/kg weekly for 2 weeks +/- 1mg/kg daclizumab days 1, 4, 8 and 15. Patients who received etanercept were given 25mg subcutaneously twice a week for 4 weeks. In a subgroup of patients, this was followed by weekly etanercept to maintain response. Patients who had at least 2 weeks of therapy were included in the analysis. Primary outcomes include 100-day mortality and median overall survival.

Results

16 patients with SR-GVHD were treated with infliximab from 2003-2006 - 4 patients received infliximab only and 12 patients were treated with infliximab and daclizumab. From 2005-2015, 42 patients with SR-GVHD were treated with etanercept. A median of 10 doses (range 4 to 33) of etanercept was given. Grade III-IV acute GVHD were noted in 100% (infliximab) and 90% (etanercept). 100-day post transplant mortality was 25% (infliximab) and 48% (etanercept). Median overall survival was 169.5 days (infliximab) and 113.5 days (etanercept) (p=0.84). 1-year overall survival was 25% (infliximab) and 26% (etanercept) (p=1).

Conclusion

In this study, treatment of SR-GVHD with TNF inhibitors was associated with modest response regardless of the combination used.

P039. Autologous stem cell transplantation (ASCT) in relapse refractory (RR-MS) and secondary progressive multiple sclerosis (SP-MS): an Australian-based prospective phase II trial

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Aim
ASCT is a promising strategy for the management of patients with MS who do not respond to conventional measures. We report the prospective outcomes of ASCT in patients with RR-MS and SP-MS in a single centre.

Methods
From 2010 to 2016, patients with RR-MS or SP-MS who were fit to undergo ASCT were recruited. Patients were conditioned with Carmustine, Etoposide, Cytarabine and Melphalan (BEAM) before autologous stem cell infusion, followed by anti-thymocyte globulin (Atgam) on days 1 and 2 post-infusion. Outcome measures include Expanded Disability Status Scale (EDSS), serial magnetic resonance imaging (MRI) studies, and MS quality of life measurements (MSQOL).

Results
29 patients were recruited and underwent ASCT, with a median follow up period of 12.8 months (range 3-57 months). Eighteen (62%) and 11 (38%) patients had RR-MS and SP-MS respectively. ASCT was performed at a median of 96 months after onset of MS (range 12-252 months). Patients had a median EDSS score of 6.0 (range 2.0-7.0), and received 4 lines of treatment (range 2-7 lines of treatment) prior to ASCT. After ASCT, the EDSS score improved to a median of 5.00 at 6 months ($p=0.004$) and 5.25 at 12 months ($p=0.02$). 7 patients (25%) experienced relapse – 5 experienced deterioration of clinical function, and 2 had increased lesions in MRI. There was no transplant related mortality in this cohort and the 1 year relapse-free survival was 86%.

Conclusion
ASCT is effective in improving disability in patients with RR-MS and SP-MS, with majority of patients remained relapse-free at 12 months. Further information on clinical and laboratory effects of ASCT will be presented at the meeting.

P040. Outcome of Mismatched Unrelated Donor (MMUD) allogeneic stem cell transplantation for acute leukaemia, MDS/MPN: A single centre experience

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Aim

To determine the outcome of mismatched unrelated donor (MMUD) allogeneic stem cell transplantation for acute leukaemia and MDS/MPN performed at our institution.

Method

Patients who had undertaken MMUD between January 2004 and December 2014 were identified from an institutional data-base. Overall survival (OS), relapse rates (RR), non-relapse mortality (NRM), acute GVHD incidence and GVHD-free, relapse free survival (GRFS) were determined retrospectively by review of individual patient medical records. All grafts were T-replete, with CsA +/- TCZ with day 1-11 MTX used as standard GVHD prophylaxis. All donor / recipient pairs were typed for HLA A, B, C, DRβ1 and DQ loci.

Results

In total 52 patients underwent MMUD during the time period under review. Median age was 45yrs (range 17-65yrs). Indication for SCT included AML in 28patients (54%), MDS/MPN in 8(15%) and ALL in 16 (31%). 41 donor / recipient pairs (79%) were mismatched at 1 loci only, including 24 with mismatches at class I and 17 at class II. 16 patients had >1 mismatched loci. SCT conditioning included myeloablative protocols in 32 (62%) and RIC protocols in 20 (38%). Platelet non-engraftment occurred in 3 patients (6%). Overall incidence of grade II-IV and III-IV acute GVHD was 50% and 17% respectively with 74% of patients surviving post D100 developing extensive stage chronic GVHD. At median follow-up of 63mths (range 10.6 – 168.7mths) 5yr OS, PFR, NRM and GRFS is 55.7%, 52.5%, 29.7% and 6% respectively. At last follow-up, 81% of patients remained on long-term immunosuppression. On univariate analysis, the only factor predictive of OS was development of grade III-IV GVHD (2yr OS 71.1% vs 44.4 respectively, p=0.009).

Conclusion

MMUD for acute leukaemia and MDS / MPN is associated with reasonable OS probability though significant risk of chronic GVHD / ongoing requirement for immunosuppression. Novel immunosuppression approaches are required for this group of patients.

P042. Preemptive plerixafor use results in significantly higher CD34+ yield than during remobilisation: retrospective analysis of South Australian data

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Aims

Autologous stem cell transplantation (ASCT) is a standard of care for multiple myeloma (MM) and relapsed lymphoma patients but 5-40% patients mobilise inadequately with G-CSF ± chemotherapy. Plerixafor increases CD34⁺ cell yield in patients failing previous mobilisation or at high risk of failure. However there is limited data comparing the efficacy of pre-emptive and remobilisation plerixafor and the efficacy of plerixafor with chemotherapy is not well known.

This study aims to compare plerixafor efficacy in: 1) preemptive and remobilisation settings 2) patients mobilised with or without chemotherapy.

Methods

MM, lymphoma and germ cell tumour (GCT) patients receiving plerixafor during 2010 to 2015 in South Australia were studied.

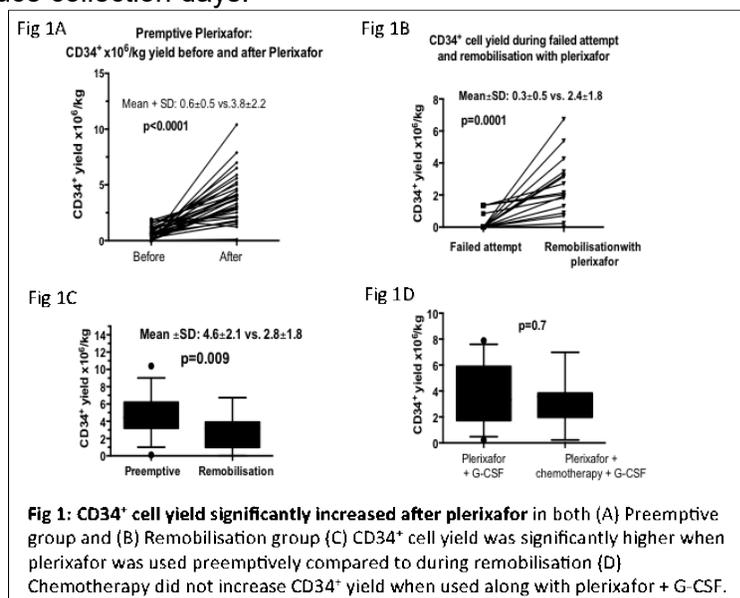
Result

47 patients of MM (n = 27), lymphoma (n = 19) and germ cell tumour (GCT; n=1) receiving plerixafor + G-CSF ± chemotherapy were analysed. The median age was 60 years (25-69) and 24 (51%) male. Plerixafor was used preemptively in 31/47 (66%) and during remobilisation in 16/47 (34%). All but 2 patients underwent stem cell collection with a mean CD34⁺ yield of $4.08 \pm 2.1 \times 10^6/\text{kg}$. In all patients PB-CD34⁺ counts and CD34⁺ yield was significantly higher after the use of Plerixafor ($p < 0.001$; Fig 1A-B). CD34 $\geq 2 \times 10^6/\text{Kg}$ body weight, adequate for single ASCT, was collected in 79% (37/47) of all patients - 90% (28/31) after preemptive treatment, 75% (12/16) after remobilisation. CD34⁺ yield was significantly higher in Preemptive compared to Remobilisation group (4.6 ± 2.1 vs. 2.8 ± 1.8 , $p = 0.009$; Fig.1C).

Overall 19 (40%) and 28 (60%) patients were mobilised with and without chemotherapy respectively with no significant difference in CD34⁺ cell yield (3.7 ± 2.1 vs. 3.4 ± 1.7 , $p = 0.7$; Fig 1D) or collection days ($p = 0.2$).

Conclusion

Preemptive use of plerixafor results in significantly higher CD34⁺ yield, adequate for single ASCT in 90% cases. In contrast to G-CSF + chemotherapy studies, addition of chemotherapy to plerixafor + G-CSF neither increased yield nor reduce collection days.



P043. Allogeneic Haematopoietic Stem Cell Transplantation in Non-Hodgkin Lymphoma: The Western Australian Experience

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Aim

The aim of our study was to assess the long term outcomes of patients who underwent allogeneic haematopoietic stem cell transplantation (AHSCT) for Non-Hodgkin Lymphoma (NHL) in Western Australia (WA).

Method

The WA AHSCT database was used to perform a retrospective audit of all patients in WA who underwent an AHSCT for NHL from 2004 to 2015. Patients were broadly grouped as high or low grade B-Cell lymphoma, or T cell lymphoma based on histological subtype at time of transplant. Disease status and chemosensitivity at time of AHSCT was defined as per the Cheson criteria (Cheson et al., JCO 2015). Overall survival (OS) was calculated from date of AHSCT, while cumulative incidence of transplant-related mortality was calculated using relapse as a competing risk, and vice versa for relapse.

Result

A total of 39 patients were identified (19 female). Median age at AHSCT was 45yrs (32-60yrs). Median follow-up of survivors post-transplant was 4 yrs (0.5-11yrs). Nineteen patients underwent AHSCT for high grade B-NHL (17 DLBCL), 15 for low grade B-NHL (7 Follicular), 5 for T-NHL, with chemosensitivity of 80%, 65% and 80% respectively. Thirty-one patients (80%) received myeloablative conditioning, of whom 23 (74%) received 12Gy TBI/Cy. Eight patients (20%) received reduced intensity conditioning, of who 5 received Flu/Mel. OS at 4yrs was 42% [95% confidence interval (CI) 26 – 58%]. Cumulative incidence of non-relapse mortality was 36% (95% CI 21-51%) at 6 months and of relapse was 16% (95% CI 6 – 29%) at 4 years.

Conclusion

In this cohort of patients who mostly received high-dose, myeloablative conditioning, transplant-related death was the dominant cause of treatment failure. While AHSCT offers patients with NHL the potential for long term survival, measures to reduce transplant toxicity warrant further investigation in these heavily pre-treated patients.

P044. Low initial ciclosporin level is not associated with increased risk of acute graft versus host disease – 12 years' experience from a single centre

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Aim

To determine the optimal initiation dose and timing of ciclosporin (CsA) for graft versus host disease (GVHD) prophylaxis.

Method

A retrospective review of all recipients of allogeneic HSCT at our centre between January 2004 – August 2015 was conducted. All patients received the standard GVHD regimen of CsA 1.5mg/kg IV twice a day and methotrexate 15mg/m² IV on day +1, 10mg/m² on day +3, +6, and +11 were included.

Results

A total of 207 patients were reviewed. CsA was commenced on transplant day -1 (n = 93) or earlier (n = 109). Trough CsA levels are summarised in the Table. Target CsA levels (200-300µg/L) were achieved in 12% of patients by day +3 and 42% of patients by day + 5, while 26% had low CsA levels (< 100 µg/L) by day +3 and 5% by day +5. The cumulative incidence of grade II-IV aGVHD was 56% by day 180 (95% CI 49 – 62%). Neither the timing of CsA initiation nor the CsA level by D+3 or D+5, nor time to achieve target CsA levels were associated with the risk of aGVHD (Cox regression). Interestingly, patients with low (<100 µg/L) CsA levels by day +3 had a lower risk of grade II-IV aGVHD (HR 0.60, 95% CI 0.38 – 0.95, p = 0.029). On multivariate analysis adjusting for donor type (related versus unrelated) and conditioning regimen intensity, early CsA level <100 µg/L was independently associated with a lower risk of aGVHD (HR 0.61, 95% CI 0.38 – 0.97, p = 0.035).

Table

Time to achieve target cyclosporine level (from commencement of CsA)		
Trough CsA level:	Median	Range
≥150 µg/L	5 days	1 – 342 days
≥200 µg/L	8 days	1 – 342 days
Trough CsA levels after stem cell infusion		
D+ 0 – 3	127 µg/L	34 – 594 µg/L
D+ 3 – 5	187 µg/L	58 – 435 µg/L
At engraftment	255 µg/L	78 - 561 µg/L

Conclusion

Our study showed that early achievement of target CsA levels did not confer a lower risk of aGVHD. Rather, patients with a lower level of CsA had a reduced risk of aGVHD. While this observation requires replication and further investigation, our analysis provides no rationale for more intensive initiation of CsA after allogeneic HSCT.

P045. Tumour associated antigen gene expression in AML as a basis for immunotherapy

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Background

The anti-tumour effect of donor-derived immune effectors plays an important role in controlling disease in acute myeloid leukaemia (AML) patients undergoing allogeneic haemopoietic stem cell transplantation. Infusion of expanded antigen specific T cells targeting tumour-associated antigens (TAAs) may be able to augment the graft versus leukaemia effect and minimise the risk of relapse following transplantation. We sought to determine the expression of tumour antigens in patients with AML to identify potential targets for immunotherapy.

Method

We measured mRNA expression of 19 TAAs using quantitative (q)PCR and Nanostring nCounter digital RNA expression in diagnostic bone marrow samples of 75 patients with AML. Expression levels were first normalised to expression of housekeeper genes (ABL1, GUSB, GAPDH), then calculated as a fold change relative to normal bone marrow samples (NBM). Comparison of AML and NBM expression levels was performed using the Mann-Whitney U test. Linear regression was used to compare results from qPCR and Nanostring nCounter.

Results

At least one TAA was found to be overexpressed in 68/75 cases using either analysis method. There was a positive correlation between qPCR and Nanostring for all TAAs (17/19) measured on both platforms. TAAs found to be frequently overexpressed by qPCR and Nanostring analysis, respectively, were WT1 (50% and 77%), CCNA1 (40% and 75%), HOXA9 (67% and 52%), HOXB4 (42% and 37%), FLT3 (20% and 53%) and MSLN (33% and 13%). PRAME was measured using Nanostring analysis only and was overexpressed in 32% of patients. Overexpression of WT1, CCNA1, FLT3 and HOXA9 in AML compared with NBM were found to be statistically significant ($p < 0.05$). There was considerable variability between individuals and no single gene was overexpressed in all cases.

Conclusion

Antigens WT1, CCNA1, HOXA9, HOXB4, FLT3, MSLN and PRAME were overexpressed in AML and may be potential targets for immunotherapy using an individualised approach.

P046. Excellent safety with myeloablative oral busulfan and IV cyclophosphamide conditioning for adult allogeneic stem cell transplantation in Western Australia

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Over the last decade, many transplant units have switched from oral to IV busulfan for myeloablative conditioning before allogeneic haemopoietic stem cell transplantation (HSCT), based on pharmacokinetic principles suggesting a lower risk of toxicity. At our centre, oral busulfan and intravenous cyclophosphamide (PO Bu / IV Cy) is still used for fit patients undergoing myeloablative conditioning. We have previously reported low toxicity for 31 patients treated with this regimen. Here, we update our experience with an additional 18 patients.

Patients treated under our transplant programme with busulfan (4mg/kg oral suspension in solution daily for 4 days) and cyclophosphamide (60mg/kg IV daily for 2 days) for allogeneic HSCT between January 2005 and April 2016 were included. An observational trial incorporating measurement of busulfan levels opened in February 2016 with the intention of recruiting 15 patients.

Forty-nine patients received PO Bu / IV Cy conditioning out of a total of 328 undergoing allogeneic HSCT during this period (15%). Other patients with myeloid malignancies received PO busulfan / fludarabine or fludarabine / melphalan. Patient characteristics and outcomes are described in the table. The median follow-up was 14 months (range, 3 - 75 months). One patient suffered severe sinusoidal obstruction syndrome of the liver and none died of transplant-related causes within 6-months of transplant. A case of graft failure occurred in a cord blood unit recipient who was salvaged with another graft. For the five patients with available data thus far, the mean busulfan area under the curve is 1365 micromol/min/L (range, 1026 – 1569) per dose.

At our centre, the PO Bu/ IV Cy regimen has been associated with remarkably little toxicity. While patient selection may contribute to this result, the lack of any early transplant-related deaths with this myeloablative regimen justifies continued use of oral busulfan with ongoing monitoring to confirm adequate exposure.

Characteristics of n = 49 patients who received PO Bu/IV Cy		
Median age, years (range)		44 (17 – 58)
Sex, n	Male	30 (61%)
	Female	19 (39%)
Diagnosis, n	AML in CR1	17 (34%)
	AML in CR2	15 (31%)
	AML not in remission	9 (18%)
	Myelodysplasia	6 (12%)
	Myeloma	1 (2%)
	NHL	1 (2%)
Outcomes		
Acute graft versus host disease		16 (33%)
Day 180 transplant-related mortality		none
Overall survival at 12 months (Kaplan Meier)		72 ± 7%
Cause of death		
	Relapse	13 (27%)
	Other	2 (4%)

P047. Autologous Stem Cell Transplantation (ASCT) in patients with HIV-Related Lymphoma at Sir Charles Gairdner Hospital (SCGH)

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Aim

To report our experience in patients with HIV-related lymphoma undergoing Autologous Stem Cell Transplantation (ASCT).

Method

Patient A is a 63 year old man diagnosed with Burkitt's lymphoma in April 2015 on the background of HIV infection, diagnosed in 2012. Highly Active Antiretroviral Therapy (HAART) of Dolutegravir + Lamivudine + Abacavir achieved undetectable viral load. He obtained complete metabolic response (CMR) following 6 cycles of DA-EPOCH-R (dose adjusted Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin Rituximab), but relapsed within six months. Hi-DICE salvage and mobilisation (G-CSF 10ug/kg/d) yielded 12.1×10^6 /kg CD34+ cells. Post salvage PET scan (January 2016) showed CMR. ASCT was delayed due to concerns over respiratory function and new diagnosis of pulmonary embolism. He proceeded to ASCT in April 2016 on modified HAART (50% dose reduction Lamivudine) without dosing interruptions. Modified BEAM conditioning with dose reduced Melphalan (impaired creatinine clearance) was administered (Carmustine 300mg/m², Etoposide 200mg/m², Cytarabine 200mg/m², Melphalan 100mg/m²). Routine post ASCT supportive medications with Pegfilgrastim was administered.

Patient B is a 45 year old man concurrently diagnosed with HIV infection and gastric plasmablastic lymphoma in February 2016, currently on HAART (Raltegravir, Tenofovir, Emtracitibine) with stable viral load (87 copies/mL). Lymphoma treatment was commenced with HyperCVAD regimen (Cyclophosphamide, Adriamycin, Vincristine, Prednisolone alternating with Methotrexate, Cytarabine). Mobilisation following cycle-2A Hyper-CVAD with G-CSF 10ug/kg/d yielded 5.8×10^6 /kg CD34+ cells. ASCT is scheduled soon using modified BEAM conditioning.

Results

Continuation of HAART throughout the transplant in patient A was not associated with significant side-effects. Neutrophil recovery was day +10 with platelet recovery day +15. This correlates with our 2015 engraftment data (n=52); 12 days and 14 days (mean time to neutrophil and platelet recovery respectively).

Conclusion

ASCT in patients with HIV-related lymphoma differs little from ASCT in patients without HIV infection, except that HAART requires some management due to potential drug-drug interactions.

P049. Dectin-1 chimeric antigen receptor T-cells - a potential antifungal therapy

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Background

Fungal infections cause significant morbidity and mortality in immunosuppressed patients after bone marrow transplantation or high dose chemotherapy. Adoptive T-cell therapies offer a novel method to reconstitute pathogen specific immunity in this context.

Aims

To generate a chimeric antigen receptor T-cell expressing Dectin-1, optimise its expression, expansion and functional activity upon exposure to fungal antigens.

Method

A dectin-1 chimeric antigen receptor (D-CAR) was designed by combining the extracellular domain of dectin-1 with a human IgG1 CH2CH3 hinge, CD28 transmembrane domain and intracellular CD3 ζ and sourced commercially. This was cloned into a Piggybac transposon vector using standard DNA digestion, gel extraction and ligation. The D-CAR was electroporated into 4×10^6 peripheral blood mononuclear cells (PBMCs) from healthy donors utilising a two plasmid Piggybac transposon/transposase system. D-CAR T-cells were stimulated with irradiated autologous PBMCs on days 1, 8 and 15 and IL-15 on alternate days. Dectin-1 expression and functional activity through interferon- γ release upon exposure to zymosan (an inactivated component of fungal cell walls) was assessed through multiparameter flow cytometry.

Results

D-CAR expansion and functional activity data was available for two donors. D-CAR T-cells expanded on average 3.5 fold from day 1 to 15. Both CD4+ and CD8+ D-CAR T-cells exhibited functional activity on exposure to zymosan, with 17.9% and 31.3% releasing interferon- γ respectively.

Conclusion

This study shows that D-CAR T-cells can be effectively produced and expanded *in vitro* while still retaining specificity of activity against fungal antigens. This can potentially be utilised as a novel treatment for resistant fungal infections in immunocompromised hosts.

P050. Plerixafor for Stem Cell Mobilisation: A Review of Plerixafor Use at Mater Adult Hospital between 2010-2015

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Aim

This audit aimed to evaluate usage and efficacy of plerixafor in haemopoietic stem cell (HSC) mobilisation for patients with haematological and non-haematological malignancies planned for autologous transplantation. Stem cell mobilisation is usually undertaken using GCSF alone or in combination with chemotherapy however this is not always successful. Failure to mobilise stem cells limits treatment options and plerixafor is used to increase the success rate of collection. Plerixafor is indicated in patients with prior or predicted HSC mobilisation failure.

Method

We conducted a retrospective review of all cases at Mater Adult Hospital Brisbane between 2010-2015 where plerixafor was prescribed. Charts and pathology results were audited for disease type, prior mobilisation attempts, previous chemotherapy treatments, dose and timing of plerixafor, peripheral CD 34 count, total apheresis yield and engraftment parameters post reinfusion.

Results

A total of 8 patients received between 1 and 4 doses of Plerixafor (0.24mg/kg). Six patients achieved our minimum collection yield of 2×10^6 /kg CD34+ cells and went on to successfully engraft. Of the 2 patients that failed, the first patient already had a previous mobilisation failure and had been heavily pre-treated for follicular lymphoma. The second patient failed mobilisation with plerixafor in the context of infection. This patient was successfully collected at a later date indicating that the infection compromised initial mobilisation efficacy. Of 23 apheresis procedures, 13 yielded collections of $< 1 \times 10^6$ /kg and 10/13 of these correlated with peripheral CD34 counts $< 10/\mu\text{L}$ prior to collection.

Conclusion

Plerixafor use was successful in the majority but not all patients requiring stem cell harvest at our institution during this time. Peripheral CD34 counts $< 10/\mu\text{L}$ prior to collection were in our experience a good predictor of poor yield.

P051. Transplant versus non-transplant outcomes in Philadelphia-positive acute lymphoblastic leukaemia

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Aim

the role of stem cell transplantation (SCT) in Philadelphia chromosome positive Acute Lymphoblastic Leukaemia (Ph+ALL) in the tyrosine-kinase inhibitor (TKI) era has been the subject of recent debate. This study compares the outcomes of Ph+ALL patients who received SCT and those treated with chemotherapy and TKI alone in a large adult haematology unit.

Method

A retrospective analysis of all patients of transplant-eligible age (defined as up to 69 years of age) diagnosed with Ph+ALL between January 2000 and May 2016 in our institution was performed. Data regarding demographics, chemotherapy and TKI use, transplantation and outcomes were collected. Primary outcome assessed was overall survival (OS).

Results

27 patients with Ph+ALL were identified between 1/00 - 5/16. 55% were male. Median age at diagnosis was 49 (25-68) years. Median follow up was 17 (2-175) months. Ten patients were treated with standard chemotherapy and/or TKI therapy alone, 16 patients received 18 allogeneic transplants, 1 patient autologous transplant, and 1 patient received both. Of those transplanted, 7 received reduced-intensity conditioning and the remainder received myeloablative conditioning. Eleven patients received a transplant in complete remission, however; only 1 patient had undetectable BCR-ABL at time of transplantation. Four patients died from causes attributed to the transplant or conditioning. OS was significantly better in the transplant group, 2682 vs 527 days ($p=0.03$), with CR at transplantation ($p=0.006$), and the development of acute and chronic GVHD ($p=0.02$ and $p=0.008$ respectively) impacting positively on OS in multivariate analyses.

Conclusion

In this small single centre study, transplantation significantly improved OS for patients with Ph+ALL in the TKI era.

P052. Case study: medical management of a patient presenting with pneumatosis intestinalis – a rare complication of chronic graft versus host disease (cGVHD) of the bowel

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Introduction

Pneumatosis intestinalis is a poorly understood and rare entity considered as a result of primary mucosal insult to the bowel. It is usually identified on abdominal computed tomography (CT) scanning, and is associated with a spectrum of diseases including infective, coeliac, connective tissue disorders, primary immunodeficiency and transplantation associated GVHD.[1]

Case report

We report a case of severe pneumatosis intestinalis in a patient with cGVHD, seven years post matched unrelated allogeneic stem transplantation for secondary AML. The patient's symptoms included frequent watery diarrhoea and abdominal pain. A subsequent intestinal biopsy was suggestive of chronic GVHD, requiring systemic immunosuppression with tacrolimus, mycophenolate and prednisone. Due to refractory symptoms a CT scan was performed which showed severe pneumatosis coli with free air under the diaphragm. In the absence of peritonism, and with negative stool cultures and deconditioning the patient was managed conservatively with bowel rest, total parenteral nutrition (TPN) and optimisation of immunosuppression. The patient successfully avoided surgical intervention and was managed with conservative methods. Serial imaging showed progressive improvement of his pneumatosis intestinalis.

Conclusion

In review of the relevant literature, conservative management of patients presenting with pneumatosis coli in the context of GVHD with bowel rest and TPN is better than surgical management which can be fatal in up to 33-44% of cases [1, 2]. The presence of pneumatosis intestinalis is a poor prognostic sign. The presence of free air does not represent true bowel ischaemia or perforation. In the absence of secondary complications, a conservative approach should be the treatment employed when treating patients with this complication of cGVHD.

P053. Peripheral blood stem cell collection: comparing spectra optia MNC and CMNC procedures

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Background

Apheresis collections of haematopoietic progenitor cells (HPCs) have become a principle source of stem cells for transplantation around the world. The Spectra Optia Apheresis System has two procedures for collection HPCs, the mononuclear cell procedure (MNC) and the continuous mononuclear cell (CMNC) procedure.

Aim

The aim of this study was to compare the two procedures identifying specific performance parameters, primarily collection efficiencies as well as total inlet volume, product volume, duration of collection, absolute CD34⁺ in bag, CD34⁺ x 10⁶/kg collected, total white cell count (WCC), and red blood cell (RBC) volume.

Method

For this study we performed a retrospective data analysis on 27 apheresis based peripheral blood stem cell collections (PBSCC) on granulocyte-colony stimulating factor (G-CSF) mobilised related or unrelated healthy donors. 17 MNC apheresis collections and 10 CMNC apheresis collections were performed between September 2015 and May 2016.

Result

Key parameters analysed included median percentage collection efficiency CE%, total inlet volume, product volume, duration of collection, absolute CD34⁺ in bag, CD34⁺ x 10⁶/kg collected, total WCC and RBC volume. For the MNC procedures, the CE% was 51.1, total inlet volume 9635 ml, product volume 278 ml, duration of collection 205 min, absolute CD34⁺ 497x10⁶, 6.3x10⁶/kg CD34⁺ cells collected, WCC of 214 x 10⁹/L, and a RBC volume of 8.3 ml. For the CMNC procedures, the CE% was 49.5, total inlet volume 9765 ml, product volume 177 ml, duration of collection 187 min, absolute CD34⁺ 368 x10⁶, 4 x 10⁶/kg CD34⁺ cells collected, WCC of 194 x 10⁹/L, and a RBC volume of 6.9 ml.

Conclusion

These preliminary data collected from this population of healthy donors indicate that both the MNC and CMNC procedures are statistically comparable.

P054. A multicentre study investigating the pharmacokinetics and pharmacodynamics of busulphan when combined with melphalan as conditioning in adult autologous transplant recipients

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Aim

Busulfan (Bu) is an alkylating agent used in conditioning prior to allogeneic stem cell transplantation (SCT). Despite many decades of Bu use, pharmacokinetic (PK) and pharmacodynamic (PD) evidence-based guidelines are lacking, including recommendations to optimise systemic drug exposure for both efficacy and safety of Bu. We investigated Bu PK in patients undergoing Bu-melphalan (mel) autologous SCT, aiming to establish factors influencing Bu exposure and assess the utility PK assessment in achieving desirable drug exposure. PD analysis was performed to establish if drug exposure correlated with transplant-related toxicity.

Method

Sixty-four adult patients with haematological malignancies were prospectively enrolled. PK analysis was performed in patients on a test dose of Bu (1.6mg/kg) on Day-7 who then received a second 1.6mg/kg dose on D-6. Twenty-seven patients subsequently received three daily doses of therapeutic Bu at 3.2mg/kg (irrespective of PK results) while the remaining 37 patients received an adjusted dose according to initial PK results, targeting a desired Bu exposure of area-under-the-concentration-versus-time curve (AUC) between 4500-5000umol.min/day. PK analyses were subsequently repeated in all patients after 'full' dose Bu.

Results

Multivariate analysis, performed on all significant covariates ($p < 0.05$) in a univariate analysis, demonstrated actual weight was the only independent variable influencing Bu-exposure ($p = 0.02$). Patients receiving PK-guided Bu dosing (5 patients received 3.2mg/kg/day, 24 greater than this and 8 less than this), received a higher mean therapeutic Bu dose, 3.4mg/kg (2.6-4.8mg/kg, $p = 0.007$) and trended toward higher mean AUC than the weight-based cohort. On average D-5 AUCs were 11% higher than anticipated for the dose received. 97% of patients in the PK-guided group achieved AUCs within 3500-6000umol.min/day, a range found to be safe and effective in previous studies (vs 81% in the weight-based group, $p = 0.03$). No correlation between AUC and transplant-related toxicities was observed, however, only three patients had AUCs > 6000 umol.min/day.

Table 1. Busulfan Pharmacokinetics

	Whole cohort n=64	Weight-based dosing n=27	PK-guided dosing n=37	P value
Mean D-5 Busulfan dose, mg/kg (range)	3.3 (2.6-4.8)	3.2 (2.7-3.2)	3.4 (2.6-4.8)	0.007
Mean D-5 Busulfan dose, mg (range)	242 (150-350)	244 (175-350)	241 (150-310)	0.789
Mean D-5 AUC (range)	4905 (3251-6305)	4785 (3251-6305)	4995 (3639-6157)	0.306

Conclusion

These results suggest that PK-directed Bu dosing may be of benefit in achieving a target level of drug exposure, but larger studies are needed to determine the clinical significance of this strategy.

P055. Increased Dose of Fludarabine/Cyclophosphamide Conditioning Prior To Allogeneic Stem Cell Transplantation Increases Toxicity Without Enhancing Donor T- and Myeloid Cell Engraftment Kinetics

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Background/Aim

Non-myeloablative (NMA) conditioning regimens pre-alloSCT balance reduced toxicity with sufficient host immunosuppression for donor engraftment. The Royal Melbourne Hospital uses two variations of fludarabine and cyclophosphamide pre-alloSCT: high-dose (FluHDCy) and low-dose (FluLDCy) (Table 1). We analysed donor chimerism kinetics to identify if increased intensity of FluHDCy improved donor engraftment thereby enhancing graft-versus tumour-effect (GVT) compared to FluLDCy.

Methods

Patients who received FluHDCy or FluLDCy prior to T-cell replete matched-sibling alloSCT from 2000-2016 were analysed retrospectively. Graft-versus-host disease (GVHD) prophylaxis was uniform between groups. Peripheral blood donor chimerism (CD3-positive and CD3-negative) was performed at days 30, 60, 100.

Results

98 patients were analysed (Table 1) with median follow-up 38.4 months. The FluHDCy group had a greater proportion of myeloid neoplasms (42.2% vs 11.8%; $p=0.002$). There were no statistically significant differences between groups in age, sex, graft type, recipient/donor CMV status, transplant era or disease risk index (DRI).

Table 1. Demographics

	FluHDCy Fludarabine 125mg/m2 Cyclophosphamide 120mg/kg	FluLDCy Fludarabine 90mg/m2 Cyclophosphamide 2250mg/m2
N	64	34
Female	28 (43.8%)	18 (52.9%)
Age (median years)	48	51
Disease (n, %)		
AML	23 (36.0)	1 (2.9)
MDS	3 (4.7)	1 (2.9)
CML	1 (1.6)	2 (5.9)
ALL	1 (1.56)	1 (2.9)
NHL/CLL	17 (26.6)	20 (58.8)
HL	6 (9.4)	4 (8.8)
MM	13 (20.3)	6 (17.7)

All patients engrafted. There was no significant difference between FluHDCy and FluLDCy in median days to neutrophil engraftment (17 vs 16; $p=0.24$). More FluLDCy patients had platelet nadir $>20 \times 10^9/L$ (58.8% vs 17.2%; $p<0.0001$). Acute GVHD occurred in 42.2% of FluHDCy and 17.6% of FluLDCy patients. On univariate and multivariate analyses FluLDCy was significantly associated with a lower rate of acute GVHD (OR 0.30, 95% CI 0.1-0.85; $p=0.024$) and chronic GVHD (HR 0.53, 95% CI 0.29-0.96; $p=0.037$) compared to FluHDCy. There were no significant differences in median CD3+/CD3- chimerism at days 30, 60, 100 or achievement of complete donor T-cell chimerism (FluHDCy 49.2% vs FluLDCy 63.9%; $p=0.14$).

Conclusion

FluHDCy increases acute and chronic GVHD compared to FluLDCy without improving chimerism kinetics suggesting that FluHDCy is only indicated for persistent disease pre-alloSCT to reduce tumour burden while awaiting GVT. The differences in GVHD despite identical T-cell kinetics suggest host tissue priming by the more intensive conditioning regimen.

P056. Use of ibrutinib in patients with b-cell lymphoid malignancies: adverse events and management in the community setting

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Aims

To better understand the side effect profile and management of ibrutinib in the community.

Methods

A cohort of 44 participants, all refractory to at least two lines of therapy or with del 17p, were entered into this single centre study: 29 patients with CLL, 10 with SLL, 5 with MCL.

All were treated with ibrutinib at either 560mg/day (MCL) or 420mg/day (all others) until disease progression or death with a median treatment time of 12 months.

Response and adverse event data were recorded at 1-2 monthly intervals for these patients and subsequently analysed.

Results

Fourteen participants experienced dermatologic complication, including two with skin boils. Seven experienced symptomatic gastro-oesophageal reflux. Bruising and platelet dysfunction was noted in eight participants. Four developed solid malignancies including sarcoma (1), melanoma (1), SCC (1), renal cell carcinoma and adenocarcinoma of lung in 1 participant. We had one participant who experienced transaminitis. One developed hypertension and AF.

No participant experienced cytopenias or renal impairment or significant diarrhoea or nausea related to therapy.

A majority of participants have achieved at least very good partial response, with only eight requiring cessation for progressive disease, other malignancies, or intolerance of therapy (all with CLL/SLL).

Conclusion

In our cohort, the side effects from ibrutinib treatment were different from those recorded in clinical trials.

Cutaneous reactions and acne like lesions, as well as staphylococcal infections appear to be more common than appreciated in our experience. We have found topical treatment of acne lesions with antibiotics to be mostly effective and oral or intravenous antibiotics required for extreme cases.

Ibrutinib therapy is a well-tolerated and effective therapy option for patients with high-risk CLL/SLL or MCL.

Therapy can be managed in the community without requiring hospitalisation. However, there are concerns of solid malignancies in these patients.

P057. Mutation detection, copy number assessment, translocation characterisation and IGH sequence analysis using a novel single next generation sequencing workflow in the diagnostic laboratory

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Aim
Detection of genomic changes is central to the diagnosis, prognostication and treatment of haematological malignancy. We aimed to develop a novel single next generation sequencing (NGS) based workflow able to comprehensively characterise haematological malignancy including mutations, copy number variations (CNV), translocations/structural variants and sequencing of the IGH repertoire in order to identify genomic changes of clinical relevance and actionability in patients with haematological malignancy.

Methods
Patient samples and selected cell lines were sequenced using an Agilent SureSelect Custom Capture Panel and an Illumina NextSeq with 2x75PE reads. Bioinformatic analysis was performed using a mixture of open source and in-house tools (see Results).

Results
The capture was designed to target entire coding regions of ~300 genes chosen for diagnostic, prognostic and therapeutic relevance in haematological malignancy. The IGH locus was also captured (including non-coding regions) to enable IGH translocation detection. Four bioinformatic processes were implemented including (i) an SNV/INDEL detection pipeline (BWA-mem/GATK) (ii) CNV assessment using a pooled reference approach (iii) translocation detection (GRIDSS) (iv) IGH sequence repertoire determination using read-assembly (PEAR) followed by blasting against the IMG T reference set. The output is viewed by the reporting scientist/molecular pathologist using clinical informatics software (Path-OS) and novel browser-based visualisation tools.

Approximately 35 samples have been analysed to date including patients with newly diagnosed and relapsed/refractory multiple myeloma, chronic lymphocytic leukaemia, acute myeloid leukaemia and T-cell lymphoma. All variants, translocations and CNVs known to be present by orthogonal methodologies (Sanger sequencing, fragment analysis, amplicon-based NGS, conventional metaphase cytogenetics and FISH) were successfully detected using the new NGS workflow. In addition, multiple new variants and CNVs were detected in these samples providing further biological insights and clinically relevant information on this cohort.

Conclusion
We have developed an NGS-based workflow able to perform simultaneous mutation detection, translocation characterisation, copy number assessment and IGH sequencing focussed on detecting clinically relevant genomic lesions in the diagnostic laboratory. This novel workflow has the potential to both complement and replace existing conventional methodologies to efficiently provide clinically relevant information on patients with haematological malignancy

P058. Outcomes With Ibrutinib by Line of Therapy in Patients With CLL: Analyses From Phase 3 Data

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Aim

Ibrutinib, a first-in-class, once-daily inhibitor of Bruton's tyrosine kinase, is indicated by the TGA for the treatment of patients with CLL/small lymphocytic lymphoma who received at least 1 prior therapy, and allows for treatment without chemotherapy. We evaluated outcomes with ibrutinib based on prior lines of therapy (LoT), and following discontinuation (DC) of ibrutinib in CLL patients.

Method

Data were analysed from two phase 3 randomized trials of ibrutinib: PCYC-1115/16 (RESONATE-2) in patients aged ≥ 65 years with treatment-naïve (TN) CLL and PCYC-1112 (RESONATE) in previously treated (PT) CLL, excluding patients with del17p for a more homogeneous dataset. Progression-free survival (PFS) and overall response rate (ORR) were assessed by investigator.

Results

In 271 patients included for analysis, median age was 73 years for TN patients and 66 years for PT patients. Most common therapies in PT patients included CD20 antibody (93%), alkylating agents (93%; bendamustine 41%), or purine analogue (87%); median number of prior therapies was 3. Median PFS or OS was not reached (NR) for TN or PT patients. For patients treated with ibrutinib in first or second line, 89-92% were progression free at 2 years (Table). ORR was high regardless of LoT (91% in TN, 92% in PT patients). The adverse event (AE) profile was similar for both groups, and most patients continued ibrutinib. Patients treated with ibrutinib in first or second line were less likely to DC ibrutinib due to progression. Median OS post-ibrutinib DC is NR for patients treated with ibrutinib in first or second line (n=23) vs 7-9 months in third line and beyond (n=34).

Conclusion

In patients with CLL, ibrutinib treatment showed favourable PFS and OS, and high ORR regardless of LoT. Patients treated with ibrutinib in first or second line were less likely to experience progression and had better post-ibrutinib survival outcomes.

Table. Outcomes with Ibrutinib by Prior Lines of Therapy				
	Subgroups by Prior Lines of Therapy (N=271)			
	0 (n=136)	1 (n=27)	2 (n=41)	≥ 3 (n=67)
Median age, years (range)	73 (65-89)	64 (30-85)	66 (46-86)	67 (44-83)
Median follow up, months (max)	22 (32+)	30 (36+)	31 (34+)	30 (37+)
24-month PFS rate, %	92	89	80	69
30-month OS rate, %	97	93	83	82
Patients evaluated for outcomes post ibrutinib DC*	0 (n=18)	1 (n=5)	2 (n=12)	≥ 3 (n=22)
Median time post DC, months				
Follow up	9	5	9	10
OS	NR	NR	9	7
OS: DC due to progression	NR (n=4)	NR (n=2)	8 (n=5)	6 (n=11)
OS: DC due to AEs	NR (n=13)	NR (n=1)	16 (n=6)	NR (n=7)
*5 patients who discontinued study ibrutinib to receive commercial ibrutinib are not included. Includes all patients who remained in study follow-up after discontinuation due to progression, AE, or investigator decision.				

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P059. The use of Ibrutinib in the acute management of bulky lymphadenopathy causing respiratory compromise – a case report

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Aim/Background

Extensive and bulky lymphadenopathy can cause significant morbidity in patients with Chronic Lymphocytic Leukaemia (CLL). Ibrutinib is a Bruton's tyrosine kinase (BTK) inhibitor used in the treatment of CLL. A case history is described of a patient presenting with respiratory compromise secondary to extensive lymphadenopathy, and in whom Ibrutinib was effectively used in the acute setting, to provide significant and rapid improvement in respiratory status.

Results

An 86 year old lady presented with type one respiratory failure, on a background of CLL diagnosed in 2002. She had received previous treatment for progressive lymphocytosis with Chlorbambucil in 2011 and subsequent FCR chemotherapy in 2013, with good response.

The patient presented in late 2015 with dyspnoea and lethargy over the preceding four days. At presentation she had a progressive lymphocytosis of 98.62×10^9 and type one respiratory failure (oxygen saturations of 85% on room air). CT imaging revealed significant interval disease progression with extensive conglomerate mediastinal lymphadenopathy with regional mass effect to the superior vena cava and bilateral bronchi. The patient was initially treated with three days of IV methylprednisolone. Urgent radiotherapy was considered, however application was made to commence Ibrutinib therapy. On the 4th day of admission Ibrutinib was commenced at a dose of 420mg daily.

The patient showed a very rapid response to therapy and after 2 days of treatment no longer had an oxygen requirement. She was able to be discharged on day three of therapy. After an initial, expected increase in lymphocytosis, the patient's lymphocyte count decreased to 10.75×10^9 after 6 months of therapy, with ongoing resolution of the respiratory symptoms.

Conclusion

In patients presenting with extensive lymphadenopathy causing respiratory compromise, the use of Ibrutinib in the acute setting showed a rapid and effective treatment with resolution of type one respiratory failure secondary to compressive lymphadenopathy.

P060. Case Report: Type 1 Cryoglobulinaemia complicated with severe acute renal failure, peripheral neuropathy and purpura on a background of chronic lymphocytic leukaemia

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Background

Cryoglobulinaemia is a rare disorder characterized by the presence of either immunoglobulins or a mixture of immunoglobulin and complement components that precipitate with reduced temperature of serum and plasma and redissolve on warming. Clinically significant cryoglobulinaemia is often associated with chronic infection, inflammation, autoimmunity and chronic lymphoproliferative disorders.

Case Report

We describe an 86 yo man with longstanding stage 1 chronic lymphocytic leukaemia complicated by Type 1 cryoglobulinaemic vasculitis. Presenting features included purpuric rash, fatigue, rapid deterioration of renal function (creatinine 200umol/L) and peripheral neuropathy.

Serum protein electrophoresis showed a monoclonal IgM 7.07g/L with Kappa free light chains 1021mg/L, and a Kappa/Lambda ratio of 31.9. Cryocrit was 53%. Renal biopsy showed type 1 diffuse membranoproliferative glomerulonephritis with arteritis, hyaline arterial and glomerular capillary luminal thrombi consistent with cryoglobulinaemia strongly staining with IgG and IgM. Nerve conduction studies showed a length dependent axonal sensorimotor neuropathy.

Treatment was directed at the underlying CLL with Rituximab 375mg/m², Chlorambucil 10 mg/m² daily, and Prednisolone 1mg/kg for 5 days. The white cell count rapidly decreased, from 41.5 to 10.6. Patient remains stable and further courses of Rituximab and Chlorambucil are planned.

Discussion

Renal biopsy was instrumental in making the diagnosis of this rare complication of CLL. The presence of isolated monoclonal immunoglobulin (typically IgG or IgM, less commonly IgA) is the criterion for classification as type 1 cryoglobulinaemia. The association of cryoglobulinaemia with CLL is rare and a search for an alternative cause including HIV, Hepatitis B, C, EBV, CMV, Parvovirus B 19, autoimmune disorders, and other haematological malignancies was negative with bone marrow biopsy confirming CLL.

Management should be directed at the underlying cause (in this case CLL) with a randomized control trial demonstrating survival benefit for rituximab based therapy over other therapy including plasma exchange for patients with severe cryoglobulinaemia.

P061. Clinicopathological features and outcome of progression of chronic lymphocytic leukaemia during treatment with the BCL2 inhibitor venetoclax

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Aim

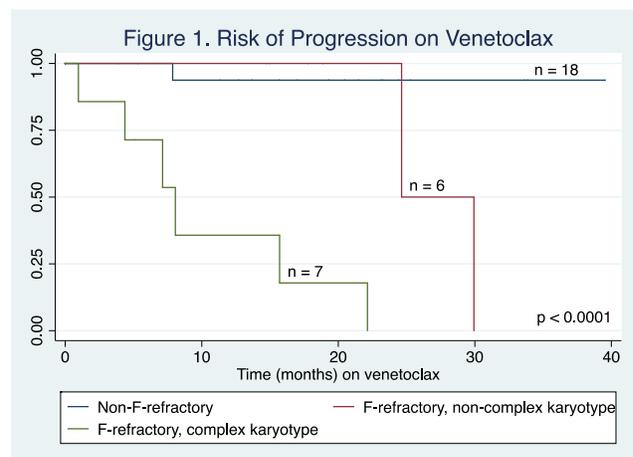
The BCL-2 inhibitor venetoclax achieves objective responses in ~80% of patients with relapsed/refractory CLL; however, some patients' disease progresses on therapy. We report the clinicopathological features, outcomes and putative predictors of progression on venetoclax.

Methods

We retrospectively reviewed 68 patients treated with venetoclax for relapsed/refractory CLL on clinical trials at Royal Melbourne Hospital and Peter MacCallum Cancer Centre from June 2011 – August 2015. We recorded baseline characteristics, responses, outcomes, and the clinicopathological features at progression. Univariate Kaplan Meier and Classification and Regression Tree (CART) analyses were used to interrogate potential risk factors for progression on therapy.

Results

The median age was 67.5 (20–87) years, median prior therapies 3 (1–12), and disease was fludarabine refractory (F-refractory) in 49%. With median follow up of 23 (2–46) months, 24 patients (35%) progressed; 17 (71%) with Richters transformation (RT) (14 DLBCL, 3 Hodgkin-like) and 7 (29%) had progressive CLL. Time-to-progression was closely related to best iwCLL response (median: not reached, 25 and 6 months for CR, PR and SD, respectively; $p < 0.0001$). In patients who received $\geq 400\text{mg/day}$ ($n=50$), F-refractoriness and complex karyotype were associated with risk of progression ($p=0.004$ and $p=0.006$, respectively). On CART analysis, complex karyotype was most predictive of progression in F-refractory disease ($p=0.0027$), whereas non-F-refractory disease had a low incidence of progression irrespective of complex karyotype ($p=0.5637$). This suggests a three tier risk score: low = non-F-refractory; intermediate = F-refractory, non-complex; and high = F-refractory and complex ($p < 0.0001$) (Figure 1). Median survival after progression was 13 months, with three cases of Hodgkin-like RT alive at a follow up of 24–46 months.



Conclusion

RT is a common mechanism of progression on venetoclax for patients with CLL. F-refractoriness and complex karyotype increase the risk of progression. Although outcomes for progressors overall are poor, some patients with RT can have durable responses to salvage chemotherapy.

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P062. Fludarabine, cyclophosphamide and rituximab (FCR) treatment improves outcome in a comatose patient with chronic lymphocytic leukemia (CLL) and cerebral leukostasis with marked hyperleukocytosis

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Case

We describe a case of 44 y.o man, with RAI stage II CLL and a lymphocyte count (ALC) of $830 \times 10^9/L$ since 2013, who declined therapy despite extensive lymphadenopathy and massive splenomegaly. In January 2016, he was brought in with a decreased conscious state (GCS=3) requiring emergency intubation and a persistent lymphocytosis of $818 \times 10^9/L$. MRI brain demonstrated lepto-meningeal enhancement and multiple foci of white matter diffusion restriction, however, no evidence of cerebral involvement with CLL. His cerebrospinal fluid examination was bland. In the absence of proven infection or other causes for decreased GCS, cerebral leukostasis was considered to be the most likely diagnosis. Graduated doses of fludarabine, cyclophosphamide and rituximab (FCR) was commenced with significant neurological improvement observed after reduction of ALC below $400 \times 10^9/L$ and with complete neurological recovery observed with $ALC < 100 \times 10^9/L$ (Figure.1). Leukopheresis was not available in our institution. Our patient achieved a complete normalisation of his lymphocyte count after one cycle of FCR.

Discussion

Leukostasis is a rare complication of CLL and is more commonly associated with acute leukemias, with cerebral and pulmonary involvement being the two most common sites. Only few case reports describe cerebral leukostasis in CLL; all of these cases have $ALC > 1,000 \times 10^9/L$ and were associated with concurrent pulmonary complications. Optimal treatment for CLL leukostasis is unknown, however, fludarabine-containing regimen has been used in CLL patients with lepto-meningeal involvement due to the ability to cross blood-brain barrier.

Conclusion

In CLL patients with marked hyperleukocytosis and neurological symptoms, cerebral leukostasis should be considered as a possible diagnosis after exclusion of other causes such as CNS infections. Prompt treatment of CLL with FCR chemotherapy appears effective in this setting.



Figure 1: Lymphocyte count vs GCS

P063. Incidence of Cutaneous Squamous Cell Carcinoma in a Population of New Zealand Chronic Lymphocytic Leukaemia Patients

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Background and Aim

Chronic lymphocytic leukemia (CLL) increases the incidence and aggressiveness of skin cancers, in particular cutaneous squamous cell carcinoma (cSCC), but little is known of cSCC incidence in Australasian CLL patients. In this retrospective study we analysed the incidence of cSCC in patients seen at a tertiary hospital in New Zealand.

Method

We retrospectively assessed the clinical history and histology data of CLL patients (n=371) who were seen in the Haematology Department, Christchurch Hospital, during the period 1996-2015. Baseline characteristics, incidence of second cancers, treatment details and overall survival were analysed.

Results

During follow-up (median = 11.8 years) 221 second cancers were recorded in 88 patients. Of these cancers, 185 were cSCC removed from 61 patients. In 56% of these patients >1 cSCC was removed with the majority of cSCC occurring following treatment for CLL. The cumulative incidence of a first cSCC was 11% at 5 years. Analysis of the cumulative incidence of a second cSCC indicated that virtually all patients who develop one cSCC will subsequently develop another cSCC. From the time of the first recorded cSCC the median time to the development of a second was 29 months (95% CI 12-46) and the cumulative incidence at 5 years was 88%. The incidence of cSCC in male patients was substantially higher than that reported for the general NZ population.

Conclusions

New Zealand CLL patients have a high incidence of cSCC, even relative to the levels observed in the general population which are themselves among the highest in the world. Careful monitoring of CLL patients is warranted, particularly those with progressive disease or who have already undergone excision of a first cSCC.

P064. Incidence and description of cytopenias with ibrutinib: a retrospective cohort analysis of 40 patients

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Background

Ibrutinib is an oral Bruton's tyrosine kinase (BTK) inhibitor with demonstrated efficacy in B-cell malignancies including chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL). Ibrutinib is generally well tolerated, and haematologic toxicity reported to be uncommon. In initial clinical trials, <20% of patients experienced cytopenias (any grade). We report and describe the incidence of cytopenias, defined as anaemia (Hb drop \geq 2g/L from baseline), neutropenia ($<1.0 \times 10^9/L$) and thrombocytopenia ($<100 \times 10^9/L$), in a cohort of patients from our centre receiving ibrutinib monotherapy.

Method and Results

We retrospectively identified a cohort of 40 patients at our institution who received ibrutinib for CLL/SLL, MCL or Waldenström macroglobulinaemia (WM), from January 2013 to March 2016. 22 of 40 (55%) patients developed cytopenias as defined above. The majority (18) of these had single cell lines affected only, although 3 patients had 2 cell lines affected, and one patient had pancytopenia. 10 patients (25%) developed neutropenia, at a median time of 9.5 weeks (range 1 – 30); the median neutrophil nadir was 0.49 (range 0.03-0.90). The majority were managed with growth factor support and/or withholding ibrutinib, and all patients recovered their neutrophil count. Infective complications were seen in 6 of these 10 patients. 13 patients (32.5%) developed thrombocytopenia at a median time of 10 weeks (range 2-24), with a median platelet nadir of 86 (range 61-94). No patients experienced severe bleeding complications, although 6 had Grade 1 ecchymoses.

Conclusion

Ibrutinib-associated cytopenias occurred in over half the patients in our cohort, which is higher than previously reported rates. The timing of the cytopenias was variable. While the frequency of thrombocytopenia was higher than neutropenia (32.5% vs. 25%), the neutropenias observed were more severe and clinically significant than the thrombocytopenias. However, the majority of patients in both groups recovered to normal levels after the event. The pattern of neutropenia observed with ibrutinib was similar to the late-onset neutropenia previously described with rituximab.

P065. Distinct molecular and immunological features of chronic lymphocytic leukemia patients with certain molecular mutations

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Background

Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western hemisphere. Next-generation sequencing technologies revealed previously unknown molecular alterations such as *NOTCH1*, *MYD88* and *SF3B1*, which may prognostic value. About 30% of CLL patients with stereotyped B-cell receptor can be grouped into distinct subsets which are characterized by biological and clinical features.

Aims

Characteristics of CLL patients with *NOTCH1*, *MYD88* and *SF3B1* mutations with regard to molecular and immunological prognostic markers in CLL.

Methods

DNA was isolated from peripheral blood mononuclear cells of 371 CLL patients. *NOTCH1* c.7544_7545delCT in PEST domain (exon 34) and *MYD88* L265P mutations were investigated by ARMS PCR. Screening for *SF3B1* mutations in exons 14-16 and *IGHV* gene mutations were performed by Sanger sequencing.

Results

NOTCH1 c.7544_7545delCT mutation was found in 19/316 (6.0%) patients. Patients harboring *NOTCH1* mutation prevalently have unfavorable prognostic factors including unmutated *IGHV* gene status, expression of CD38 (>30%) and expression of ZAP-70 (>20%) (Table 1). Analysis of *IGHV* subsets in patients with *NOTCH1* mutation revealed frequent presence of subset #1 in n=2/19 (10.5%), which is associated with particularly poor prognosis. Patients belonging to subsets #5, #6, #201 and #202 were also present, each in single *NOTCH1* mutated CLL case (5.2%). *MYD88* L265P (exon 5) mutation occurred in 12/323 (3.7%), of whom one patient was characterized as subset #2 and another as subset #4. *MYD88* mutations were nearly equally distributed in patients with mutated/unmutated *IGHV* status (5 vs. 7). *SF3B1* mutations occurred in 5/72 (6.9%) patients, furthermore two of them carrying negative prognostic features of subsets #2 and one subset #1.

Summary

NOTCH1 and *SF3B1* mutations accompany certain biological markers of unfavorable prognosis, while *MYD88* has no association with them.

Table 1. Correlation between NOTCH1, MYD88 and SF3B1 mutations and prognostic markers in CLL

	<i>NOTCH1</i> mutated		<i>NOTCH1</i> wild-type		Significance
	N=	%	N=	%	
<u>Unmutated</u> <i>IGHV</i> status	18/19	94.7	146/293	49.8	p<0.0001
CD38 positive	10/18	55.0	66/250	26.4	p=0.0132
ZAP-70 positive	12/18	66.0	77/242	31.8	p=0.0041
Subset #1	2/19	10.5	9/298	3.0	ns.
Subset #2	0/19	0.0	10/298	3.4	ns.
	<i>MYD88</i> mutated		<i>MYD88</i> wild-type		
<u>Unmutated</u> <i>IGHV</i> status	7/12	58.3	162/308	52.6	ns.
CD38 positive	1/12	8.3	78/265	29.4	ns.
ZAP-70 positive	4/12	33.3	89/257	34.6	ns.
Subset #1	0/12	0.0	9/265	3.4	ns.
Subset #2	1/12	8.3	11/265	4.2	ns.
	<i>SF3B1</i> mutated		<i>SF3B1</i> wild-type		
<u>Unmutated</u> <i>IGHV</i> status	2/5	40	27/67	40.3	ns.
CD38 positive	1/2	50	16/58	27.5	ns.
ZAP-70 positive	1/2	50	12/54	22.2	ns.
Subset #1	1/5	20	4/65	6.2	ns.

P066. Analysis of Quality of Life and Well-being in Older Patients With Treatment-naïve CLL: Results From the Randomized Phase 3 Study of Ibrutinib Versus Chlorambucil (RESONATE-2™)

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Aim

CLL is primarily a disease of older patients for whom health-related quality of life (QOL) is an important consideration. We evaluated QOL parameters and patient well-being in older patients with treatment-naïve CLL from the recent phase 3 randomized PCYC-1115 study (RESONATE-2).

Method

Treatment-naïve patients age ≥65 years received ibrutinib 420 mg once daily until progression or chlorambucil (0.5 to 0.8 mg/kg days 1, 15) up to 12 cycles. Patient-reported QOL was measured by change from baseline in FACIT-Fatigue and EORTC QLQ-C30 scores.

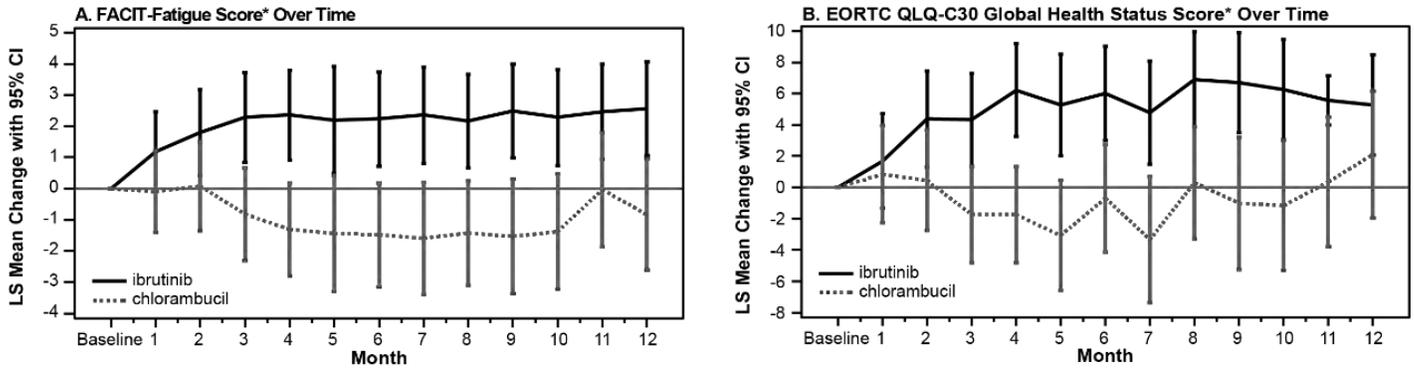
Results

Among 269 patients randomized, median age was 73 years and 69% had comorbidities at baseline. Median follow-up was 18.4 months. Ibrutinib patients reported greater improvements over time in FACIT-Fatigue ($P=0.0004$) and QLQ-C30 global health ($P=0.0002$) by repeated measures versus chlorambucil (Figure). In ibrutinib patients, sustained improvement in hematologic function occurred in 84% with baseline anaemia and 77% with baseline thrombocytopenia versus 45% and 43% for chlorambucil patients. Median time to first sustained improvement in haemoglobin occurred earlier with ibrutinib (4 months versus 12 months with chlorambucil; $P=0.0003$), with similar results for platelets (3 months with ibrutinib versus 11 months with chlorambucil; $P=0.0269$). Reduction ≥50% in lymph nodes and spleen enlargement occurred in 95% and 95% of ibrutinib patients versus 40% and 52% chlorambucil patients; complete resolution of lymphadenopathy and splenomegaly occurred in 42% and 56% versus 7% and 22%, respectively. Improvements in disease symptoms including fatigue, night sweats, and weight loss were more frequent with ibrutinib. Adverse events resulting in ibrutinib discontinuation were infrequent. At study completion, 87% of ibrutinib patients continued treatment.

Conclusion

Ibrutinib was associated with greater increase in QOL, improved haematologic function, and reduced disease burden versus chlorambucil in older treatment-naïve CLL patients. The favourable safety profile of ibrutinib allowed 87% of these patients to continue therapy after median 1.5 years of follow-up.

Figure. Change in Patient-reported QOL Scores Over Time



*Least square mean change from baseline

The research was supported by Pharmacyclics LLC, an AbbVie Company. The company provided statistical analyses of the data, and worked in collaboration with the lead author in preparing the abstract. Editorial support was funded by Pharmacyclics LLC, an AbbVie Company.

P067. Metastatic Non-Melanoma Skin Cancer in patients with Chronic Lymphocytic Leukaemia/Low Grade LPD: A Pilot Study

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Aim
CLL is associated with more locally advanced and/or metastatic non-melanoma skin cancer (NMSC), increased rate of recurrence, and death. Being a non-reportable cancer, the epidemiology of NMSC is notoriously difficult to study. Aiming to better inform study design for future research efforts, we undertook a Pilot Study to identify patients with CLL/indolent LPD and NMSC in order to evaluate baseline characteristics and survival outcomes. To our knowledge this is the first evaluation of this challenging cohort in the Australian context.

Method
A retrospective review of databases from Calvary Mater Newcastle, NSW, was undertaken to identify patients with CLL or indolent LPD and invasive NMSC. Data pertaining to age, gender, dates for diagnosis and treatment of CLL and NMSC, death and cause of death was obtained.

Result
Merging the databases of 546 patients treated with Radiotherapy for node positive NMSC between 2005-2015, and 1416 patients coded as having an LPD between 1996-2016, a total of 23 cases with both conditions were found. Of the 17 CLL/SLL and 6 cases of indolent NHL, 19 patients received treatment prior to development of NMSC. With an average follow up of 10.7 years, the overall survival from time of diagnosis of LPD was 7.76 years for the CLL/SLL patients and 3 years for the indolent NHL patients. The average survival from diagnosis of invasive NMSC was 1.25 years for the CLL/SLL patients and 0.83 years for the indolent NHL cases.

Conclusion
Recognising the significant limitations owing to small study size and retrospective database review subject to selection bias, this study generated numerous valuable insights. Survival of this overall low to intermediate risk cohort was worse than historically reported. There remains scope for substantial advancement of the scientific understanding and clinical service provision for people with both CLL/indolent LPD and NMSC.

P068. The dermatological manifestations of chronic lymphocytic leukaemia – data from a combined chronic lymphocytic leukaemia / dermatology clinic

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Aim/Background

Chronic Lymphocytic Leukaemia (CLL) patients often have cutaneous manifestations from their disease and are at increased risk of dermatological conditions, including malignant and infective processes. In addition to increased skin cancer incidence, clinical evidence suggests that skin cancers progress more rapidly in CLL patients, particularly following chemotherapy for their CLL. It is not uncommon for patients to die from a progressive dermatological malignancy while control of their CLL is maintained, particularly since the advent of immunochemotherapy and targeted therapies for CLL which have improved CLL-related survival. Distinguishing these dermatological processes in the correct clinical context is vital in the management of these patients. To address this area of need, a combined CLL Haematology / Dermatology tertiary referral clinic was established.

Method

Retrospective audit of presentations to the combined CLL/dermatology clinic from May 2013 to June 2016.

Results

From May 2013 to June 2016, 75 CLL patients with skin lesions were reviewed in 258 separate encounters which resulted in 133 distinct diagnoses. Cutaneous manifestations of CLL accounted for 3.8% of the diagnoses – these ranged from leukaemia cutis to life-threateningly severe paraneoplastic pemphigus ([PNP] or paraneoplastic autoimmune multi-organ syndrome [PAMS]). A significant number of dermatologic malignancies were encountered in these patients (46.6% of diagnoses), facilitating early specialist treatment including referral for surgical and/or radiotherapy. Infective skin conditions, which may often be exacerbated or atypical due to immunoparesis or as a consequence of therapy, accounted for 13.5%% of diagnoses. Exaggerated insect bite reactions are well recognised in CLL. Non-malignant dermatological conditions including drug exanthems and eczema accounted for the remaining diagnoses and appeared significantly more common during and following therapy for CLL, both with immunochemotherapy and targeted therapies.

Conclusion

Provision of a combined Haematology/Dermatology referral service allows for timely triage, and more targeted management and follow-up of the myriad of skin conditions that may occur in the often complex clinical setting of CLL patients.

P069. Methyl Tetrahydrofolate Reductase (MTHFR) genotyping and its correlation with methylation status of CDKN2A & SnK/PIK2 gene in hematological malignancies (CML, AML, MM, MDS, ALL)

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Introduction

Folate and methionine metabolism pathways plays essential role in DNA biosynthesis and methylation. DNA methylation is catalyzed by MTHFR of which two most commonly studied variants (677CT, 1298AC) are associated with decreased enzymatic activity. PIK2 (Snk/PIK2) is involved in check point mediated cell cycle arrest to ensure genetic stability thereby inhibiting accumulation of genetic defects. Snk/PIK2, tumor suppressor gene in B lymphocytes. However no study has examined Snk/PIK2 epigenetic changes in plasma cell dyscrasias.

Materials & Methods

Study included, 200 patients with hematological malignancy (CML, AML, MM, ALL, CLL) and 200 healthy subjects. DNA was extracted from both individuals. Genotyping PCR was done for MTHFR 1298 and 677 gene and product were digested with MBOII and HinfI enzymes respectively. Further, DNA had used for bisulfide conversion. Product obtained after bisulfide conversion was used for analysis of methylation status of CDKN2A & SnK/PIK2 genes. Clinical parameters were correlated with MTHFR genotyping and methylation status.

Results

Results showed insignificant statistical difference in MTHFR 1298AC and 677CT genotypes. MTHFR polymorphism have unequal role in different blood malignancy (AML, CML, ALL, MM). Moreover, MTHFR 1298AC and 677CT correlation was observed between CDKN2A methylation and MTHFR genotypes. We observed CDKN2A promoter hyper methylation in 27% of patient. Methylation in the Snk/PIK2 CPG island was detected in 60% of patients but in control tissues. The presence of methylation did not correlate significantly with any clinical parameter. No correlation found between CDKN2A and Snk/PIK2 hepermethylation and 1298AC genotypes.

Conclusion

PCR-RFLP considered as a simple and sensitive method to analyze the genotypic polymorphism in leukemia. Moreover, MTHFR considered as marker for the detection of disease complication. Patients with *MTHFR* mutation are associated with adverse outcome, poor response to therapy and shorter survival. Snk/PIK2 as a novel methylated gene in some hematological malignancies.

P070. Evaluation of the Cepheid Xpert BCR-ABL ultra assay sensitivity with clinical specimens

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RPA Haematology

Aim
The Xpert BCR-ABL Ultra assay has been released as a sensitive quantitative BCR-ABL test what will replace the existing Cepheid Xpert BCR-ABL Monitor test on the GeneXpert instrument. Our laboratory sought to ascertain whether claimed improvements in sensitivity of the Ultra assay over the Monitor could be validated and to determine whether the Ultra assay could be used to test external quality assurance specimens distributed as RNA.

Method and Results

A pre-release version of the Ultra assay was used to test blood from 39 patients with previously confirmed CML as per the manufacturer's instructions and compared to results obtained using the Monitor. Concordant results were obtained on 32 patients (22 detectable and 10 not detectable). Seven patient specimens were only detectable using the Ultra assay with BCR-ABL IS levels ranging between 4.57 and 5.44 log reduction. RNA from clinical specimens was loaded into Ultra cartridges in lysis buffer following an existing validated laboratory procedure used to test RNA with the Monitor assay. The Ultra assay was found to require at least 2.5 times more RNA than the Monitor when loaded directly in order to detect BCR-ABL transcripts in a positive specimen. Subsequent testing of the released version of the Ultra assay with RNA obtained from other clinical specimens and RCPA QAP material consisting of lyophilised cell line mixtures was used to compare the two assays. At a BCR-ABL transcript level equivalent to MR5 on the IS scale, at least 1000ng of RNA was required for detection on the Ultra assay whereas 500ng was sufficient for detection using the Monitor assay.

Conclusion

Preliminary evaluation of the Ultra assay with a limited number of clinical specimens indicated that it was able to detect BCR-ABL transcripts in patients with MR 4.5 or greater when transcripts could not be detected with the Monitor assay. External quality control material could be also be utilised to allow the assay to be adopted for routine diagnostic testing. The deeper sensitivity of the Ultra assay is promising in the context of selecting and monitoring patients for kinase inhibitor cessation studies.

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

P071. The Impact of Early Molecular Response on Long-Term Outcomes in Patients With Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated With Dasatinib or Imatinib From the DASISION Trial

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Aim
To evaluate the impact of EURO risk score on achieving *BCR-ABL1* ≤10% at 3 months and to investigate other predictive factors of survival.

Methods
Patients received dasatinib 100 mg (n=259) or imatinib 400 mg (n=260) once daily. *BCR-ABL1* transcript levels were measured on the International Scale. Risk scores ≤780 were considered low, >780 to ≤1480 intermediate, and >1480 high.

Results
Based on EURO score, 33% of patients in both treatment arms were at low risk, 48% and 47% of patients receiving dasatinib and imatinib, respectively, were at intermediate risk, and 19% in both arms were at high risk. Across all risk groups, more patients on dasatinib had *BCR-ABL1* ≤10% at 3 months vs imatinib (low: 91% vs 73%, intermediate: 80% vs 66%, high: 83% vs 44%). The largest difference was in the high-risk group: 17% of high-risk patients on dasatinib did not achieve *BCR-ABL1* ≤10% at 3 months vs 56% on imatinib. More patients on dasatinib vs imatinib had *BCR-ABL1* ≤1% at 3 months (low: 56% vs 20%, intermediate: 48% vs 12%, high: 33% vs 5%). Five-year efficacy outcomes were higher for patients with *BCR-ABL1* ≤10% vs >10% at 3 months in all risk groups. Multivariate analysis of overall survival at 5 years showed women on dasatinib were at a lower risk of death than men; no association was found between survival and other explanatory variables.

Conclusions
More patients achieved *BCR-ABL1* ≤10% at 3 months on dasatinib vs imatinib regardless of risk score, with significantly more patients on dasatinib with *BCR-ABL1* ≤1%. The largest difference in *BCR-ABL1* between the treatment arms was in high-risk patients. Long-term outcomes were superior for patients with *BCR-ABL1* ≤10% at 3 months in all risk groups. These analyses suggest that dasatinib should be considered as first-line therapy for CML-CP patients across risk groups.

This research was supported by Bristol-Myers Squibb. The company sponsored both data analysis and abstract preparation.

P072. Long-term Results from the Ponatinib Phase 2 PACE Trial

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Aims

Evaluate long-term outcomes with ponatinib in heavily-pretreated CML and Ph+ALL patients from the pivotal PACE (NCT01207440) trial.

Methods

Multinational trial; n=449. Ponatinib starting dose 45 mg/day; dose reductions were instructed in Oct-2013 to mitigate arterial occlusive events (AOEs). Outcomes: 4-year efficacy (cytogenetic/molecular response) and safety. Exposure-adjusted incidence of new AOEs reported as number of events/100 patient-years.

Results

59% of patients received ≥ 3 prior TKIs. As of 3-Aug-2015, 30% (133/449) of all patients (median follow-up 37.3, range 0.1–58.5 months) and 41% (110/270) of chronic phase (CP)-CML patients (48.2, 0.1–58.5 months) remained on study. Primary discontinuation reasons: disease progression (22.5% overall/10.4% CP-CML), AEs (16.0%/18.5%). Response by disease phase is shown in the Table. In CP-CML, among responders, estimated maintenance of MCyR and MMR at 4 years was 82% and 61%, respectively. Estimated 4-year PFS/OS rates were 56%/77%. For accelerated phase patients, estimated 4-year OS: 51%; median OS for blast phase/Ph+ALL patients: 6.9 months (95% CI, 5.0–9.2). Treatment-emergent AEs in $\geq 30\%$ of all patients: thrombocytopenia 44%, abdominal pain 43%, rash 42%, constipation 37%, headache 37%, dry skin 36%, fatigue and hypertension 30%. AOE/serious AOE rates: 23%/19%. New AOE exposure-adjusted incidence rates fell after 2 years: 15.5 Year 1, 15.7 Year 2, 10.4 Year 3, and 9.6 Year 4. Nearly 2 years after prospective dose reductions were introduced, irrespective of whether patients underwent dose reductions or not, 87% (114/131) and 74% (70/95) of CP-CML patients maintained MCyR and MMR, respectively, and 8% (6/75) of all dose-reduced patients without a prior AOE on trial had a new AOE. Regional Asia-Pacific data will be presented.

Conclusions

After 4 years, heavily pretreated leukemia patients continue to show deep, lasting responses on ponatinib; approximately 2 years post recommended dose reductions, maintenance of response is high and the incidence of newly-occurring AOE's has not increased.

Response to ponatinib at any time in the treated population, %				
	MCyR	CCyR	MMR	MR ⁴ /MR ^{4.5}
CP-CML (n=267)	59	54	39	29/23
	MaHR			
AP-CML (n=83)	61% (MMR = 22%)			
BP-CML (n=62)	31%			
Ph+ALL (n=32)	41%			

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P073. High gene expression of HIST1H2AG and HIST1H4A reduces imatinib uptake into CML cells and predicts poor response to frontline imatinib therapy

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Aim

We have previously demonstrated that the OCT-1 activity (OA) assay, (which measures the cellular uptake of IM at diagnosis), is highly predictive of subsequent treatment outcomes in chronic phase chronic myeloid leukemia (CP-CML) patients. OA assay requires radiolabelled ¹⁴C-imatinib, thus it is not widely transferable. We aim to identify a set of genes (based on gene expression) measured at diagnosis that predicts patients who have low OA and inferior clinical outcome.

Method

RNA was isolated from total white cells collected from patients on TOPS, TIDEL-I and TIDEL-II trials. The OA associated genes were identified based on a pilot microarray study (n=14), and were validated by qRT-PCR in 110 diagnostic CP-CML samples. Patients were divided into low and high gene expression groups using the recursive partitioning and regression trees in the training cohort (n=55), and validation in an independent cohort (n=55).

Results

We identified high expression of 2 histone genes (*HIST1H2AG* and *HIST1H4A*) was significantly associated with low OA in the training cohort, and this was validated in the independent patient cohort (Figure 1A). Significantly, in the validation cohort, patients with high expression of these 2 histone genes (histone^{high}) demonstrated inferior rates of major molecular response (MMR: 43% vs 79%, p=0.007), and inferior rates of MR^{4.5} (18% vs 52%, p=0.005) compared to patients with low expression of these genes (histone^{low}; Figure 1B-C). There were 6 patients with blast crisis (BC) progression and/or mutations that classified as low OA, and all 6 were in the histone^{high} group. Furthermore, overexpression of *HIST1H2AG* using lentiviral transduction significantly reduced the IM cellular uptake in K562 cells (p=0.001, Figure 1D), supporting our clinical data observation.

Conclusion

Histone gene expression likely plays a role in regulating OA, and possibly BC progression. Patients in the histone^{high} group are highly likely having inferior clinical outcomes on IM.

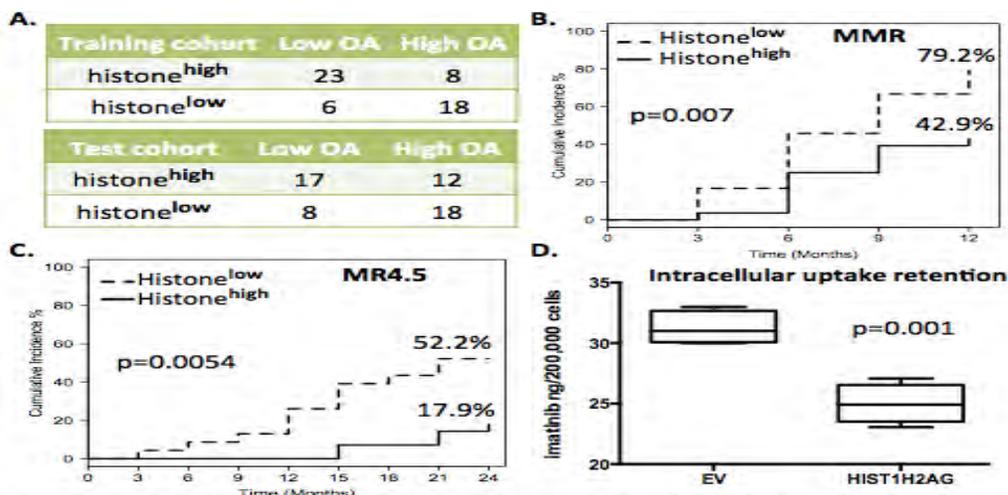


Figure 1. Low *HIST1H2AG* and *HIST1H4A* gene expression predicts high OCT-1 activity (OA) and favorable clinical outcomes. (A.) Patients were divided into low and high *HIST1H2AG* and *HIST1H4A* gene expression group using the classification tree method in the training cohort (n=55). Histone^{low} patient group was significantly associated with high OA (p=0.0003). This result was validated in an independent test cohort (p=0.038). (B-C.) In the test cohort, histone^{low} patient group (dotted line) demonstrated superior rates of MMR and MR4.5 compared to histone^{high} patient group (solid line). (D.) Overexpression of *HIST1H2AG* gene significantly reduced cellular uptake of imatinib compared to empty vector control (EV) in K562 cells.

P075. Ponatinib facilitates treatment-free remission by inducing deep molecular responses

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Multiple trials now demonstrate the potential for treatment-free remission (TFR) in patients with sustained deep molecular response to tyrosine kinase inhibitors (TKIs). We describe two patients who remain in treatment-free remission after achieving undetectable BCR-ABL with ponatinib on the phase II PACE trial, despite being multiply resistant/intolerant to other agents or carrying the T315I mutation.

Patient 1 previously had a suboptimal response to imatinib (BCRABL >10% at 15mo) and then was intolerant of nilotinib and dasatinib due to grade 3 liver enzyme abnormalities. He achieved undetectable BCR-ABL (Molecular MD laboratories) by six months on ponatinib. His ponatinib dose was gradually reduced from 45mg/d to 15mg/d due to vascular events (myocardial infarction requiring stenting, carotid stenosis requiring endarterectomy, recurrent ischaemic colitis). At the time of his last episode of colitis, his BCR-ABL had been undetectable for 47 months and hence a decision to attempt TFR was made. He remains in TFR 10 months post-cessation.

Patient 2 progressed on imatinib and was found to carry the T315I mutation. He achieved undetectable BCR-ABL by nine months on ponatinib. His ponatinib dose was also gradually reduced from 45mg/d to 15mg/d due to vascular events (popliteal stenosis requiring stenting, embolic stroke, coronary artery disease requiring stenting). At the time of his last event, his BCR-ABL had been undetectable for 30 months and hence a decision to attempt TFR was made. He remains in TFR 17 months post-cessation.

This series adds to the one prior case report of ponatinib facilitating treatment-free remission and these remissions are notable because they occur in patients not considered for other TFR studies because of refractoriness to other agents or known T315I mutations. This is an alternative avenue to dose reduction to reduce the risk of vascular events in ponatinib-treated patients.

This research was supported by Ariad Pharmaceuticals. The company sponsored the trial providing Ponatinib to the two patients but had no role in abstract preparation.

P076. A CML-like haematological malignancy with t(8;22) and BCR-FGFR1: a case report and literature review of a rare phenomenon

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Case

MD was a 67 year old lady referred in November 2013 for the investigation of progressive neutrophilia noted on routine blood tests. Bone marrow aspirate and trephine morphology was suggestive of CML in chronic phase, however cytogenetic analysis demonstrated no evidence of t(9;22) and instead showed t(8;22). MD was commenced on hydroxyurea and had a partial haematological response, remaining clinically well for the next two years.

In early 2016, MD developed RUQ pain and was found to have progressive hepato-splenomegaly and generalised lymphadenopathy. Biopsies of lymph node and bone marrow revealed transformation to T precursor lymphoblastic leukaemia associated with *FGFR1* rearrangement. Treatment with chemotherapy plus ponatinib was initiated, however MD rapidly clinically deteriorated and was palliated.

Discussion

The 8p11 myeloproliferative syndrome (EMS) is a rare entity with less than 100 reported cases published. EMS is characterised by disruption of the *FGFR1* gene at the 8p11 locus, resulting in a fusion gene producing a chimeric protein with constitutive activation of the FGFR1 tyrosine kinase. Over 14 translocations and one insertion involving *FGFR1* have been described, with variable presentations. In general EMS is an aggressive condition with high rates of progression to acute leukaemia.

A subset of EMS is characterised by the t(8;22) translocation with *BCR-FGFR1* fusion gene, and distinct clinical and pathological features. To the best of our knowledge, only 20 cases are reported within the published literature. Here we review the published literature and report the 21st case of myeloproliferative disease with t(8;22), and only the second case with transformation to T-ALL. Management of this condition presents a significant challenge, and allogeneic stem cell transplantation remains the only curative option. However, there is emerging evidence that novel multi-kinase inhibitors, such as ponatinib, with activity against FGFR1 may have therapeutic potential.

P077. Characterization of the thrombin generation potential of haematologic and solid cancer microvesicles by calibrated automated thrombography

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Introduction

The CAT assay is emerging as a reliable tool for real time estimation of thrombin generation (TG) potential. We recognise that the pathways underlying the thrombotic phenotype for different malignancies may be driven by factors of the coagulation cascade with TG has a common denominator. Two such malignancies with high venous thromboembolism incidence are haematologic histiocytic lymphoma and pancreatic cancer(PC). Understanding the underlying variations in these two distinct cancer models using tumour microvesicles(MVs) and investigating the influence of coagulation factors on TG, might potentially allow individual approaches to identifying thrombotic risk and relevant prevention strategies.

Methods

Cancer microvesicles isolated from malignant haematologic and pancreatic cancer cell lines at 1×10^6 /ml concentrations were assessed on a Thrombinoscope for the CAT assay, with the addition of platelet-free NormTrol plasma or plasma deficient in coagulation factors VII and XII. Results are mean \pm S.D of n=3 in triplicates.

Results

Compared to standard controls at 1 pmol/L TF concentration, tumour MVs of haematologic cell lines exhibited weaker TG profiles in NormTrol than MVs of PC cell lines, with longer lag times and times-to-Peak, lower thrombin peak, endogenous thrombin potential and velocity Index. The reduction in TG due to factor VII-deficiency in the plasma was 60% \pm 9% more pronounced in the solid PC lines than in haematologic lines, with 2-3 times more inhibition in lag times and times-to-Peak TG parameters. Apart from U937 lymphoma cell line, there was no significant difference between TG in NormTrol compared to Factor XII-deficient plasma in both cancer cell lines.

Conclusion

This study demonstrates that the CAT assay is a useful predictor of the thrombotic phenotype in cancer microvesicles *in vitro* as it gives a comprehensive overall coagulation profile including the effects of coagulation factor deficiency.

P078. Primary CNS lymphoma: safety, tolerability and outcomes of the R-MPV/Ara-C protocol

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Background

Treatment of primary CNS lymphoma (PCSNL) is most effective when intense combination therapy is utilised. However concerns regarding scheduling, immediate and long-term toxicities of combination regimens remain, particularly in older patients

Aim

To investigate the safety, tolerability and outcomes of patients receiving R-MPV/Ara-C at Monash Health.

Method

Patients with PCNSL diagnosed according to WHO 2008 criteria, receiving R-MPV/Ara-C at Monash Health between 1997 and 2016 were included. Only cases with adequate clinical information were included. Factors associated with overall survival, treatment-related mortality, non-relapse mortality and relapse were analysed. No patient underwent formal pre-treatment investigation for neuro-cognitive deficit. Treatment-related neurotoxicity was defined as any neuro-cognitive deficit not present prior to the initiation of treatment or explicable by alternative aetiology.

Results

35 patients met inclusion criteria. 24 (69%) achieved CR; median overall survival 4.0y [0.5-12.8]. Of nine deaths; three were attributed to MTX-related toxicity during induction, five due to refractory or relapsed disease, one due to IHD. 25 (71%) completed full treatment. Radiotherapy was administered in 13 (37%); 8 of 13 were treated prior to routine use of rituximab. Seven experienced long-term neurotoxicity confirmed by neuropsychological/neurological investigation in five cases. Neurotoxicity was not associated with pretreatment variables. Age, initial performance status, and tumour location were not associated with survival or toxicity.

Completion of treatment at full doses was associated with improved overall survival ($p=0.04$), as was the presence of only a solitary lesion ($p=0.03$). The addition of rituximab was associated with improved overall survival ($p=0.007$) as noted previously. Unanticipated readmission for treatment-related complications occurred 8 (24%) and was not significantly higher in patients aged $>70y$.

Conclusion

R-MPV/Ara-C is an efficacious regimen which should be offered to appropriately selected patients regardless of age. While generally well tolerated, further research is needed to reduce the impact of long-term toxicities.

P079. MYD88 L265P Mutation is Identified in Transformed Waldenstrom Macroglobulinemia After a Range of Previous Treatments Including Ibrutinib

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Background

Diffuse Large B Cell Lymphoma (DLBCL) in patients with WM is uncommon. It may arise from histological transformation or from synchronous lymphomas with different clonal origin.

Aim

To investigate the utility of molecular testing in patients with DLBCL and a previous diagnosis of WM to confirm transformation from the WM clone.

Method

Formalin-fixed paraffin-embedded (FFPE) sections of pre-transformed and histologically confirmed transformed tissue were obtained for four cases. DNA was extracted and MYD88 L265P PCRs performed. IgHv PCR has been performed on 2 cases.

Results

Cases 1, 2 and 3 show the heterozygous MYD88 L265P mutation on pre-transformed samples; case 4 could not be tested. Cases 1, 2 and 4 showed the heterozygous MYD88 L265P mutation on the transformed samples; case 3 showed 2 wild-type alleles suggesting that this case is either due to synchronous lymphoma or loss of MYD88 L265P mutation during clonal evolution. This is being investigated on IgHv sequencing. IgHv PCR has been performed in case 1 and 2 so far and has confirmed clonal bands. Case 1 is the first reported case of WM transformation on ibrutinib therapy; transformation occurred in the context of persistence of the MYD88 L265P mutation.

Table 1: MYD88 L265P PCR results

<i>Case number</i>	<i>Pre-transformed tissue</i>	<i>Transformed tissue</i>
1	Heterozygous	Heterozygous
2	Heterozygous	Heterozygous
3	Heterozygous	Wild-type
4	Not tested	Heterozygous

Conclusion

Molecular studies with combination of MYD88 mutation studies and IgHv PCR helps confirm transformation versus development of synchronous lymphomas, which has implications for prognosis and therapy in DLBCL patients with a prior diagnosis of WM. We also report the first case of large cell transformation on ibrutinib and show persistence of the MYD88 L265P mutation in transformed tissue.

P080. Bleeding, with associated platelet dysfunction, in the initial presentation of hairy cell leukaemia; a case report

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Hairy cell leukaemia (HCL) is a relatively uncommon lymphoproliferative disorder representing less than 1% of lymphoid neoplasms¹.

Presentation patterns are varied, but typically HCL presents with features due to underlying splenomegaly or cytopenias. Thrombocytopenia is present in up to 80% at presentation² and this can be associated with bleeding.

There have been a number of case reports in the 1970's and 1980's of associated platelet dysfunction; though this platelet dysfunction has not frequently been associated clinically with bleeding^{3,4}. There have been rare reports of patients who have both clinically significant bleeding and confirmed platelet dysfunction³. Here we present a case of platelet-dysfunction associated with the diagnosis of hairy cell leukaemia.

In this case report, a 50-year old woman presented with symptoms suggestive of a respiratory tract infection. Past history includes recurrent unprovoked VTE, treated with lifelong anticoagulation without bleeding complications, most recently with a non-vitamin K dependent oral anticoagulant (Rivaroxaban). Blood film examination lead to the suspicion of hairy cell leukaemia; which was subsequently confirmed with flow cytometry and bone marrow biopsy. Upon further clinical review large non-traumatic ecchymoses, and a new, and persisting, petechial rash were noted. Furthermore, excessive bleeding was noted at the time of bone marrow biopsy despite temporary cessation of rivaroxaban, and she developed large ecchymoses at the sites of venepuncture.

Thrombocytopenia was present on initial blood tests; however, this was only mild ($100 \times 10^9/L$) and not thought to explain the bleeding diathesis. Therefore, we performed platelet function tests, which revealed abnormal PFA-100 studies, normal vWF studies and abnormalities on formal platelet aggregometry (reduced response to collagen at 1 and $2\mu g/mL$). An acquired platelet function disorder should be considered when haemorrhagic symptoms are encountered in the context of an underlying HCL.

Follow-up platelet function studies will be performed after treatment for lymphoma.

P081. Retrospective analysis of patients with limited stage NLPHL presenting in the Auckland region

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Aim

The optimal therapy for limited stage Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is unknown. Unlike classical Hodgkin lymphoma, NLPHL is often treated with radiation alone. Considerations when treating NLPHL include its propensity to relapse late, the risk of transformation to aggressive lymphoma and minimising treatment-related toxicity. We performed a retrospective analysis of patients with NLPHL presenting in the Auckland region over the last decade to review treatments given and patient outcomes.

Method

19 patients with limited stage (1A/B or 2A) NLPHL diagnosed between April 2003 and June 2014 were identified in the Auckland Regional Lymphoma Conference Database. Data were collected on patient demographics, treatment received, survival outcome and treatment-related complications.

Results

10 patients had stage 1 disease and 9 patients had stage 2 disease. Only one patient had B symptoms. 16 patients received radiation (RT) alone, two patients received combined-modality treatment (CMT) and 1 patient had chemotherapy alone. The median age at diagnosis was 45 years (range 17 to 74 years) and most patients were male (68%). The median follow up time was 47 months (range 13 – 179 months). Of the 18 patients who achieved a complete remission, two patients (11%) relapsed. There were no cases of disease transformation or second malignancies during the course of follow up. One death occurred however this was unrelated to the diagnosis of NLPHL.

Conclusion

Our experience using RT alone to treat limited stage NLPHL shows this to be an efficacious and low-toxicity treatment. The value of combined modality therapy is yet to be established. Insufficient follow up time for some patients limits the evaluation of late relapses, disease transformation and delayed therapy-related effects.

P082. A Case Report of Hairy Cell Leukaemia: A Rare Case involving Skull, Skeletal Bone and Central Nervous System

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Introduction

Hairy cell leukaemia (HCL) is a rare lymphoproliferative disorder with typical presentation involving cytopenias, splenomegaly and marrow infiltration [1]. We describe an unusual case of HCL involving many atypical sites.

Case Report

A 68-year-old man with a longstanding history of delusional disorder presented with acute recurrence of delusional symptoms. Subsequent imaging demonstrated right base of skull lesion, with an enhancing component along the right glossopharyngeal nerve and extension into the right posterior fossa and suboccipital region. Staging PET showed further avidity within right humerus, pleura and mediastinal nodes. Full blood count showed a low platelet and monocyte count. CSF analysis did not show any infection or involvement with HCL

Biopsy of skull lesion showed infiltration with small B cells which on immunohistochemistry staining were CD20+, CD25+, DBA44+ and CD5-. Of interest was diffuse cyclin D1 expression suspicious for mantle cell lymphoma (MCL); however FISH analysis was negative for t(11;14).

Staging bone marrow biopsy revealed presence of hairy cells on aspirate. Flow cytometry analysis showed B cells with phenotype CD5-, CD20+, CD25+, CD11c+ and CD103+. Again, there was effacement with small B cells with diffuse cyclin D1 expression. FISH once again did not detect t(11;14). Subsequent PET-guided contralateral iliac bone biopsy revealed similar findings.

Given classic morphological and immunophenotypical features of HCL along with negative t(11;14), he was diagnosed with HCL with aberrant cyclin D1 expression. Treatment was commenced with cladribine.

Conclusion

This is an unusual case of HCL with uncommon presenting symptoms, atypical distribution, cyclin D1 over-expression, and lack of cytopenias or splenomegaly. Only rare cases of skeletal bone and neurological involvement have been previously reported, and while cyclin D1 can be over-expressed in HCL, the level of expression is usually less diffuse [2-4]. This case highlights that careful haematopathological assessment is required for accurate diagnosis and appropriate treatment of rare cases of HCL.

P083. The impact of salvage treatment modality in patients with positive PET after R-CHOP chemotherapy for aggressive B-cell non-Hodgkin's lymphoma

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Aim

Patients with aggressive B cell non-Hodgkin's lymphoma (NHL) who remain PET positive after chemotherapy have poor prognosis. There is limited data on the best salvage treatment, with the few studies confounded by older imaging methods and chemotherapy regimens. We compared outcomes of different salvage treatment modalities, in patients who remained PET positive after R-CHOP.

Method

Patients with diffuse large B-cell NHL or grade 3 follicular NHL were retrospectively reviewed from Prince of Wales Hospital databases. Eligible patients were: age ≥ 18 years, treated with R-CHOP, and had positive or equivocal post-chemotherapy PET. Salvage treatment was classified as radical radiotherapy (RT, dose ≥ 30 Gy), high dose chemotherapy and autologous stem cell transplant (transplant), or non-radical management. Survival was calculated from date of post-chemotherapy PET scan to last follow-up.

Result

26 patients met the inclusion criteria (85% positive PET, 15% equivocal), with post chemotherapy PET scans performed over 2003-2015. Median age was 60 (range 19-84) years. Most patients had adverse baseline features: 21 (81%) stage III-IV disease, 2 (8%) IPI 4-5, 24 (92%) bulky disease, and 9 (35%) skeletal involvement. 23 (88%) patients had ≥ 6 cycles R-CHOP.

PET positivity post chemotherapy corresponded to: single sites in 16 (62%) patients, sites of prior bulk disease in 24 of 24 patients (100%), skeletal sites in 5 of 9 patients (55.5%), and encompassable by RT in 81%.

Salvage treatment was: radical RT in 17 (65%), transplant in 4 (15%), and non-radical in 5 (20%). Median follow-up of surviving patients was 31 months. At last follow-up, overall survival was 54% (all), 65% (RT), 25% (transplant), and 40% (non-radical). PFS were respectively 42%, 53%, 25%, and 20%.

Conclusion

Patients who have PET positive disease after R-CHOP for aggressive B-cell NHL may be salvaged by radical radiotherapy or high dose chemotherapy and transplant.

P084. Reduced Phosphorylated STAT3 (pSTAT3) Expression in Diagnostic Hodgkin Lymphoma Tissue Biopsies Correlates With Poor Prognosis

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Aim

Hodgkin lymphoma (HL) has a median 5-year overall survival approaching 90%, however some patients respond poorly to chemotherapy. It has been reported that inactivating mutations in protein-tyrosine phosphatase 1B (PTP1B) which negatively regulates the JAK/STAT pathway are common in HL and may contribute to clinical outcome^{1,2}. We tested this hypothesis, and assessed the utility of immunohistochemistry staining of PTP1B and pSTAT3 as upfront prognostic markers in HL.

Method

A retrospective clinical audit of patients treated for HL from 2010 to 2015 was performed, and their respective diagnostic tissues stained to determine expression of PTP1B and pSTAT3 in CD30 positive counterstained Reed-Sternberg (RS) cells. Blinded analysis of RS cell staining (both intensity of staining and percentage of RS cells stained) was performed by an anatomical pathologist. This data was correlated with clinical outcome.

Results

Twenty-nine patients were evaluated as Cohort A (18 patients) who obtained a durable complete remission, and Cohort B (11 patients) with relapsing/refractory disease. Baseline characteristics were similar between the two groups (not shown). The estimated 5-year survival was 100% and 54.5% for Cohorts A & B respectively. No difference in PTP1B expression was observed, however pSTAT3 expression was significantly reduced in biopsies from patients in Cohort B, whether measured by either proportion of RS cells staining ($p=0.008$) or intensity of staining ($p=0.003$). pSTAT3 staining was noted to be absent or reduced in the majority (four out of five) of the patients who died.

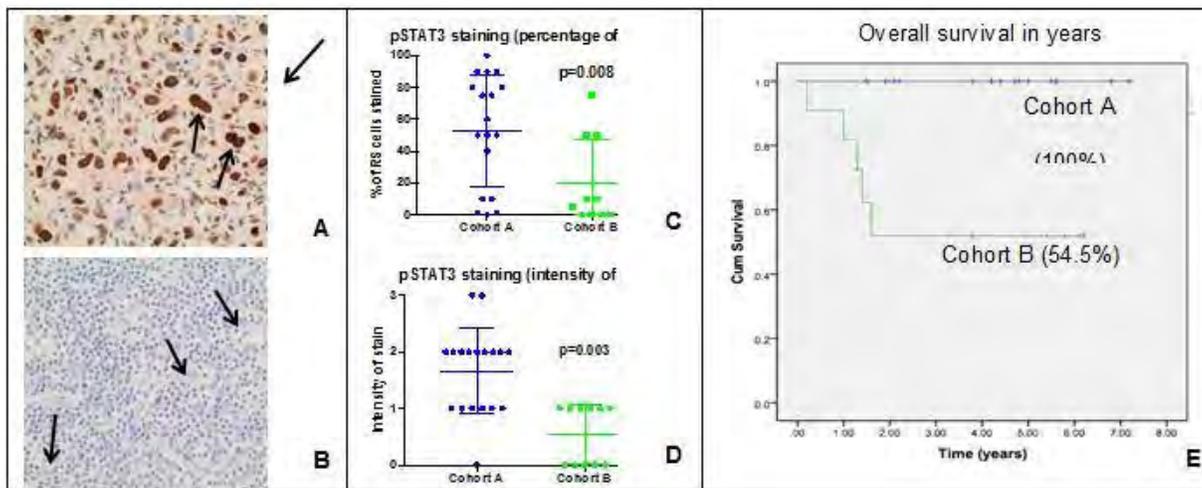


Figure 1: Representative examples of the pSTAT3 stain in HL sections, demonstrating positive staining (A) & negative staining (B) of RS cells (arrows); pSTAT3 staining by percentage of RS cells (C) and maximum intensity of stain (D); Kaplan-Meier curves of overall survival between the two cohorts (E)

Conclusion

Contrary to our anticipated findings, reduced pSTAT3 expression in RS cells from diagnostic tissue correlated with poor outcomes in our HL cohort. In other cancers, STAT3 may act as a tumour suppressor by inactivating cell dependent kinases³. These results suggest further study is needed to establish pSTAT3 as a prognostic marker in HL and opens a potential novel role for STAT3 in HL pathogenesis.

P085. Excellent outcomes for patients with Burkitt lymphoma and B-cell lymphoma, unclassifiable (Burkitt/DLBCL) using intensive chemo-immunotherapy regimens

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Burkitt lymphoma (BL) is a highly aggressive B cell Non Hodgkin's Lymphoma that is characterised by a translocation involving the *cMYC* oncogene with a rapid doubling time of 25 hours. Although prospective trials have demonstrated efficacy of multiphase high intensity chemotherapy regimens, the optimal initial therapy of BL is yet to be clearly defined. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCLU) is a poorly characterised entity. There is no recognised standard therapy.

Aim

To assess the outcomes of patients with BL and BCLU treated with different chemo-immunotherapy regimens at a single centre.

Methods

We performed a retrospective review of adult patients at Sir Charles Gairdner Hospital, who were treated for BL and BCLU between 1999 and 2016. Cases were identified by searching the departmental database. Histopathology of all patients had been reviewed by pathologists with subspecialty expertise in lymphoma. Baseline demographics, disease characteristics, treatment and survival outcomes were collected. Overall survival (OS) and progression-free survival (PFS) were determined using the method of Kaplan and Meier, with comparisons using the log-rank test.

Results

30 patients had BL. The average age was 39 years (range 18-81) with 22 males and 8 females. Treatment regimens included CODOX-M/IVAC±R (n=7), dose-adjusted EPOCH-R (n=5), hyperCVAD±R (n=14) and other (n=4). 11 patients had BCLU. The average age was 55 (36-72) with 8 males and 3 females. Treatment regimens included hyperCVAD±R (n=5), R-CHOP (n=3) and dose-adjusted EPOCH-R (n=2). Median follow-up was 46 months (range: 1-121). Evaluation of the entire cohort revealed a 4-year OS of 81% (95% CI: 64 – 91) and 4-year PFS of 85% (95%CI: 69-93%). Those who received CODOX-M/IVAC or HyperCVAD achieved the highest PFS.

Conclusion

Although patients with BCLU were older, outcomes appeared similar to BL within the limits of a retrospective analysis.

P086. Lenalidomide in Multiply Relapsed, Chemorefractory Diffuse Large B-cell Lymphoma: A Case Report

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Introduction

Prognosis is poor for patients with multiply relapsed diffuse large B-cell lymphoma, and treatment guidelines currently suggest either autologous stem cell transplant or enrolment in a clinical trial. Recent literature has demonstrated that Lenalidomide, an oral immunomodulatory agent with direct and indirect antitumor activity in B-cell malignancies, is effective and well tolerated in heavily pretreated patients with high-grade lymphoma.

Case presentation

This case report describes a 73-year-old female with multiply relapsed diffuse large B-cell lymphoma. She initially presented with stage 1 primary breast disease and achieved complete remission with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. Nine years later she was treated for a solitary cerebellar recurrence with the Sloan Kettering Central Nervous System Lymphoma protocol (high dose methotrexate, procarbazine, vincristine and cytarabine) and again achieved complete remission. She remained disease free for a further eighteen months before relapsing with extensive disease above and below the diaphragm. Initial treatment with gemcitabine, oxaliplatin and vinorelbine demonstrated progressive disease after one cycle and so lenalidomide was added to the subsequent five cycles of therapy with a striking response. A PET scan five weeks post cycle six showed a complete metabolic response. No significant treatment related adverse effects were encountered. The patient has now commenced maintenance lenalidomide, planned to continue for the next two years.

Conclusion

The addition of Lenalidomide to an oxaliplatin-based regimen was effective in our patient with multiply relapsed, chemorefractory diffuse large B-cell lymphoma.

P087. Brentuximab vedotin as first-line treatment can advent complete metabolic response in Hodgkin lymphoma-associated severe liver failure

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As a bridge to transplant in relapsed/refractory Hodgkin lymphoma and anaplastic large cell lymphoma, brentuximab vedotin has shown impressive results with reported objective responses in over half of patients^{§,¶,#}. However, the utility of this agent as first line monotherapy has been limited to case reports, which have demonstrated partial responses. We present here a 44-year-old male presenting with stage IV, Hasenclever 5 Hodgkin lymphoma co-incident with a new diagnosis of human immunodeficiency virus infection. This patient was also noted to have renal failure (eGFR of 48ml/min), and a mixed picture of hepatitis and cholestatic liver failure with a bilirubin of 589 μ mol/L. A liver biopsy showed vanishing bile duct syndrome, a recognised paraneoplastic phenomenon in Hodgkin lymphoma. Multi-organ failure precluded use of cytotoxic therapy, thus brentuximab was implemented as monotherapy. Following five cycles of brentuximab, the patient achieved a complete metabolic response with minimal side effects. This is the first report of brentuximab given in the setting of such marked hyperbilirubinaemia with the achievement of a complete metabolic response.

[§] Gopal AK et al. *Blood*. 2015;125:1236-43

[¶] Pro B et al. *J Clin Oncol*. 2012;30:2190-6

[#] Gupta A et al. *Blood*. 2015;126:5105

P088. Sequential Rituximab therapy in Post-Transplant Lymphoproliferative disorders (PTLD) is associated with increased overall survival and less infective complications: retrospective single-centre study

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Aim

To retrospectively evaluate the impact of initial rituximab monotherapy (+/- subsequent CHOP) on survival in a single-centre PTLD patient cohort.

Method

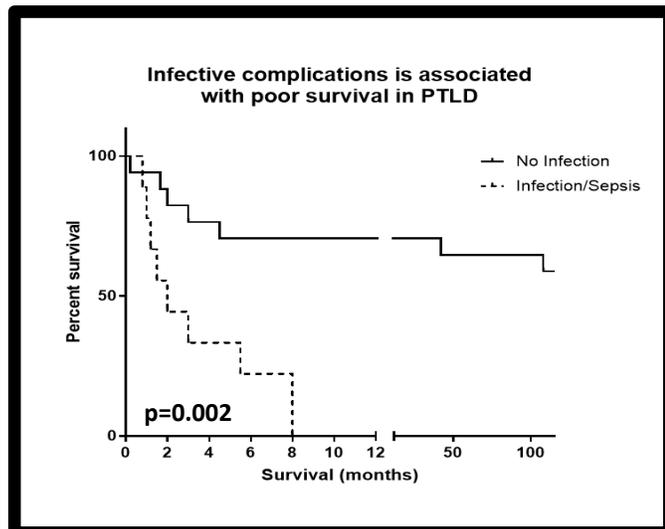
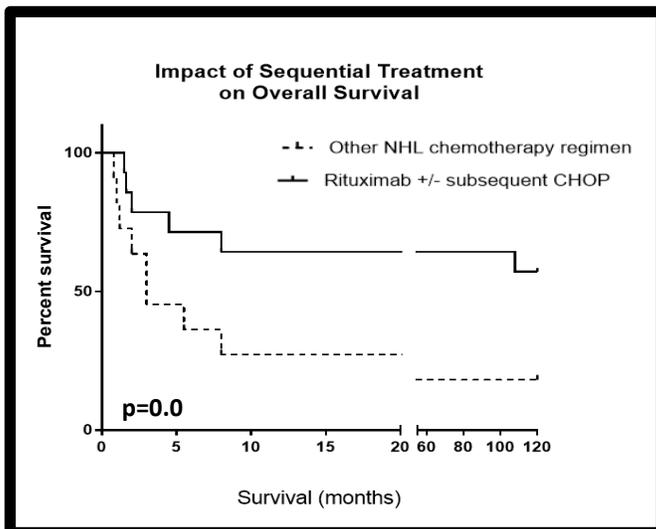
Patient medical records were used for retrospective analysis. A total of 26 adult patients were identified with PTLD diagnosis from 2005 to 2015. PTLD diagnosis was based on histology reports and made in accordance with the World Health Organisation classification. Survival was analysed with regards to time to PTLD diagnosis, Epstein Barr virus (EBV) infection status, concurrent infection/sepsis, International Prognostic Index (IPI) score and chemotherapy regimen. Rituximab therapy consisted of four cycles of rituximab (375mg/m²) weekly, and standard doses of CHOP was given for 4-6 cycles every 3 weeks. For the chemotherapy regimen analysis, patients were divided into those who received initial rituximab monotherapy (+/- subsequent CHOP) and other conventional lymphoma chemotherapy regimens. Kaplan-Meier method with Gehan-Breslow-Wilcoxon test was used for survival analysis.

Results

Median age was 49 years. Median time to diagnosis 42 months, with 33% being early PTLD diagnosis (<12 months post-transplant). The cohort consisted of mostly of heart or lung transplant patients (89%) with the remainder being kidney (7%) or bone marrow transplant (4%). Median overall survival was 8 months. OS survival was significantly increased in patients treated with initial rituximab monotherapy (+/- subsequent CHOP) (p=0.04) (Figure 1). Time to PTLD diagnosis (early vs late), EBV infection status, or IPI score had no effect on survival. Infection/sepsis complications were less likely to occur in patients who received initial rituximab monotherapy (+/- subsequent CHOP) (21% vs 55%) and were associated with increased mortality with median survival of 2 months (p=0.002) (Figure 2).

Conclusion

Although PTLD has an extremely poor prognosis, rituximab given as initial sole therapy (+/- subsequent CHOP) is associated with increased survival and less infective complications, and should be considered as first-line therapy.



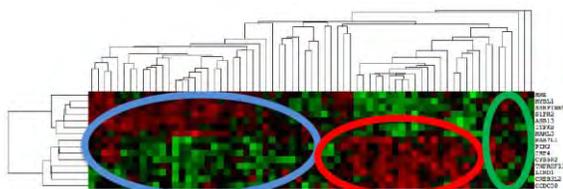
P088a. Formalin-fixed Paraffin-Embedded Core Biopsies and Decalcified Trepine Samples Permit Clinically Meaningful Gene Expression Profiling in Diffuse Large B-Cell Lymphomas

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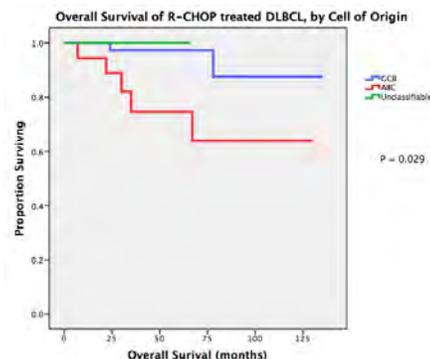
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Method: 148 cases of R-CHOP treated *de novo* DLBCL between 2003 to 2015 were identified. 120 had residual diagnostic tissue; >100ng of RNA was available for 117 cases that were genotyped using the 20-gene *Lymph2Cx* on the nanoString nCounter (Scott et al *Blood* 2014 and *J Clin Oncol* 2015). COO was assigned through a Bayesian algorithm provided by the National Cancer Institute, USA. The Kaplan-Meier method was used to estimate overall and progression free survival (OS, PFS).

Results: COO assignment was successful for 115 (98%). Median lymphoma surface area on 5µm sections was 16mm² (range 2-450mm²). 72 (62%) were biopsied from extranodal sites. 94 (81%) were core biopsies. Median time in archive was 5.6 years (range 0.4-12.3 years). 10 patients had synchronous trephine biopsy involvement and adequate RNA was extracted from 9. COO was concordant between diagnostic biopsy and trephine in 6 (67%); these trephines were diffusely infiltrated by DLBCL. The 3 discordant cases had focal trephine involvement. Median age was 67 years (range 27-92), 69 (59%) were ≤70 years. IPI was predictive of OS ($P<0.0001$) and PFS ($P<0.027$). COO assignment by *Lymph2Cx* showed the activated B-cell (ABC) subtype had inferior OS compared to the germinal centre B-cell (GCB) subtype in patients ≤70 years; the ABC subtype reached 75% survival at 2.9 years, the GCB subtype did not reach 75% at median follow-up of 6 years ($\chi^2=7.01$, $P=0.029$). Five-year OS for the GCB, ABC and unclassifiable subtypes were 97.3%, 74.6% and 100%, respectively.



Heatmap of *Lymph2Cx* genes showing COO classification for 69 patients aged ≤70 years with *de novo* DLBCL treated with R-CHOP. Blue denotes GCB, red denotes ABC, green denotes unclassifiable.



Conclusion: Small core biopsies and EDTA-decalcified trephines are useful for COO assignment. COO was predictive of OS in R-CHOP treated DLBCL patients aged ≤70. *Lymph2Cx* on the nanoString has a clinically meaningful turn-around time of 48-72 hours and is superior to immunohistochemistry-based methods for COO assignment (Gutierrez-Garcia et al, *Blood* 2011, Coutinho et al, *Clin Cancer Res* 2013). FFPE tissue should be deployed for molecular subtyping to inform prognostication and therapeutic considerations in DLBCL.

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P089. Comparative Benefit of Brentuximab Vedotin Versus Salvage Chemotherapy in Relapsed or Refractory Patients with Hodgkin Lymphoma Without a Prior Autologous Stem Cell Transplant

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Aim

Standard management for relapsed refractory Hodgkin lymphoma (RR HL) following front-line treatment is high-dose chemotherapy followed by autologous stem cell transplant (ASCT). However, many patients cannot undergo ASCT due to poor response or are contraindicated (age or comorbidities). Brentuximab vedotin (BV) is a salvage treatment for patients with RR HL following at least 2 prior therapies when ASCT or multi-agent chemotherapy is not possible. BV has demonstrated a high overall response rate (ORR) in a Phase II study of Post ASCT patients; however data in ASCT naïve patients is limited. This study aimed to assess the comparative efficacy of BV in ASCT naïve patients from the literature.

Methods

The published literature and internal databases were systematically searched to identify studies of BV and conventional chemotherapies in patients with a median of ≥ 2 previous therapies and no prior ASCT. Efficacy and safety data were pooled and confidence intervals derived using the Clopper-Pearson exact method.

Results

Seven BV studies were identified totalling 268 patients; 1 post-hoc analysis of phase I/II clinical trials and named patient programs and 6 published retrospective observational studies. Five comparator studies were identified, totalling 82 patients; 4 prospective single-arm studies and 1 retrospective observational study of gemcitabine (alone or in combination with other chemotherapies or corticosteroids).

Use of BV demonstrated substantial improvement in both ORR (66.0% vs. 29.3%) and CR (32.8% vs. 6.5%) compared to older salvage chemotherapy. Following BV treatment 35.6% (95% CI: 27.5, 44.4) of patients proceeded to ASCT. No patients were reported to have undergone ASCT in the comparator studies.

Conclusion

Despite the limitations of heterogeneity between studies, these data suggest that BV may achieve a higher response rate in at-risk patients previously ineligible for ASCT, and may provide a bridge to transplant for otherwise eligible patients not achieving a response to conventional chemotherapies.

This research was supported by Takeda Australia. The company was involved in the extraction and analyses of the data and the preparation of the abstract.

P090. Autologous stem cell transplant in follicular and diffuse large B cell lymphoma - a retrospective review

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Aim

Examine the outcomes of patients with FL or DLBCL, who received R-CHOP as primary therapy, and subsequently received salvage chemotherapy and ASCT.

Method

Retrospective single site audit of patients treated at Gold Coast Hospital and Health Service who underwent ASCT for relapsed/refractory FL or DLBCL between January 2009 and June 2015.

Results

Thirteen patients were identified. Seven patients had a diagnosis of de-novo DLBCL, 6 had a diagnosis of FL. Median age: 61y (range 34-67y). Median follow-up post ASCT: 15 months (range 4-80mo).

All patients received R-CHOP as primary therapy. Of the patients with DLBCL, 1 was primary refractory; six achieved CR - median time to relapse was 20 months (range 7-75mo). Of the patients with FL, 4 achieved CR, 2 achieved PR - median time to relapse was 43 months (range 4-110mo). Three of 6 FL patients developed histological transformation after R-CHOP; at 4, 74 and 110 months respectively.

Salvage chemotherapy was R-ICE in 12 patients; one patient (with FL) received R-ICE/R-FND.

At time of transplant, 10 patients had DLBCL – 7 de-novo, 3 transformed – 4 were in CR, 6 were in PR following salvage. Two patients with FL were in CR, 1 was in PR.

Of the 10 patients with DLBCL at time of ASCT, 9 relapsed – median time to relapse from transplant was 4 months (range 2-59mo).

None of the FL cohort (n=3) had relapsed at last follow-up.

There were 6 deaths, all related to progressive disease, all patients had DLBCL at time of ASCT.

Median PFS from time of ASCT: 4 months overall, 4 months for DLBCL/tDLBCL, 26 months for FL.

Conclusion

This audit contains small numbers, however demonstrates the limited utility of salvage therapy and ASCT in relapsed/refractory DLBCL post R-CHOP. Further prospective data is required to define the benefit of ASCT in this group.

P091. Routine Blood Investigations Have Limited Utility in the Follow-up of Aggressive Lymphomas

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Background

After attaining complete remission with first-line therapy, patients with aggressive lymphoma undergo close surveillance to detect early relapse. Current global guidelines recommend routine follow-up blood tests but this is not evidence-based. This study evaluates the utility of blood tests in detecting relapse after treatment.

Methods

We conducted a retrospective review of all patients diagnosed with Hodgkin lymphoma (HL) or aggressive non-Hodgkin lymphoma (NHL) who attained complete remission (CR) after first-line chemotherapy between 2000 and 2015. Clinical records were reviewed to determine follow-up and relapse details. An abnormal blood test was defined as any new and unexplained abnormality for FBE, white cell differential, LDH or ESR.

Results

The 246 patients generated a total of 2383 outpatient visits, and blood tests were performed at 91% of these appointments. Forty-three (17%) of the patients experienced relapse; of these, 33 (77%) were detected clinically and 8 (19%) by imaging. There were 74 early visits due to abnormal symptoms; compared to scheduled appointments, these showed a significantly stronger association with relapse (OR 50.7, $p < 0.001$). Abnormal routine laboratory test results prompted further investigations in 49 patients, however only 2 of these (5%) had an identified relapse on subsequent imaging. 47 patients who underwent further investigation had no relapses identified. Thus the sensitivity of blood testing in asymptomatic patients was 60% and PPV of 9% due to frequent false-positive results.

In addition, an abnormal result prompted a change in management for only 16% of asymptomatic patients (such as an earlier routine subsequent review), compared to 61% of those with concurrent symptoms or signs. There was no difference in survival after relapse in patients who underwent a routine blood test within 3 months prior to relapse versus those who did not ($p = 0.562$), although numbers of relapse were small.

Conclusion

The majority of relapses are still detected by patient-reported symptoms, often at unscheduled visits, and blood tests demonstrate unacceptably poor performance characteristics with no survival benefit. This suggests that routine blood tests are not useful in the surveillance of asymptomatic patients in CR after primary chemotherapy.

Eliza Hawkes has accepted travel expenses from Takeda and research funding from Merck Serono and Bristol Myer Squibb.

P092. The positive effect of deferasirox on erythropoiesis in a patient with a dual diagnosis of myelofibrosis and diffuse large B-cell lymphoma

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Introduction

Iron chelators, such as deferasirox, are the mainstay of treatment in patients with secondary iron overload. Studies have shown that some patients treated with iron chelators can demonstrate a positive erythropoietic effect resulting in reduced transfusion requirements, which can occur despite persistently elevated ferritin levels(1-3). This has prompted further investigation into the anti-neoplastic effects of iron chelators on haematological disorders.

Case Report

A 70-year-old female was diagnosed with triple-negative essential thrombocythaemia (ET), with eventual transformation to secondary myelofibrosis (MF) and transfusion-dependent anaemia. Two years after her initial diagnosis of ET, she presented with visual symptoms caused by a cavernous sinus lesion. Bone marrow biopsy at this time demonstrated complete effacement of marrow with lymphoma cells, and she was diagnosed with stage IV diffuse large B-cell lymphoma (DLBCL) (IPI score = 4).

Induction chemotherapy with R-CHOP followed by R-hyper-CVAD was commenced, which was ceased after three cycles due to ongoing marked cytopenias and recurrent episodes of neutropenic sepsis. Repeat imaging demonstrated unchanged dimensions of the cavernous sinus lesion, and repeat bone marrow biopsy was hypocellular with no evidence of lymphoma.

Due to ongoing transfusion requirements and biochemical iron overload, she was commenced on deferasirox 15mg/kg. Over three years, we have observed a positive erythropoietic effect to the extent that she is now transfusion independent and has normal ferritin levels. She has remained stable in terms of her underlying myelofibrosis and is in complete remission from her DLBCL with the most recent MRI brain demonstrating complete resolution of the cavernous sinus mass.

Conclusion

This case highlights the positive erythropoietic effect of deferasirox on patients with variable bone marrow disorders, including myelofibrosis and potentially those who have undergone myeloablative chemotherapy. It also raises the rationale that deferasirox may possess anti-neoplastic properties on lymphoproliferative disorders, including DLBCL.

P093. Presenting features and outcomes of nodal T-Cell Lymphoma in Western Australia: the critical importance of The International Prognostic Index (IPI)

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Aim

T-cell lymphomas (T-NHL) are a rare and heterogeneous group of lymphomas which have poor outcomes with current standard therapy. We aim to characterise presentation, initial management and prognostic factors of nodal T-NHL in a large Western Australian population.

Method

We retrospectively collected data for patients diagnosed with nodal T-NHL between 2005-2014 (inclusive) in the Western Australian South Metropolitan Health Service, which is the public tertiary referral network for approximately half of Western Australia.

Results

Presenting features for the 62 identified patients are described in the Table. Median follow-up was 33 months (range 1.4-108 months), and three year overall survival (OS) was 31% (95% CI 19 – 43). Nineteen (30.5%) received palliative treatment, 27 (43.5%) received CHOP and 16 (26%) received other chemotherapy regimens (6 HyperCVAD, 3 CHOEP, 7 other). Three year OS was 6% (95% CI -5.76 – 17.76), 33% (13.4 – 52.6) and 61% (37.48 – 84.52) respectively. Stem cell transplantation was performed in first remission in five (8%) patients, and in first or subsequent relapse in four (6%). International prognostic index (IPI) correlated strongly with outcomes (see Figure). After excluding the 19 patients treated with palliative therapy, IPI was strongly prognostic in multivariate analysis, while the choice of chemotherapy regimen was not.

Conclusion

While patients presenting with nodal T-NHL had poor outcomes overall, survival was strongly predicted by IPI risk group. Those with low IPI had a high likelihood of achieving sustained remission with CHOP or alternative regimens, whereas treatment for patients presenting with high-risk disease in our cohort was predominantly palliative. Survival remains poor in the intermediate group, and strategies to optimise outcomes in these patients, including transplantation and novel therapies, are required.

Clinical Characteristics	All Patients (n=62)	
Age (y), median (range)	66	(23-91)
Male/Female	36/26	58%/42%
B symptoms	35	61%
Stage III-IV	49	79%
Bone Marrow Involvement	25	49%
>1 Extranodal Sites	25	40%
ECOG >1	24	40%
LDH > ULN	31	58%
IPI		
0 to 1	12	19%
2 to 3	32	52%
4 to 5	18	29%
Histological Subtype		
PTCL-NOS	17	27%
AITL	8	13%
ALK-ALCL	9	15%
ALK+ALCL	5	8%
Other	23	37%

Table: Patient characteristics at diagnosis

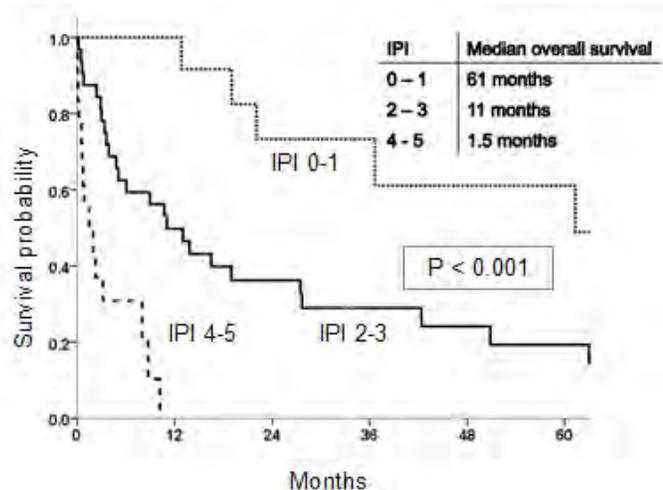


Figure: Overall survival according to IPI score at diagnosis

P094. Autologous PBSC harvest and transplant in a patient with significant cold agglutinins

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A 61 year old female with relapsed marginal zone lymphoma, being worked up for an autologous PBSCT, was found to have a positive DAT (C3d). A cold agglutinin, reacting against O_{adult and cord} RBC, was found (titres 64, 16, and 2 against O_{adult} RBC) at 4, 16, and 30°C respectively. Antibody screen at 37°C was negative. The upper limit of thermal amplitude was higher than 30 but lower than 37°C. Negative reactions with DTT-treated plasma suggested that the cold agglutinin was an IgM. Haemoglobin 89 g / L, bilirubin 34 µmol / L and haptoglobins 0.08 g/L were consistent with haemolysis. PBSC harvest and processing could have exposed patient/product to the cold agglutinin and have compromised the PBSCT.

There is little information on autologous PBSCT in patients with cold agglutinins. We used the regime of Crowther, et al.¹ Briefly, two plasma exchanges using pre-warmed 4% albumin on days -1 and 0 were followed by PBSC harvests on days 0 and +1. Re-induction and stem cell mobilisation were with fractionated ICE + G-CSF. All four procedures were carried out in a room at 28°C with pre-warmed priming fluids, warming coils on lines, and a Bair Hugger at 38°C. The PBSC collection was transported and maintained warm during processing. The cryopreservation solution was made up of 4% albumin (instead of patient's plasma) and DMSO. Collections were frozen down in a controlled rate freezer. Despite preparations clumping occurred but viable CD34+ cells pre-processing of 3.4×10^6 / kg was considered acceptable. The patient was conditioned using BEAM. Stem-cell thawing and infusion were uneventful.

Neutrophils recovered to 0.5×10^9 / L by day 16 but she remained platelet transfusion-dependent till day 38. Currently at 4 months post-PBSCT, her disease has relapsed – first indicated by the cold agglutinin returning followed by clinical and haematological relapse.

P095. Clinical and Genetic characteristics of Transformed Indolent Lymphomas

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Background

Transformation in low grade lymphoma poses high mortality and morbidity risk for patients leading to studies detailing the genetics of transformation, predominantly in transformed Follicular lymphoma (tFL).

Aims

We aimed to compare data from our local cohort to published literature for transformed follicular lymphoma (tFL). Given the limited data on non-tFL cases, we also aimed to identify clinical and genotypic characteristics in our cohort.

Methods

We screened patients with a diagnosis of DLBCL in our hospital since 2003, and identified 35 patients with transformed lymphoma on whom adequate data was available. Clinical data including demographics, treatment received, time to transformation and clinical outcome was obtained from medical records. Genotyping using a customised targeted sequencing panel was performed if fresh tissue was available from the ACT Haematology Research Tissue Bank.

Results

Thirty two patients in our cohort had a previous diagnosis of FL, 2 patients were transformed from marginal zone lymphoma (MZL) and 1 from Waldenstrom Macroglobulinaemia (WM). Nine patients had early transformation (all FL) and 26 had late transformation (defined as > 18 months from initial diagnosis). Patients received 1-8 lines of treatment prior to transformation. 6 (17%) patients were treated with chlorambucil and 9 (26%) with R-CVP for low-grade lymphoma. R-CHOP was the most commonly used regimen at transformation. Overall, 19/36 (54%) including 1 with MZL and 1 with WM, died.

Genotyping was performed on 10 transformed samples including 8 tFL, 1 transformed from MZL and 1 from WM. Number of genetic mutations ranged from 2 to 39. Mutations were identified within the NOTCH2, EP300, CREBBP, CCND3,¹ ²TNFRSF and MYD88 genes.

Conclusions

Here, we elucidate the clinical and genetic features of a local cohort of transformed lymphomas, including 3 non-tFL. Further studies to genotype pre-transformation tissues are planned to identify mutations important in determining transformation, especially in non-tFL.

¹ Rossi, Trifonov, Fangazio et al. The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. *The Journal of experimental medicine*. 2012; 209: 1537-1551

² Pasqualucci, Khiabani, Fangazio et al. Genetics of follicular lymphoma transformation. *Cell Reproduction*. 2014 16 (6) 130-140

P096. Midostaurin is an effective treatment for Aggressive Systemic Mastocytosis

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Aggressive Systemic Mastocytosis (ASM) is a rare haematologic malignancy that has a poor prognosis and has no effective treatment options. Midostaurin is a kinase inhibitor that inhibits KIT D816V. It has been understood that KIT D816V is a key component of pathogenesis in ASM. A recent open label study (Gotlib et al) and case report series (Chanderis et al) have demonstrated the response of ASM to Midostaurin with up to a 60% ORR. We report the outcome of two patients with ASM treated with Midostaurin. Both patients demonstrated a significant improvement in symptoms associated with a fall in serum tryptase (from >200 to 46 and 118). No significant toxicity from the Midostaurin has been seen. After 26 months of treatment both patients continue to respond symptomatically, although in one the tryptase has risen. This experience highlights the benefits of Midostaurin in this rare, but often fatal disease.

P097. Outcome of gut rest and total parenteral nutrition in patients undergoing chemotherapy for gastro intestinal tract lymphoma

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Aim

To assess the outcome of gut rest and total parenteral nutrition (TPN) in patients undergoing chemotherapy for gastro intestinal tract (GIT) lymphoma

Method

All patients between January 2013 and April 2016 with GIT lymphoma who were put on gut rest and received TPN were identified, and patient outcomes were determined by review of individual medical records. Survival outcome was measured from the start of chemotherapy.

Results

33 patients with GIT lymphoma (66%DLBCL, 6%BL, 9%MCL, 3%MALT, 9%EATL, 9% BCL unclassifiable) were put on gut rest and received TPN during the first cycle of chemotherapy. 23 patients received RCHOP, 8 HyperCVAD, 1 CHOP and 1 RGDP initial chemotherapy. The median age at diagnosis was 67yrs (range 19 - 85).

Majority had an IPI ≥ 3 (80%), 3 were MIPI 5,6,7 and 94% had stage 4 disease. LDH was elevated in 67% patients, 34% had B symptoms on presentation and 27% were ECOG ≥ 2 . More than 2 extra nodal sites were involved in 27 patients.

Percentages of regional involvement are as follows (individual patients had more than 1 region involved): 6%oesophagus, 27%stomach, 27%duodenum, 9%jejunum, 39%distal small intestine, 9%terminal ileum and caecum, and 15%colon. All patients were put on gut rest and received TPN during the first cycle of chemotherapy. All but 1 patient had treatment response assessed after the first cycle. One patient had a jejunal artery bleed which needed embolization. None had gut perforation.

At a median follow up of 15.2months (range 2.6-48.7), estimated progression free survival and overall survival is 66.8% and 78.5% respectively. 11patients relapsed at a median of 8.2months (range 1.5-30.1). 9 deaths occurred (5 relapsed disease, 4 non relapse mortality). There was no treatment related mortality.

Conclusion

Routine application of gut rest and TPN for patients being treated for GIT lymphoma is resource intensive and our data suggest this is not required given the low perforation risk.

P098. Radiotherapy in the management of gingival infiltration by CMML – a case report

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Chronic myelomonocytic leukaemia (CMML) is a malignant hematopoietic stem cell disorder with clinical and pathological features of both a myeloproliferative neoplasm (MPN) and myelodysplastic syndrome (MDS). Gingival hyperplasia with gingivitis secondary to infiltration of gingival tissue by leukaemic cells is a well described complication of the leukaemia. It typically responds to systemic treatment.

We describe a case of 67 year old female who developed leukaemic gingivitis 3 months after diagnosis of CMML. She was mildly symptomatic of anaemia at presentation (Hb 77 g/L, WCC $14.36 \times 10^9/L$, platelet count of $149 \times 10^9/L$). Bone marrow showed CMML with 1% blasts and normal karyotype. Within 3 months of presentation she developed leucocytosis, thrombocytopenia, moderate splenomegaly and gum infiltration. Gingival bleeding resulted in anorexia, nausea, and weight loss. WCC and splenomegaly improved with chemotherapy; however gingival infiltration and associated symptoms progressed. The bleeding did not respond to Tranexamic acid (mouth wash or tablets).

She was treated with low dose palliative radiotherapy 4Gy in 2 fractions to the entire oral cavity. There was a slow response such that by 12 weeks post RT there was resolution of oral discomfort and anorexia, reduced bleeding, and 75% reduction in gingival hypertrophy. Patient was much happier with her appearance and smile. There was no radiotherapy related toxicity. Response was maintained for a further 7 months; she was treated with a further 4Gy in 2 fractions to the oral cavity without toxicity but died 6 weeks later from disease progression

Low dose radiotherapy has been shown to be an effective and un-morbid treatment in the setting of indolent non-Hodgkin's lymphoma. It should be considered for patients with symptomatic chemotherapy resistant leukaemic deposits, such as leukaemic gingivitis.

P099. Real World Management of Lymphoma: Development of the Australian and New Zealand Lymphoma and Related Diseases Registry (LaRDR)

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Aim

To describe the rationale, aims, methodology and recruitment strategy for the new Australian and New Zealand (ANZ) Lymphoma and Related Diseases Registry.

Method

Lymphoma is the sixth most common cancer in terms of prevalence and primary reason for cancer hospitalisation in Australia. Incidence is increasing, however, reasons are poorly understood. Survival is also increasing, in part due to new approaches including availability of targeted therapies.

Although lymphoma represents a significant disease burden to the community and the health budget, few national Australian/NZ data are available on patterns of treatment, variations in outcomes (both survival and quality of life). LaRDR has been established to address questions about lymphoma epidemiology variation in practice, process and outcome measures; access to care; and uptake of new therapies.

Result/progress to date

Registry inclusion criteria comprise patients ≥ 18 years, diagnosed with non-Hodgkin lymphoma, Hodgkin lymphoma, chronic lymphocytic leukaemia or a related disease. LaRDR uses an "opt-off" consent model. A database, with tailored fields according to diagnosis, has been constructed and is available for secure online data entry. Data quality will be ensured via a central pathology review subcommittee. The registry has ethics approval for six pilot sites and began collecting data in July 2016. An ANZ multidisciplinary Steering Committee oversees registry operational and research activities.

Conclusion

LaRDR will provide important Australian/NZ epidemiological, clinical and outcomes data and will support the translation of research findings into clinical practice. Hospital data reports will allow sites to review their practice and compare against national aggregate data. Variations in practice and factors relating to differences in approach to lymphoma diagnosis and management will be able to be evaluated, and compared against national and international guidelines, as has proven very valuable in other clinical registry settings. Site participation and research project ideas using LaRDR are welcomed.

This project is supported by Bristol-Myers Squibb, Novartis Pharmaceuticals, Roche Australia and Takeda Pharmaceuticals. These companies have no role in data analysis or abstract preparation.

P100. Successful Maternal and Fetal Outcome in a Rare Case of Rectal Diffuse Large B Cell Non Hodgkin's Lymphoma diagnosed at 22 weeks of Pregnancy

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Introduction

Diffuse large B cell non Hodgkin's lymphoma is extremely rare in pregnancy and cases reported run a more aggressive course. Management is complicated by the conflict between optimal maternal therapy, fetal well being and the medical and ethical issues surrounding this. A multi-disciplinary approach is essential in providing optimal care. We report a rare case of rectal diffuse large B cell non Hodgkin's lymphoma diagnosed in a 29 year old pregnant woman of 22 weeks gestation, who was successfully treated with R-CHOP 21 chemotherapy throughout her pregnancy and delivered at full term a healthy baby with no complications.

Case Discussion

29 year old pregnant woman of 22 weeks gestation presented with three months history of constipation, overflow diarrhoea and rectal bleeding. Endoscopic investigations confirmed almost complete obstruction with an ano-rectal circumferential mass with was confirmed diffuse large B cell non Hodgkin's lymphoma on biopsy. MRI staging confirmed rectal extra-nodal involvement and staged 1E. She was commenced on RCHOP 21 chemotherapy and tolerated 6 courses with minimal side effects. No GCSF or prophylactic antimicrobials were given in view of potential pregnancy category D risks. The birth was electively planned a week after her last cycle to allow maximal fetal drug excretion via the placenta to avoid neonatal myelosuppression. She spontaneously ruptured her membranes at 38 weeks + 4 days and progressed to a normal vaginal birth delivery a healthy baby boy of 2.64kg. Post treatment staging confirmed clinical, endoscopic and histological remission. She proceeded to consolidation radiotherapy after fertility preservation options were explored.

Conclusion

This case highlights some of the pertinent management issues in treating haematological malignancies during pregnancy. We report a rare case of extra-nodal diffuse large B cell NHL with successful maternal and fetal outcome.

P101. Diagnostic Utility of CD200 in Mature B cell Neoplasm

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Background

CD 200 [Membrane MRC OX-2 (MOX2)], is a type I immunoglobulin super family membrane glycoprotein, which is expressed in several mature B cell neoplasm (MBN). Objective: This study was aimed at observing the expression pattern of CD200 by flow cytometry immunophenotyping (FCI) and to evaluate its utility in narrowing down the differential diagnosis of MBN.

Methods

A total of 136 samples, including 87 peripheral blood, 44 bone marrow, three fine needle aspiration material and two ascitic fluids, were evaluated by 6 color FCI over a period of 20 months. The mean fluorescence index (MFI) of CD200 in the neoplastic population was noted and compared amongst several groups of MBN.

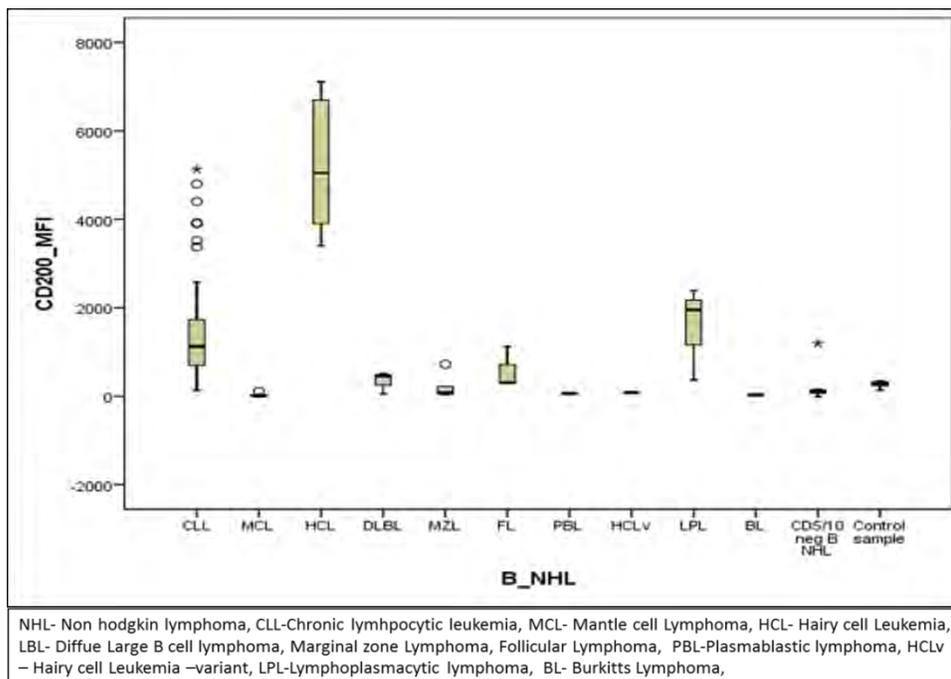
Results

CD200 was expressed in all CLL (n=84), even those with atypical immunophenotyping, while all the cases of mantle cell lymphoma (MCL, n=20) were consistently negative. All six cases of hairy cell leukemia (HCL), including those with atypical clinical and morphological features, expressed CD200 and that too with brightest intensity amongst all the MBN. Other MBN also expressed CD200, but in a proportion of their cases and with variable intensity.

Conclusion

This study, probably the first from this country, confirmed that CD200 has a definite valid role in differentiating CLL from MCL, and also showed its consistent and brightest expression in HCL. This antibody should be included in the primary panel for MBN immunophenotyping, as it can be of great diagnostic help in difficult cases with clinical, morphological and immunophenotypic overlap.

Figure 1: Box plot showing difference in the intensity of CD200 expression amongst different mature B cell neoplasm



P102. The Role of SMG1 and ATM in B cell Lymphoproliferative Disorders

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Background

SMG1 and ATM belong to the family of PIKK proteins which are known tumour suppressors. They have well described roles in cellular stress responses including DNA damage and nutrient deprivation which are important pathways for cancer survival. Loss of expression from one or both alleles of these genes leads to increased lymphoma and leukaemia development in mice. Data from mouse studies indicate that SMG1 and ATM co-regulate pathways which control haemopoietic cancer development and also pathways targeted by chemotherapeutics including DNA damage response and mTOR signalling.

Aim

1. To correlate SMG1 and ATM expression in human B cell lymphoproliferative disease with clinical characteristics, histology, karyotype, response to therapy and prognosis.
2. Decipher the relationship between the DNA damage control response and the mTOR and NF-κB pathways in haematological malignancy.

Method

Patients are currently being recruited through Liverpool Hospital. Peripheral blood or bone marrow aspirate samples from 70 B-NHL and CLL patients were purified via Ficoll Paque separation and immunomagnetic beads into malignant and non-malignant cell types. Total SMG1 and ATM levels and mTOR and NF-κB substrate phosphorylation was measured.

Results

Sub-group analysis of patients with CLL, Follicular Lymphoma, DLBCL and Mantle Cell Lymphoma including clinical response to therapy such as chemotherapy and ibrutinib will be presented. There is a trend for CLL patients to have reduced expression of SMG1 with complete absence of SMG1 in CLL being rare. Preliminary results demonstrate that low expression of SMG1 correlates with increased mTOR activity as measured via phosphorylated mTOR substrate AKT, and increased NF-κB activity as measured via phosphorylated NF-κB substrate p65. A more detailed analysis will be presented at the meeting.

Conclusion

SMG1 and ATM may be biomarkers for predicting outcomes in NHL patients. They may also predict responses to mTOR and NF-κB inhibitors as novel treatments for NHL patients.

P103. DA-EPOCH-R chemotherapy is well tolerated and effective - experience from a regional cancer centre

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Aim

Assess the tolerability and outcome of DA-EPOCH-R at Waikato Hospital, Hamilton, New Zealand.

Method

All patients treated with DA-EPOCH-R at Waikato Hospital 2011 to March 2015 were retrospectively identified and reviewed from databases and clinical records. Data (to 30 June 2016) included diagnosis (WHO 4e, revision 2016), cell of origin (COO; by Hans algorithm), stage, International Prognostic Index (IPI), performance status (PS; ECOG), chemotherapy tolerability (treatment stopped for toxicity, admissions for neutropenic fever or infection), response (CR, PR) / progression / relapse (Lugano Classification 2014) and death.

Results

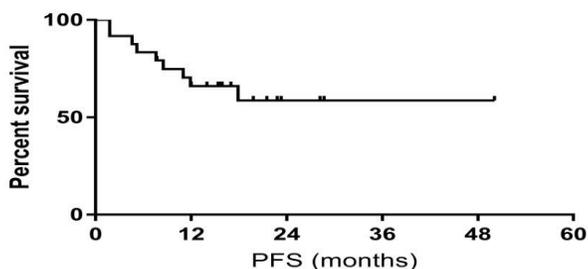
24 pts (15 male, median 58yo [range 25-85]) were identified. The majority had high risk features including stage 4 (19) and IPI ≥ 3 (17). PS was median 1 (range 0-4). Diagnoses, COO, response and remission status are detailed in the Table:

Diagnosis	N	Cell of origin			Response			Relapse
		GCB	NGCB	U	PR	CR	Refractory	
PMBCL	4	0	4	0	1	3	0	0
Double Hit	8 ¹	7	0	1	0	5	2	1
DLBCL (gray zone)	5	3	2	0	0	5	0	3
DBLCLNOS	4	2	0	2	1	2	1	0
Other ²	3	2	0	1	1	2	0	1
TOTAL	24	14	6	4	3	17	3	5

¹ 1 pt abandoned therapy at cycle 2 ² Follicular Lymphoma (1), Plasmablastic (1), Burkitt (1)

PR= partial response, CR= complete response, GCB= germinal centre B cell, NGCB= non-GCB, U= unclassifiable.

Of the 4 non responders, 3 progressed while on treatment and 1 abandoned treatment due to chemotherapy side effects. COO was associated with diagnosis but neither influenced response. Remissions appear reasonably durable in this poor risk group (Figure), PFS 60% at 2 yrs. Therapy was moderately well tolerated; 3 pts ceased chemotherapy following cycle 2 (cytopenias / sepsis), 12 (50%) pts had a total of 26 hospital admissions for neutropenic fever.



Conclusions

DA-EPOCH-R produced excellent response rates and durable remissions in a poor risk cohort. Toxicity was moderate.

P104. Serial comprehensive genomic characterisation dissects the complex underlying biology of a case of synchronous Burkitt lymphoma and myeloid malignancy with shared haematopoietic ancestry

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Background and Aim

Comprehensive genomic profiling has the ability to reveal novel information about fundamental mechanisms underlying tumour biology. We aimed to comprehensively characterise a patient with two episodes of Burkitt lymphoma (BL) and a treatment associated myeloid neoplasm to illustrate the role of next generation sequencing (NGS) in elucidating the pathology of the sequential haematological malignancies in this unique patient.

Methods

Genomic analysis was performed using the Peter Mac PanHaem Panel (Agilent SureSelect) designed to detect gene mutations, genome-wide copy number variations, translocations and IGH family usage in a single diagnostic NGS assay.

Results

A 55-year-old man initially presented with extensive Stage IV BL and achieved a complete remission after treatment with CODOX-M/IVAC. Five years later he presented with a large appendiceal mass, histologically consistent with BL, and was treated to remission again with R-CODOX-M/IVAC. He developed pancytopenia 12 months later and was diagnosed with treatment associated myelodysplastic syndrome (t-MDS). Highly typical and specific mutation, copy number and structural variations were detected in both episodes of BL, however the lack of shared genomic events and different IGH family usage indicated that they were clonally unrelated (see table). His t-MDS displayed genomic lesions characteristic of a myeloid malignancy and importantly shared a *TP53* mutation with his second BL. Retrospective testing identified this *TP53* mutation in the uninvolved bone marrow at diagnosis of his second BL but absent from the uninvolved bone marrow at diagnosis of his first BL.

	1st Burkitt lymphoma	2nd Burkitt lymphoma (appendix)	t-MDS
Mutations	<i>TP53</i> (Arg209Lysfs*6), <i>ID3</i> , <i>ARID1A</i> , <i>SALL3</i> , <i>MYC</i>	<i>TP53</i> (Arg282Trp), <i>DDX3X</i> , <i>KMT2D</i> , <i>PIK3R1</i>	<i>TP53</i> (Arg282Trp)
Translocations	t(8;14) MYC-IGH	None detected	None detected
Copy number changes	Loss: 6q Gain: 2p, 11q [#] , 13q [#] 19p	Gain: 4q, 7p, 8q, 11q [#] , 13q [#] , 18q	Loss: 5q, 7q, 17p
IGH rearrangement	IGHV4-4*07 F IGHJ4*02 F IGHD6-19*01 F	IGHV3-72*01 F IGHJ5*02 F IGHD3-3*02 F	

[#]The breakpoints of gain(11q) and gain(13q) were different in the 1st and 2nd Burkitt lymphomas

Conclusion

Using serial comprehensive molecular assessment we infer that *TP53* clonal haematopoiesis arose following treatment for the initial episode of BL and an ancestral clone from this compartment subsequently sequentially gave rise to both a second clonally-unrelated episode of BL and an aggressive myeloid malignancy. This case demonstrates the usefulness of comprehensive NGS based genomic characterisation for delineating disease biology in complex clinical cases.

P105. Routine Bone Marrow Cytogenetics in Initial Staging of Lymphoma

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Aim

To assess the utility of conventional cytogenetic analysis of bone marrow aspirates in initial staging of lymphoma.

Method

Bone marrow aspirates were reviewed at a single institution from 1/1/15 to 31/12/15. 50 included a request for conventional cytogenetics as part of initial staging of lymphoma. For each case, data was collected on: indication, final diagnosis, results of aspirate, trephine, flow cytometry, conventional cytogenetics, FISH (when performed) as well as whether diagnosis had been aided via other specimens.

Result

8 abnormalities were noted in 7 of 50 cases (see table). 2 related to translocations pathognomonic for mantle cell lymphoma. Another was a complex karyotype in a diffuse large B cell lymphoma. Morphological involvement and an extramedullary biopsy had already established the diagnosis in these 3 cases. 3 abnormalities were age related loss of Y and another was a small hyperdiploid clone in a T Cell lymphoma. The final abnormality was a balanced t(9;22) with a breakpoint appearing around the centromere in a follicular lymphoma whose marrow appeared neither involved nor myeloproliferative. None of these abnormalities altered management. BCL2 rearrangement was also detected via FISH in one case with normal conventional cytogenetics, which did aid a diagnosis of follicular lymphoma.

Final Diagnosis	Aspirate	Trephine	Flow	Cytogenetics	Other Pathology
Mantle Cell	Suggestive	Positive	Positive	t(11;14)	Lymph Node
Mantle Cell	Positive	Positive	Positive	t(11;14)	Lymph Node
Diffuse Large B Cell	Suggestive	Positive	Negative	Complex	Lymph Node
Follicular	Negative	Negative	Negative	t(9;22), Loss Y (15%)	Lymph Node
T Cell	Positive	Positive	Positive	2/20 hyperdiploid	Nil
Splenic Marginal Zone	Positive	Positive	Positive	Loss Y (45%)	Peripheral Flow
Splenic Marginal Zone	Positive	Positive	Positive	Loss Y (60%)	Peripheral Flow

Conclusion

Conventional cytogenetics did not alter management in any of the 50 patients reviewed. Its routine request during initial staging of lymphoma should be reviewed.

P106. Bleomycin whipping up trouble: Flagellate dermatitis in two patients treated for Hodgkin Lymphoma

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Aim

Bleomycin, an anti-tumour antibiotic that induces genomic instability through DNA fragmentation, remains an important agent in standard protocols for treatment of Hodgkin lymphoma (HL) as well as many non-haematological malignancies. In addition to typical chemotherapeutic side effects (mucositis, nausea, vomiting), pulmonary toxicity and cutaneous reactions are well recognised. We report two cases of a rare but significant cutaneous toxicity.

Method

A 53 year old female developed urticarial erythematous lesions affecting the groin, trunk, lower back and flexural upper limb surfaces after Cycle 1 Day 15 ABVD therapy (adriamycin, bleomycin, vinblastine, dacarbazine) for nodular lymphocyte predominant HL. Clear clinical progression occurred following further ABVD. The second case is a 32 year old male who developed pruritic raised plaques affecting his hands, arms and anterior and posterior trunk, following Cycle 1 Day 15 ABVD for classical HL.

Result

Although dacarbazine is also reported as causing cutaneous adverse reactions, given the efficacy of AVD for HL and the nature of skin rashes a therapeutic trial of bleomycin withdrawal was undertaken. Bleomycin was omitted from day 15 Cycle 2 of ABVD onwards, with both patients continuing with AVD chemotherapy. Incidentally, interim PET scan confirmed complete metabolic response for both patients. The rash was treated conservatively with antihistamines and topical emollients with case 1 also receiving oral prednisolone. The pruritus promptly responded, with slower resolution of the rash itself following cessation of the bleomycin. Both patients developed significant post-inflammatory pigmentation in the affected areas, typical of bleomycin induced flagellate dermatitis, which remains months following omission of bleomycin.

Conclusion

We report two cases of bleomycin induced flagellate dermatitis in patients being treated with ABVD chemotherapy for HL. Presentations were typical, with urticarial lesions that developed into hyperpigmented streaks, occurring weeks after commencement of therapy. The omission of bleomycin, continuing therapy with AVD, has not appeared to compromise treatment of HL.

P107. Outcome of patients with Follicular Lymphoma managed at Royal Darwin Hospital and Northern Territory

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Aim

To compare the outcome of patients treated with follicular lymphoma at our hospital to published data.

Method

The medical records of patients with FL diagnosed and managed within the Northern Territory over the last 10 years were assessed. Specifically, we investigated the effect of various treatment regimens on the progression free survival (PFS) and overall survival (OS).

Result

In total, our cohort comprised of 32 patients with a slight male (68.8%) preponderance. Average follow up was 63.7 months. The mean age at diagnosis was 54.9 years, and only 2 (6.6%) patients were Aboriginal. Most of our cohort presented with advanced stage disease (Ann Arbor 3 or 4; 71.9%). Two patients were excluded due to insufficient data. Maintenance rituximab was given to all patients (N=23, 72%) who responded to their initial chemotherapy. PFS was 86%, 81.5%, and 59.9% at 2, 5 and 8 years, respectively. OS was 96.6% at 2 and 5-years, respectively, and 84.5% at 8 years. Median PFS and OS was not yet reached. Most of these patients were treated with chemotherapy (81.3%), predominantly R-CHOP (42.3%) and R-CVP (38.5%). R-CHOP had slightly worse 5-year PFS than R-CVP, but this was not statistically significant (66.7% vs 80%, p=0.325).

Conclusion

The outcomes of patients with follicular lymphoma treated at Royal Darwin Hospital compared favourably to those of published studies¹⁻³. Differences in PFS and OS between our cohort and these others may relate to our centres' almost global use of rituximab maintenance after chemotherapy. The low proportion of Aboriginal patients in our cohort may warrant further investigation.

This research was approved by the Menzies School of Health Research Human Research Ethics Committee.

P108. Defibrotide use in vincristine induced hepatic sinusoidal occlusion syndrome

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Aims/Background

Hepatic sinusoidal obstruction syndrome (HSOS) is typically associated with allogeneic haemopoietic stem cell transplantation, but may occur after chemotherapy, including gemtuzumab and vincristine. We report a case of HSOS in a 70 year old man who received vincristine for diffuse large B cell lymphoma (DLBCL) in the setting of existing cirrhosis, successfully treated with defibrotide.

Case report

A 70 year old male presented with obstructive jaundice and a bilirubin level of 420umol/L. Biopsy of a large duodenal mass demonstrated DLBCL. His medical history included alcoholic cirrhosis (abstinent for over 15 years), and hypertension. Percutaneous biliary drainage, enteroscopic biliary stenting and pre-phase prednisolone reduced his bilirubin by 100umol/L but this was not sustained. He then received 2 cycles of Rituximab 375mg/m², Cyclophosphamide 750mg/m², vincristine 1.4mg/m² and Prednisolone 100mg (R-COP) 14 days apart. (Doxorubicin was withheld due to persisting hyperbilirubinemia (450umol/L).) He subsequently developed features of HSOS including a progressive elevation in his bilirubin to 632umol/L, weight gain and ascites, despite MRCP demonstrating marked reduction in the duodenal mass and no ongoing biliary obstruction. Liver biopsy was consistent with possible HSOS. After exhibiting no response to ursodeoxycholic acid he was treated with defibrotide, which led to a 4-fold reduction in his bilirubin over 7 days. The patient was subsequently treated with 3 cycles of Rituximab and Bendamustine (BR). With this he achieved a complete metabolic response and suffered no further biochemical hepatotoxicity.

Conclusions

This patient appeared to have develop HSOS following administration of vincristine given without dose-adjustment in the setting of existing liver damage and hyperbilirubinemia. This was successfully managed with use of defibrotide. This case illustrates the necessity for dose adjustment of vincristine in liver disease and adds to the growing literature supporting use of defibrotide for HSOS and Bendamustine in treatment of lymphoma in patients with hepatic impairment.

P109. Primary gastric DLBCL: management and clinical outcomes – a single tertiary hospital experience

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Aim

The optimal therapy for Primary Gastric DLBCL (PG-DLBCL) is yet to be defined with current approaches largely based upon case series, rather than randomized clinical trials. This study examines the clinical management of PG-DLBCL in an Australian tertiary care centre with the aim of identifying the role of various therapeutic modalities.

Methods

Patients with PG-DLBCL diagnosed between 2002-2015 according to 2008 WHO criteria were identified from hospital databases. Cases with adequate information, including baseline characteristics, treatment regimens and outcome, were included. Overall survival (OS) and progression free survival (PFS) were modelled using Cox regression.

Results

26 patients were identified, of which n=16 (62%) had limited stage and n=10 (38%) advanced stage disease (involving oesophagus, small bowel, pancreas, liver or serosal tissues). No cases showed positive immunohistochemistry for *Helicobacter pylori*.

Treatment with curative intent was delivered to 24 patients with 23 receiving R-CHOP. Combined modality chemotherapy and radiotherapy was delivered to 8/16 (50%) and 1/10 (10%) patients with limited stage and advanced stage disease, respectively, with a median dose of 30.6 Gy (range 30-36 Gy). Treatment was generally well tolerated, however three deaths occurred during induction chemotherapy due to neutropenic sepsis (1), gastrointestinal haemorrhage (1) spontaneous intracranial haemorrhage not associated with CNS lymphoma (1). There were no gastric perforation events.

Induction therapy was highly efficacious with 21 (88%) of the patients treated with curative intent achieving complete remission. The 3Y-PFS was 73% and 42% for limited and advanced stages respectively (P=0.08). The 3Y-OS was 73% and 46% respectively (P=0.13). The addition of consolidative radiotherapy was associated with a trend to improved PFS (P=0.09) as noted in the IELSG4 study.

Conclusions

Outcomes of patients with PG-DLBCL receiving R-CHOP+/-RT, while similar to those seen in nodal DLBCL, are suboptimal. Rational approaches based upon an understanding of tumour biology are warranted.

P110. WhiMSICAL (Waldenström's Macroglobulinemia study involving CArt-wheel): empowering patients internationally to contribute patient-derived data for observational research

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Aim

Waldenström's Macroglobulinemia (WM), a rare cancer, is difficult to study in large trials, resulting in paucity of well-founded evidence. Clinical trial data does not reflect real-world dilemmas of funding and availability. Patient-derived data is an attractive option to gain better breadth of knowledge.

This study utilises CART-WHEEL, a global, online rare cancer database for patient-derived data, to gain a better, real-world understanding of WM. It aims to gather data on symptoms, correlation to pathology results, triggers for therapy, different treatments, their efficacy, tolerance and disparities within countries and internationally.

Method

An HREC-approved, WM-specific extension to the CART-WHEEL questionnaire was developed by clinician and patient investigators, going online in June 2016. Continuous participant recruitment is through liaison with WMozzies, Australian affiliate of the IWMMF, Leukaemia Foundation Australia, and members of HSAZ. International promotion, assisted by the IWMMF, will be undertaken once feasibility has been demonstrated locally. Participants are encouraged to print out their personal CART-WHEEL report and review any missing data with their haematologist. Data analysis will be conducted utilising independent samples t-test, cross-tabulation and Pearson Chi-squared.

Results

Feasibility of the project was demonstrated early with excellent participant feedback received by WMozzies. Consent for use of entered data was received from 30/43 participants in the first five weeks, from Australia (76%), USA (16%), Wales (4%) and Belgium (4%), with median age 66.5 years (51-83) and male predominance (73%). Of listed symptoms, 73% of patients listed fatigue and 18% listed night sweats, weight loss >10%, fever, epistaxis and/or sensory disturbance. Further data will be available to present at the meeting.

Conclusion

Having established a robust data-collection platform for WM patient-derived data, upon international roll-out, the information gathered will expand knowledge of the range of patient experiences. Demonstration of any treatment disparities, coupled with information regarding treatment efficacy may facilitate access to subsidised novel therapies.

P111. Paraneoplastic manifestations of Hodgkin Lymphoma - two case reports and a brief literature review

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Aim

Hodgkin lymphoma (HL) may manifest atypically and although rare (<1% of cases), may lead to delays in management (1). We describe two cases of HL manifesting with paraneoplastic neurological and renal disorders, specifically cerebellar degeneration with limbic encephalitis, and nephrotic syndrome secondary to minimal change nephropathy. These conditions have been described as manifestations of HL (1-3).

Method

We present two cases of HL presenting de novo with paraneoplastic syndromes. Case One is a 47-year-old female with diplopia, dysarthria, delirium and progressive cerebellar dysfunction with a painless inguinal enlarged lymph node (LN). Case Two is a 24-year-old female with gross pitting oedema, nephrotic range proteinuria, night sweats, and exertional dyspnoea with a large mediastinal mass.

Result

Both patients underwent investigation of the presumed involved organ, as well as a tissue biopsy showing HL. Case One underwent positron emission tomography (PET), lumbar puncture and right inguinal LN biopsy. The LN biopsy was diagnostic for classic HL, and brain biopsy showed anti-mGluR5 positivity consistent with limbic encephalitis. Case Two presented with marked hypoalbuminaemia (9.0 mg/L) and nephrotic range proteinuria, with minimal change nephropathy on renal biopsy. Chest X-ray showed a large anterior mediastinal mass, and supraclavicular LN biopsy was diagnostic for nodular sclerosing HL. In case One, treated HL remains in PET negative complete remission (CR) at 3 years after ABVDx4; however, disabling neurological manifestation persisted requiring full-nursing care despite the use of rituximab for limbic encephalitis. Case Two, had rapid resolution of nephrotic syndrome after 2 cycles of ABVD for stage IIb disease and is in PET negative CR.

Conclusion

Paraneoplastic manifestations of HL are rare, but important to recognise. Resolution may not necessarily occur following the attainment of CR in HL. A summary of paraneoplastic presentations of HL will be presented, based on the available published literature.

P112. Comparative Benefit of Brentuximab Vedotin Versus Salvage Chemotherapy in Relapsed or Refractory Hodgkin Lymphoma Patients Following Prior Autologous Stem Cell Transplant

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Aim

Standard management for relapsed or refractory Hodgkin lymphoma (RR HL) in patients with progressive disease after autologous stem cell transplant (ASCT) is salvage chemotherapy ± radiotherapy. Depending on response and suitability, a second SCT (usually allogeneic) may be considered. However, current salvage chemotherapy regimens either do not achieve substantial survival gain; or their efficacy is compromised by early onset and cumulative toxicity, particularly haematological in nature. Brentuximab vedotin (BV) is a salvage treatment registered in Australia for patients with RR HL following ASCT ('post ASCT'). In the Phase II single arm registration trial (Study SG35-0003) BV demonstrated high response rates (including complete response) and gains in overall survival (OS). Being single arm, the Study 0003 data were not comparative. This analysis aimed to assess the comparative efficacy of BV versus other salvage treatments used post ASCT.

Methods

The published literature was searched to identify large studies of post ASCT patients receiving either BV or conventional chemotherapy.

The search retrieved Chen et al, 2015 - Study 0003's final survival analysis (N=102) and two large retrospective analyses: Martinez et al, 2013, a study of patients in the European Bone Marrow Registry (N=511), and Arai et al, 2013, an international study including European and North American transplant centres (N=756).

Results

For BV, the estimated 5-year OS rate was 41% (95% CI: 31, 51), and the median OS was 40.5 months (95% CI: 28.7, 61.9 [range, 1.8 to 72.9+]). In Martinez, median OS was 32% at 5 years and median OS was ~28 months. In Arai, median OS was 30 months.

Conclusion

Despite the limitations associated with comparing heterogeneous populations and the possibility of overlapping patients between the Arai and Martinez studies, these data suggest that BV may improve OS in post ASCT patients compared to that seen with conventional chemotherapies.

This research was supported by Takeda Australia. The company was involved in the extraction and analyses of the data and the preparation of the abstract.

P113. Azacitidine induced sweet's syndrome

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Azacitidine induced sweet's syndrome A 54 year old female with a new diagnosis of myelodysplasia (RAEB-2) was commenced on subcutaneous azacitidine. Within 48 hours, exquisitely tender violaceous lesions developed at the injection site. The patient presented to hospital on day 5 of azacitidine treatment with fevers and an extensive rash surrounding the sites of injection. Despite the discontinuation of azacitidine and the commencement of broad spectrum antibiotics for possible superimposed infection and necrotising fasciitis, the rash progressed to target like lesions (Fig 1), the fever continued and the patient developed acute kidney injury.

Histopathology of the lesions revealed a neutrophilic dermatosis (Fig 2) and a diagnosis of azacitidine-induced Sweet's syndrome was made. Prednisolone (1mg/kg) was commenced and within 24 hours her fever resolved and skin lesions and renal function started to improve.

Sweet's syndrome is characterised by tender erythematous skin lesions, fever and a diffuse infiltrate of neutrophils, most commonly in the upper dermis. Extracutaneous manifestations, including renal involvement, can occur. The condition responds rapidly to steroid therapy. While injection site reactions and pain are common with subcutaneous azacitidine administration, Sweet's Syndrome is a rare complication which needs to be considered.

P114. The use of Flow Cytometry in the Diagnosis of Low Grade Myelodysplasia

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Aim

The diagnosis of low grade Myelodysplastic Syndrome (MDS) can be challenging. By definition these patients do not have increased numbers of blasts, morphological changes can be subjective and confirmatory cytogenetic changes are often absent. Whilst flow cytometry may assist in the diagnosis, established guidelines are extremely complex requiring both expert interpretation and at least 20 markers in 5 separate tubes (1). Other studies have shown that just 4 key parameters can be used to help diagnose low-grade MDS ($P < 0.001$) (2). We aimed to validate the utility of these characteristics in our patient cohort.

Methods

We used flow cytometry to assess side scatter, CD34 and CD45 expression in the bone marrow of 48 patients being investigated for cytopenias. These characteristics were used to establish 4 parameters: myeloblast-related cluster size, B-progenitor-related cluster size, myeloblast CD45 expression and granulocyte side scatter. Abnormalities in at least 2 of these parameters were considered supportive of the diagnosis of MDS, whilst a score of < 2 meant MDS was unlikely. The final diagnosis of MDS was confirmed after 2-36 months of follow-up by the treating haematologist. We compared the initial flow assessment of the patient with their final diagnosis.

Results

Of the 48 patients, the final diagnosis remains unclear in 6 and they were excluded from the analysis. There were 29 patients who were found to have probable or definite MDS and 12 patients in whom MDS was deemed unlikely. The sensitivity of the flow score was 72%, specificity 83% with a positive predictive value of 91% and negative predictive value of 56%.

Conclusion

There appears to be a role for flow cytometry in the diagnosis of low grade MDS. This relatively simple assay may help guide which patients without blasts or cytogenetic changes are more likely to have true clonal MDS.

P115. Reduced survival in low IPSS-R risk myelodysplasia: a single centre retrospective review

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Aim

To assess the outcomes in myelodysplastic syndrome (MDS) in a single centre and compare to the IPSS-R cohort (Greenberg et al, 2012).

Method

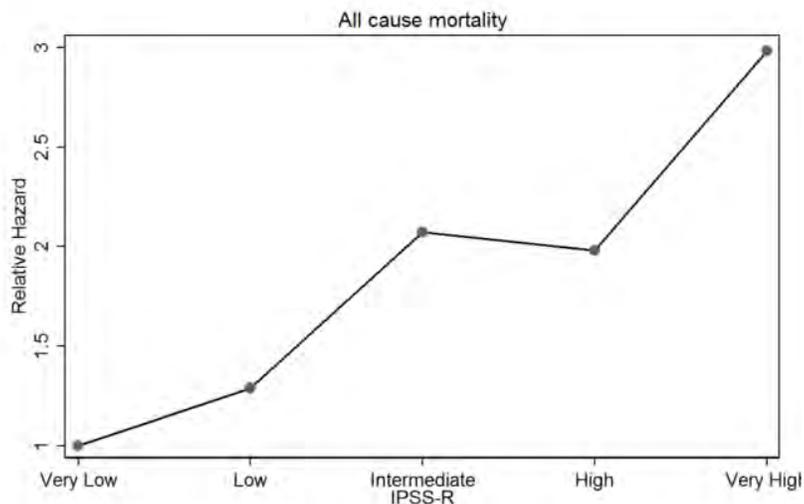
A retrospective review was performed by searching the electronic database for an admission code of MDS. Identified patients were confirmed for a MDS by review of the bone marrow. IPSS-R risk was assigned. Overall survival (OS) was calculated from date of diagnosis and censored at 1st of May 2016. Statistical analysis was by Cox Regression.

Results

Between 2008 and 2015, 160 patients were identified and 31 cases were excluded due to incorrect coding or non-MDS marrow. Median age was 72 (28-90) years. Median follow-up was 20 (1-178) months. Median OS was 19 (0 -178) months. The IPSS-R risk group was associated with mortality (Figure, HR 1.49, 95CI 1.19-1.87, p=0.001). The median OS for very low, low, intermediate, high and very high risk groups were 2.17, 2.04, 1.33, 1.58 and 0.96 years, respectively. There was a trend to improved median OS in the low risk (very low, low, intermediate) group cf. high risk (high, very high) (p=0.094). 62% were deceased with median OS of 16 months and 63% of deaths were MDS-related. The Cox regression model demonstrated a mortality association with bone marrow blast % (HR 1.40, 95CI 1.08-1.43, p=0.012), cytogenetic risk group (HR 0.66, 95CI 0.52-0.83, p=0.001), haemoglobin (HR 0.25, 95CI 0.07-0.93, p=0.038) and ferritin (HR 3.00, 95CI 1.75-5.16, p<0.001). Progression to AML was associated with mortality (HR 2.95, 95CI 1.66 –5.23, p<0.001). Age, Gender, LDH, marrow fibrosis, platelet and neutrophil counts were not significantly associated with increased risk of mortality.

Conclusion

This retrospective review found lower than predicted survival in the 'low' risk IPSS-R when compared to the Greenberg et al. cohort and therefore this represents an unmet clinical need. The IPSS-R, cytogenetics, aspirate blast percentage and ferritin were associated with OS. There was an association with elevated ferritin and AML transformation and OS. Further assessment with clinical factors, including azacitidine therapy, transfusion and iron chelation therapy is underway.



P116. Predictors of survival after MDS allograft. State-wide outcomes from a decade of MDS transplants in the azacitidine era

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Aim

To review transplant outcomes in patients with Myelodysplastic Syndrome (MDS) and identify predictors of response.

Method

A retrospective review of the transplant registry database was conducted including primary and secondary MDS from 2004 - 2016. Diagnostic pathology reports were reviewed to confirm cases and an IPSS-R score assigned (Greenberg et al, 2012). Additional clinical information including pre-transplantation bone marrow biopsy and pre-treatment with azacitidine or chemotherapy as a bridge to alloSCT was also recorded. This project was performed under governance of local quality committee (for clinical audit). Data has been censored at 28th June 2016.

Results

44 patients underwent alloSCT for MDS with a total 46 transplants and 2 patients re-transplanted due to failed of engraftment. The median age at time of transplantation was 54 years (range 22-66). Cohort characteristics are provided in Table 1. 48% were pre-treated at time of transplantation, with most of these receiving azacitidine therapy. Most transplants were myeloablative (72%), using busulfan in combination with cyclophosphamide, fludarabine or melphalan. Median follow up was 26 months (4 – 125 months) and 2-year overall survival was 50% (95% CI: 34 – 66). Acute GVHD (grades 2-4) occurred in 45% and the cumulative incidence of chronic GVHD (limited and extensive) was 56% (95% CI: 38-70). The cumulative incidences of relapse and non-relapse mortality at 2 years were 30% (95% CI: 14-47) and 24% (95% CI: 11-40), respectively. Azacitidine or other chemotherapy prior to transplantation, IPSS-R and conditioning regimen intensity were not significantly associated with survival in this cohort.

Conclusion

This retrospective review has identified median survival in keeping with other published cohorts, with high rates of chronic GVHD. Disease status prior to transplantation, pre-treatment, conditioning, IPSS-R, and cytogenetics do not appear to affect transplant survival in our cohort. Relapse and chronic GVHD are major factors affecting survival outcomes.

Table 1:

Characteristics			%
Gender	Male	33	75%
	Female	11	25%
Year of transplant	2005 – 2009	11	24%
	2010 – 2016	35	76%
Donor type	Related	17	37%
	Unrelated	29	63%
Source of graft	Peripheral blood	39	85%
	Bone marrow	4	9%
	Umbilical cord	3	7%
Cytogenetics	Good	19	43%
	Intermediate	12	27%
	Poor	4	9%
	Very poor	6	14%
	No result	3	7%

P117. Circulating microvesicles are less procoagulant but carry more miRNA cargo in Myelodysplasia

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Aim

To evaluate the number and function, including small RNA content, of circulating microvesicles (MV) in Myelodysplasia (MDS).

Background

Circulating MV are important cellular messengers, with capacity to influence normal cellular function of surrounding and paracrine tissue. Their role in MDS has not been studied.

Methods

Citrated blood samples were collected from 35 patients with Myelodysplasia and 15 age similar controls after receiving informed consent. MV subsets were enumerated by Flow cytometry (BDFACS Canto) after staining with specific antibodies for platelets (CD41), endothelial cells (CD105), white blood cells (CD45), monocytes (CD14) and red cells (CD235a) as well as tissue factor (CD142), and phosphatidyl serine (Annexin V binding). MV were also labelled with quantum dots (Qtracker 655 nanocrystals) for Nanotracking analysis on a NanoSight NS500 under scatter and fluorescent settings. Pro-coagulant function was assessed by the XaCT assay on a BCS analyser and by thrombin generation (ETP) using a Calibrated Automated Thrombogram (CAT). Small RNA was extracted and quantitated on an Agilent bioanalyzer. The statistical analysis was carried out on Prism 7 software using non-parametric tests (Mann-Witney U tests, significance at $p < 0.05$).

Results

The number of MV expressing CD105 and CD14 were significantly lower in MDS compared to age similar normal subjects ($p < 0.0001$ and $p = 0.0018$). Other MV and enumeration by nanotracking was not significantly different. The pro-coagulant function by XaCT and ETP was also significantly lower in MDS ($p < 0.0001$ for both). In contrast, the small RNA and miRNA content was significantly higher in MDS samples ($p = 0.0005$ and $p = 0.02$ respectively).

Conclusions

Circulating MV in MDS show reduced pro-coagulant functional activity but significantly increased small RNA content when compared to age similar normal controls. It is possible that the MV in MDS influence normal endothelial cells through their miRNA content even though their role in pro-coagulant activity may be limited.

P118. A case of eosinophilic pneumonia secondary to azacitidine therapy

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Introduction

Eosinophilic pneumonia is a rare disorder characterised by accumulation of eosinophils in the alveoli and lung parenchyma resulting in cough, shortness of breath and lung infiltrates detected radiologically. It is usually a diagnosis of exclusion. Eosinophilic pneumonia as a complication of Azacitidine therapy has not been previously reported in Australia. Here we report a case of eosinophilic pneumonia secondary to Azacitidine therapy.

Case Description

An 81year old gentleman was diagnosed with MDS (RAEB2) in mid 2015 and commenced on Azacitidine therapy soon after diagnosis. He tolerated the medication well without any significant complications. Halfway through his second cycle of therapy, he presented with a one month history of progressive shortness of breath, cough and bilateral lung infiltrates detected on HRCT. It was noted that he had peripheral blood eosinophilia on presentation. The azacitidine was ceased and he was initially managed as a bacterial pneumonia with IV antibiotics, without much improvement. He went on to have a bronchoalveolar lavage, which demonstrated increased eosinophils and scant growth of aspergillus. He subsequently underwent multiple treatment courses of anti-fungal agents including voriconazole, amphotericin and anidulafungin, without any improvement in symptoms.

He was subsequently commenced on oral prednisolone, which resulted in a dramatic improvement of respiratory symptoms and function, disappearance of radiological signs as well as normalisation of his eosinophil count. Unfortunately, the steroids needed to be tapered rapidly due to the development of steroid induced diabetes mellitus. Since cessation of Azacitidine and completing a short course of steroids, the patient has remained symptom free and without any recurrence of disease. Azacitidine has not been re-commenced, and he remains transfusion dependent.

Conclusion

Eosinophilic Pneumonia is a diagnosis of exclusion and a high index of suspicion is necessary to identify it in patients who are on Azacitidine therapy. It should be considered in patients who do not respond to conventional treatments and those who have a demonstrable eosinophilia.

P119. Improved survival in Myelodysplastic Syndrome (MDS) salvaged on clinical trials after Hypomethylating Agent (HMA) failure

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Aim

Survival after HMA failure in MDS is dismal, with a reported median overall survival of 4 months in high-risk disease. We sought to identify determinants of survival in patients post HMA failure, including the effects of subsequent treatments.

Method

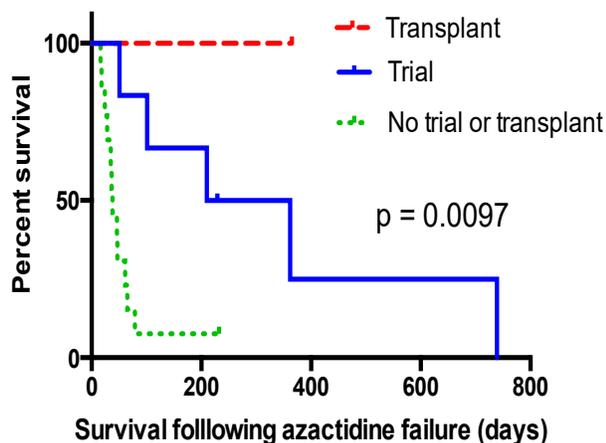
Following ethics approval, pharmacy records were utilised to identify all 5-azacitidine recipients in Monash Health between 2012 and 2015. Clinical outcomes were determined in all patients failing HMA therapy.

Result

Of 40 patients commencing azacitidine, 33 (median age 75 yrs, range 57–92; 60% male), failed treatment after a median of 7 cycles (range 1–30). 39% had very high risk IPSS-R, 45% high-risk, 9% intermediate-risk and 3% low-risk. Reasons for failure were: disease progression (n=20, including 15 AML transformations, 5 MDS progressions), death (n=8), toxicity (n=4) and 1 unknown cause. At a median follow-up of 349 days (range 1–1448 days), overall survival was 12% (n=4), with 1 patient lost to follow-up.

Patients with disease progression had a median of 1 further line of therapy (range 0–4), including 1 allogeneic transplantation and 6 patients participating in clinical trials. Median survival was significantly higher for those on clinical trial compared to those not on trial (286 vs 38 days, p=0.0097) (*fig 1*). Non-trial/transplant patients included 9 patients receiving supportive care, and 4 receiving other therapy (induction chemotherapy, low-dose cytarabine, thioguanine, melphalan, steroids). Patients with low/intermediate IPSS-R trended towards increased survival compared to high/very high risk (median 134 vs 56 days, p=0.59).

Fig 1: Outcomes following disease progression based on subsequent therapy



Conclusion

This real-world experience confirms previously reported poor survival following HMA failure in MDS. However, a subset of patients derived significant benefit from salvage as part of a clinical trial or transplantation. This may be explained by selection of the fittest patients for such salvage and/or the impact of emerging novel agents. Clinical trial participation remains imperative post HMA failure.

P120. Evaluation of both the Diagnostic and Prognostic Role of SNP/CGH Microarray Based Genomic Testing in Myelodysplastic Syndrome

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Aim

Cytogenetic testing, crucial in MDS evaluation, helps to establish the diagnosis and the prognostic stratification, as well as guiding decisions re therapy. The overall objective of this study was to demonstrate that the early detection of significant genomic lesions by SNP/CGH microarray analysis provides better diagnostic and prognostic data in comparison to routine metaphase cytogenetic (MC) testing.

Methods

Microarray testing was performed in 28 cases including 18 confirmed MDS, five suspected MDS with borderline dysplasia, three AML with myelodysplasia related changes, one CMML and one MDS/MPN unclassifiable. According to the MC results, the study cohort was subdivided into two main groups, Group A: 15 cases with normal MC, and group B: 11 cases with a wide range of MC detected chromosomal abnormalities. The detection rate of genomic abnormalities was compared between MC and microarray testing. Further analysis including IPSS scores, survival data and disease outcome was performed on the 18 confirmed MDS cases.

Results

The concordance rate between the two testing methods is 57.1%. In group A, array analysis produced a higher diagnostic yield by detecting cryptic lesions in 5 (33.3%) cases. Group B showed a good correlation between the two methods, with the array analysis detecting additional lesions in 3 (27.2%) of cases. Genetic abnormalities were determined by microarray in 8 cases, two with significant prognostic implications (NF-1 and TP53). The identification of the TP53 gene abnormality in particular explained the extremely progressive course and poor survival in one MDS case with a relatively low risk IPSS score.

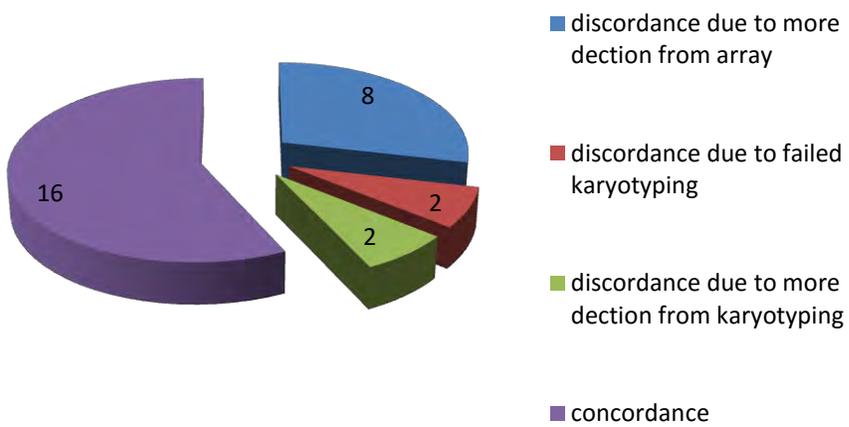
Conclusions

Although the results of the microarray analysis did not alter the IPSS scores in this small series, the identification of clonality and/or poor prognostic genetic lesions may well be critical in situations of diagnostic uncertainty and/or when planning therapies such as transplantation.

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

Table 2. The cross tabulation of karyotype and micro array

Array * Karyotyping Crosstabulation												
Count												
Array	Karyotyping	Karyotyping										Total
		-20	-7	-y	+20	complex	del(20q)	del(20q)+t(4;7)	Del(7q)	karyotype-failed	Normalkaryotype	
-7		0	1	0	0	0	0	0	0	0	0	1
-y		0	0	1	0	0	0	0	0	1	0	2
complex		0	0	0	0	2	0	0	0	0	1	3
complex(incl del 7q)		0	0	0	0	0	0	0	1	0	0	1
complex-additional		0	0	0	0	1	0	0	0	0	0	1
Del(13q)		0	0	0	0	0	0	0	0	0	1	1
Del(17q11.2)		0	0	0	0	0	0	0	0	0	1	1
del(20q)		0	0	0	0	0	2	1 (additional de	0	0	1	4
Del(5q13.2)		0	0	0	0	0	0	0	0	0	1	1
dic(17;20)		1	0	0	0	0	0	0	0	0	0	1
NormalArray		0	0	0	1 (abnormal	0	0	0	0	1	10	12
Total		1	1	1	1	3	2	1	1	2	15	28



P121. Spontaneous improvement of cytopenias in a patient with myelodysplastic syndrome with complex cytogenetics

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Cytogenetic abnormalities have important prognostic significance in patients with Myelodysplastic Syndrome (MDS) and complex cytogenetics have been shown to confer a very poor prognosis. We report a case of spontaneous improvement of cytopenias in a patient with myelodysplastic syndrome with complex cytogenetics.

A 67 year-old woman presented with one month's history of general lethargy and easy bruising. She has a background of breast cancer 17 years prior for which she underwent a lumpectomy followed by radiotherapy. She did not receive chemotherapy but regularly consumed colloidal silver as a supplement. Initial full blood count at presentation demonstrated pancytopenia with haemoglobin level of 91 g/L, total white cells of 3.1×10^9 /L and platelet count of 4×10^9 /L. Bone marrow examination revealed a hypocellular marrow with dyserythropoiesis and dysgranulopoiesis with a blast population of 8%, consistent with refractory anaemia with excess blasts-1 (RAEB-1). Cytogenetic studies showed multiple abnormalities including del(5q), del(7q), i(17q) and trisomy 8. Revised IPSS score was 8, consistent with very high-risk disease with a median overall survival of 0.8 years. As she declined chemotherapy with Azacitadine, she was provided transfusion support and advised to cease consuming colloidal silver. Her blood counts were noted to have gradually improved without treatment over the following three months. On the fifteenth week after her diagnosis, her haemoglobin was 121 g/L, white cell count was 4.5×10^9 /L and platelet count was 106×10^9 cells/L in absence of any transfusion support for eight weeks. A repeat bone marrow examination has been organised on a future date for prognostic purpose.

We postulate that her cytopenia on peripheral blood and dysplastic morphologic picture on marrow aspirate were a result of silver toxicity. Although the phenomenon of spontaneous blood count recovery could be explained by the cessation of colloidal silver use, the complex cytogenetic changes are unlikely to resolve and their prognostic significance in this case remains uncertain. As cytogenetic abnormalities are given increasing weight in predicting prognosis in MDS, further study into their relevance in this case would be of value.

P122. The Impact of Autophagy on the Progression of the Myelodysplastic Syndromes

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Background

75% of myelodysplastic syndromes fall into the lower risk categories with no cure and only supportive management with blood transfusions with the complication of iron overload requiring iron chelation. A number of studies including ours have demonstrated that the iron chelator, Deferasirox (DFX) additionally improved haemoglobin levels in a subset of MDS patients. We went on to demonstrate that DFX blocked growth of myeloid leukaemia cell lines, sparing normal stem cells.

Iron chelation is thought to degrade ferritin via autophagy, a catabolic cellular recycling pathway clearing redundant and damaged organelles to sustain cellular metabolism. Autophagy initiated by the Atg1-Atg13 protein complex and can be upregulated in cancer facilitating the propagation of the malignant clone.

Aims

1. To demonstrate that enhanced levels of autophagy are driving disease progression.
2. To explore the impact of iron modulation on autophagy.

Methods

The human monocytic cell line (THP-1) expressing the autophagy reporter LC3-GFP was treated with DFX in the presence/absence of chloroquine (to visualise autophagy) and analysed using Flow Imaging.

Acute myeloid leukaemia cell lines were treated with DFX in the presence/absence of chloroquine and subjected to Western blotting using anti-LC3-II antibody.

CRISPR/Cas9 gene editing was used to delete essential autophagy genes, ATG 5 and 7 in THP-1 cells which were then subjected to cell proliferation assays and Western blotting using ATG5, 7 and LC3 antibodies.

Results

Reduced cell growth was seen in ATG5/7 deleted cells. Inhibition of autophagy with DFX was demonstrated with flow imaging in the chloroquine plus DFX treatment in comparison to chloroquine only, as well as with Western Blotting that demonstrated lower LC3b II levels, also in the DFX and chloroquine treatment.

Conclusion

The impact on cell growth demonstrated the role of autophagy in cell proliferation. The inhibition of cell growth and proliferation with DFX is through autophagy and may have an impact on disease progression.

P123. Multiple Mutations In The Same Gene Is Associated With Clonal Diversity And Poor Prognosis In MDS

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Aim

To identify and determine the frequency of multiple somatic mutations in patients with primary MDS by next generation sequencing.

Method

Targeted massively parallel (TMP) sequencing on a custom 29-myeloid gene panel (coding regions) was performed on 142 MDS bone marrow (BM) samples using Illumina HiSeq2500. SNP-A was also performed on 138/142 BM samples.

Results

BM cytogenetics and SNP-A karyotype was normal in 57% and 42% of MDS cases respectively. However, TMP sequencing revealed at least one mutation in 82% of cases. Mutations in the spliceosome complex were detected in 44% of MDS cases, with *SF3B1* mutation being most frequent. Overall survival (OS) was better in patients with *SF3B1* mutations and poorer in patients harbouring *U2AF1*, *NRAS*, and *KRAS* mutations.

Multiple mutations in several genes were found in 64% of the cases. Importantly, of those with *TET2* mutations (n=48), multiple mutations were detected *in the same gene* (60%). Multiple mutations within the same gene were also observed in other genes (Figure 1A). Somatic variants were confirmed in matched germline control samples (MSC and hair) for 22 cases by Sanger sequencing. In patients harbouring three *TET2* somatic variants, variant allele load (VAL) was lower for one mutation compared to the other two suggesting sub-clonal evolution of the third variant. In cases harbouring two *TET2* somatic variants, VAL of both variants was similar in most cases, suggesting a biallelic clonal origin (Figure 1B). Compared to single *TET2* mutations, patients with multiple *TET2* mutations have more severe cytopenias and tend to have shorter OS. Interestingly, patients with multiple *TET2* mutations also have fewer mutations in other genes (Figure 1C).

Conclusions

TMP sequencing detected mutations in 82% MDS cases. This report demonstrates multiple mutations in the same gene. Multiple mutations in *TET2* are associated to severe cytopenias and fewer mutations in other genes.

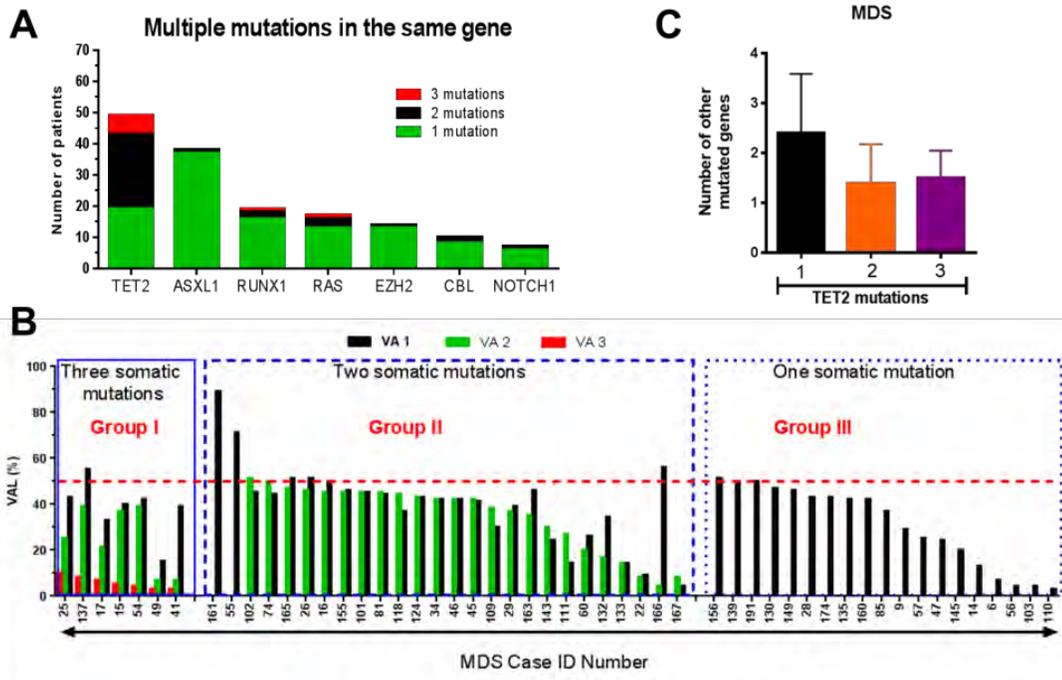


Figure 1. Multiple mutations in the same genes
 A) Multiple mutations in TET2, ASXL1, RUNX1, EZH2, CBL, RAS and NOTCH1,
 B) VAL of TET2 mutations in MDS, and
 C) number of mutations in other genes for cases with single and multiple TET2 mutations.

P124. Patient and disease characteristics of myelodysplastic syndromes in Singapore

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Aim

There is a lack of national registry data on myelodysplastic syndromes (MDS) in Singapore. We aim to describe the baseline patient and disease characteristics of MDS in our patients.

Method

In our institution, we established a hospital-based MDS registry to collect clinical data using an online electronic database application. From January 2008 to December 2015, we have identified 176 patients who were diagnosed to have MDS in our institution, according to the 2008 World Health Organization (WHO) classification. Their baseline disease and clinical data were extracted from the clinical records and stored in the MDS registry.

Results

In this cohort of 176 MDS patients, the median age at diagnosis was 68 years (range 17-86). There were 105 males and 71 females (M:F ratio approximately 3:2). These patients were Chinese (83.5%), Malay (9.7%) and Indian (6.8%). The WHO categories were refractory cytopenia with unilineage dysplasia (RCUD) (11.4%), refractory anaemia with ring sideroblasts (RARS) (5.1%), refractory cytopenia with multilineage dysplasia (RCMD) (43.2%), refractory anaemia with excess blasts, type 1 (RAEB-1) (18.8%), RAEB-2 (17.6%), MDS with del(5q) (3.4%) and MDS, unclassifiable (0.6%). Cytogenetic risk categories were very good, good, intermediate, poor and very poor in 5.1%, 56.3%, 15.9%, 5.1% and 17.6% respectively. The revised international prognostic scoring system (IPSS-R) risk groups were very low, low, intermediate, high and very high in 11.4%, 29.5%, 20.5%, 17.6% and 21% respectively. Disease progression and transformation to acute myeloid leukaemia (AML) occurred in 29.5% and 20.5% of this cohort.

Conclusion

Nearly half of our MDS patients had higher-risk MDS at the time of diagnosis. In this higher-risk group, beside best supportive care, disease-modifying treatment such as hypomethylating agents and/or allogeneic stem cell transplant (for eligible patients) should be suggested as the upfront treatment in the current standard practice.

P125. Hypereosinophilia: Three Divergent Presentations

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Aim

To describe three distinct cases of eosinophilia demonstrating the heterogeneity of clinical presentation and pathophysiology.

Method

Clinical presentation, investigation and management data were obtained from hospital medical records.

Results

Case 1: A 76 year-old female presented with severe neutropenia ($0.09 \times 10^9/L$). Bone marrow biopsy showed a cellular marrow with megakaryocytic hyperplasia and dysplasia, absent neutrophils and marked eosinophilia (35% of nucleated cells). BCR-ABL and FIP1L1-PDGFR α were negative. CSF3R and ASXL1 mutations were detected. Favoured diagnosis of myeloproliferative neoplasm with eosinophilia or myelodysplastic/myeloproliferative neoplasm.

Patient was treated with regular G-CSF, however after 3 years she became unresponsive to G-CSF and developed marked thrombocytosis ($1700 \times 10^9/L$) and peripheral eosinophilia ($1.75 \times 10^9/L$). Repeat biopsy showed increased bone marrow blast count (8%). Thrombocytosis responded to cytoreduction and patient has remained asymptomatic throughout.

Case 2: A 68 year-old man presented with severe anaemia (Hb 57g/L), peripheral eosinophilia ($12 \times 10^9/L$) and leucoerythroblastic blood film, without splenomegaly. Bone marrow biopsy showed granulocytic and megakaryocytic hyperplasia and moderate fibrosis. Cytogenetics revealed trisomy 19 and 21. FIP1L1-PDGFR α mutation was not detected, thus allowing classification as chronic eosinophilic leukaemia. The patient subsequently developed myocardial infarction, acute kidney injury and multiple brain infarcts, attributed to hypereosinophilia-related end-organ damage. The eosinophilia resolved with prednisolone and hydroxyurea however the patient remains transfusion-dependent.

Case 3: A 24 year-old man with Crohn's disease (inactive at presentation), presented with haematuria and marked peripheral eosinophilia ($77 \times 10^9/L$). He subsequently developed warm autoimmune haemolytic anaemia. Bone marrow biopsy showed marked eosinophilia. All other relevant investigations were non-diagnostic, thus allowing classification as idiopathic hypereosinophilic syndrome. Symptoms resolved with prednisolone and patient remains well following steroid cessation.

Conclusion

These cases highlight the wide range of clinical presentations of hypereosinophilia. Diagnosis can often be challenging, as aetiologies are extensive. Overlapping features may preclude specific diagnosis, while some are diagnoses of exclusion.

P126. Pitfalls of CALR Mutation Testing: Is There Still a Role for Bone Marrow Biopsy in the Diagnosis of Myeloproliferative Neoplasms?

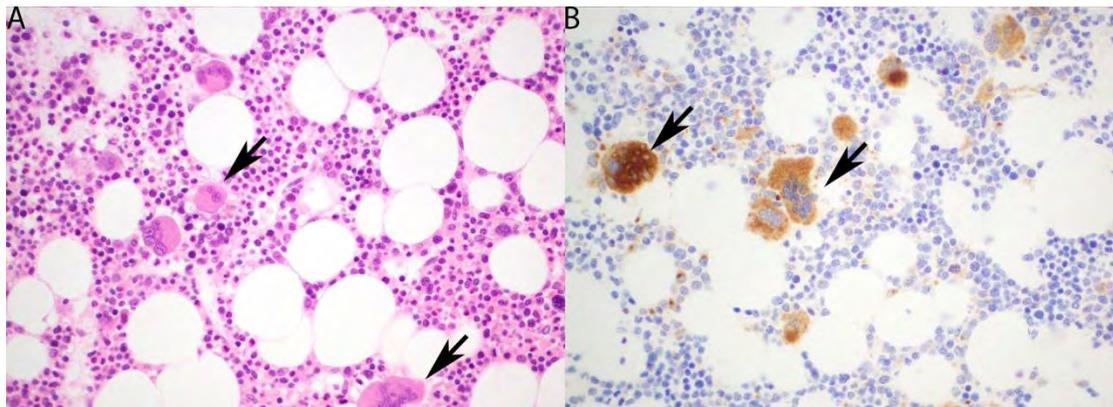
Cromer N, Koleth M, Lane J, Buss T, Lee L, Gill A, Stevenson W

Royal North Shore Hospital

Aim
Peripheral blood genetic testing of *JAK2*, *MPL* and *CALR* is increasingly used to diagnose myeloproliferative neoplasms (MPN) with the avoidance of a bone marrow biopsy. *CALR* is a recently identified mutation in these disorders that appears to alter JAK-STAT signalling and promote megakaryocyte proliferation in the bone marrow. Interestingly, the reported mutations in *CALR* are all indels that cause specific changes to the carboxyl terminal of *CALR* causing a gain of function interaction with the thrombopoietin receptor (*MPL*). It has been suggested that different indel sizes have prognostic significance and accurate sizing of amplicons is an important feature of the assay. We aimed to accurately diagnose *CALR* mutations via molecular and immunohistochemical methods including accurate sizing of the mutation via molecular methods.

Method
We have developed a sensitive PCR-based fragment analysis assay to detect indels in the *CALR* gene as well as an immunohistochemistry assay (IHC) that identifies mutant megakaryocytes in bone marrow. 105 samples with suspected MPN which were *JAK2* negative were studied.

Result
27 *CALR* mutated cases were identified including 13 cases of 52bp deletion, 9 cases of 5bp insertion and small numbers of 31bp del, 34bp del, 19bp del and 1 bp del. Three of these indels are novel to the reported literature and have been confirmed with sanger sequencing. Problems with the PCR-based assay include validation of indels that fall outside the normal size markers and small clones that show minimal amplification compared to wild-type sequence. We have used IHC as a second diagnostic technique to both validate novel mutations, demonstrating that they confer a mutant protein phenotype, as well as identify small numbers of mutant megakaryocytes on a background of non-MPN haematopoietic cells.



Serial H&E (A) and calreticulin IHC (B) stained sections of the bone marrow trephine. The megakaryocytes (arrows) are significantly increased and show some clustering and increased nuclear complexity. The megakaryocytes demonstrate diffuse strong positive immunohistochemical staining for calreticulin. (Original magnifications 400x).

Conclusion
The combination of PCR based testing on peripheral blood and IHC of bone marrow specimens provides better diagnostic accuracy than molecular tests alone and bone marrow biopsy may still be indicated in small numbers of patients with diagnostic complexity.

P127. Use of Ruxolitinib in a Patient with Myelofibrosis with Severe Thrombocytopenia

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Aim and Method

Ruxolitinib is usually not indicated in patients with platelet count $< 50 \times 10^9/L$. Here we report the successful use of Ruxolitinib in a patient with myelofibrosis (MF) and severe thrombocytopenia.

Result

A 59 year-old indigenous woman was diagnosed with JAK-2 mutated MF in 2013 (low risk DIPSS at diagnosis) and initially treated with Hydroxyurea. She presented with marked anaemia (Haemoglobin 65g/L), thrombocytopenia (Platelet count $9 \times 10^9/L$) and distended abdomen caused by massive splenomegaly. Bone marrow biopsy did not reveal leukaemic transformation. She was started on Ruxolitinib 5mg twice daily. The platelet count gradually increased and 2 months later was $135 \times 10^9/L$. The dose of Ruxolitinib was increased to 10 mg twice daily when her platelet count reached $80 \times 10^9/L$. The splenomegaly gradually improved.

	25/02/16	06/03/16	17/03/16	26/04/16	24/05/16	31/05/16	28/06/16
Haemoglobin g/L	65	72	85	95	58	76	81
White cell count $\times 10^9/L$	2.9	2.3	3.5	12.7	33	15.2	26.9
Platelet $\times 10^9/L$	9	9	23	80	135	87	83

↑ ↑ ↑

Hydroxyurea Ruxolitinib Ruxolitinib
ceased 5mg BD 10mg BD

Conclusion

Although available data suggests that Ruxolitinib causes thrombocytopenia and therefore it is not recommended in patients with very low platelet count, our experience suggests that reduction in splenic volume with Ruxolitinib can eventually lead to rise in platelet count. We found that an initial dose of 5 mg twice daily can be safely used when platelet count is $< 50 \times 10^9/L$.

P128. The importance of accurate reporting in patients with potentially low JAK2 V617F allelic burden myeloproliferative neoplasms (MPNs)

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Aim

A collective group of haematopoietic stem cell disorders, polycythaemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are known as Philadelphia-negative MPNs. The JAK2 V617F mutation is a standard molecular diagnostic criterion, exceeding 95% of individuals with PV and seen in approximately 60% of those diagnosed with ET or PMF. This study aims to elucidate the significance of inconclusive results and to exclude low allelic burden of JAK2 V617F mutation.

Method

We have tested a cohort of 553 individuals for JAK2 V617F at Liverpool Hospital over a period of 18 months using a semiquantitative commercial kit. Twelve percent of cases were positive and 3% were borderline or inconclusive.

The test, using genomic DNA from peripheral blood, was repeated on recollected samples of initially inconclusive patients.

Subsequent retesting and Sanger sequencing was performed using granulocytic and T lymphocytic DNA.

Results

Quantification of PCR product to generate genotype results is due to the calculated quantitation value ($\Delta\Delta C_T$) in a reaction where fluorescence is proportional to target amplification.

The majority of cases reported as inconclusive showed a negative $\Delta\Delta C_T$ value and on retesting were wild type.

Other samples were reported inconclusive twice, one with both positive $\Delta\Delta C_T$ values, two individuals with both negative $\Delta\Delta C_T$ values and one indicating a positive and negative $\Delta\Delta C_T$ value on different occasions. Sanger sequencing revealed no JAK2 V617F mutation.

These cases prompted cell sorting preceding Sanger sequencing to exclude potentially low allelic burden.

Conclusion

The elucidation of borderline results is pertinent to further patient care and molecular testing. Molecular testing of the calreticulin (CALR) gene exon 9 is appropriate only if an individual is JAK2 V617F negative.

Furthermore, low JAK2 V617F allelic burden has been increasingly associated with mutations in the thrombopoietin receptor (MPL) gene exon 10, necessitating accurate JAK2 V617F reporting for alternate clinical care.

P129. Masked Polycythaemia Vera is Genetically Intermediate between JAK2V617F Mutated Essential Thrombocythaemia and Overt Polycythaemia Vera

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Background

Polycythaemia vera (PV) is commonly defined by the presence of an elevated haemoglobin (Hb) or haematocrit (Hct), but it is recognized that 10-15% of PV patients have a normal haemoglobin, so-called 'masked' PV (mPV). This poses a problem for accurate diagnosis, and may lead to under-diagnosis and under-treatment of PV. We hypothesized that the *JAK2* clonal pattern of patients with mPV would resemble that of overt PV, and that this could have diagnostic utility for mPV.

Method

We compared the *JAK2* genotype in erythroid colonies of mPV ($n=17$) with overt PV ($n=20$) and essential thrombocythaemia (ET; $n=12$). We defined mPV as a *JAK2*-positive myeloproliferative neoplasm (MPN) with Hb below the 2008 WHO cut-off for PV and either: Hb above the sex-specific normal range; or Hb above the middle of the normal range despite iron deficiency; or bone marrow (BM) panmyelosis. Erythroid burst-forming units (BFU-E) grown from either peripheral blood (PB) or BM mononuclear cells were genotyped by single nucleotide primer extension assay for *JAK2V617F*. A total of 2984 colonies (28-127 per patient) were genotyped.

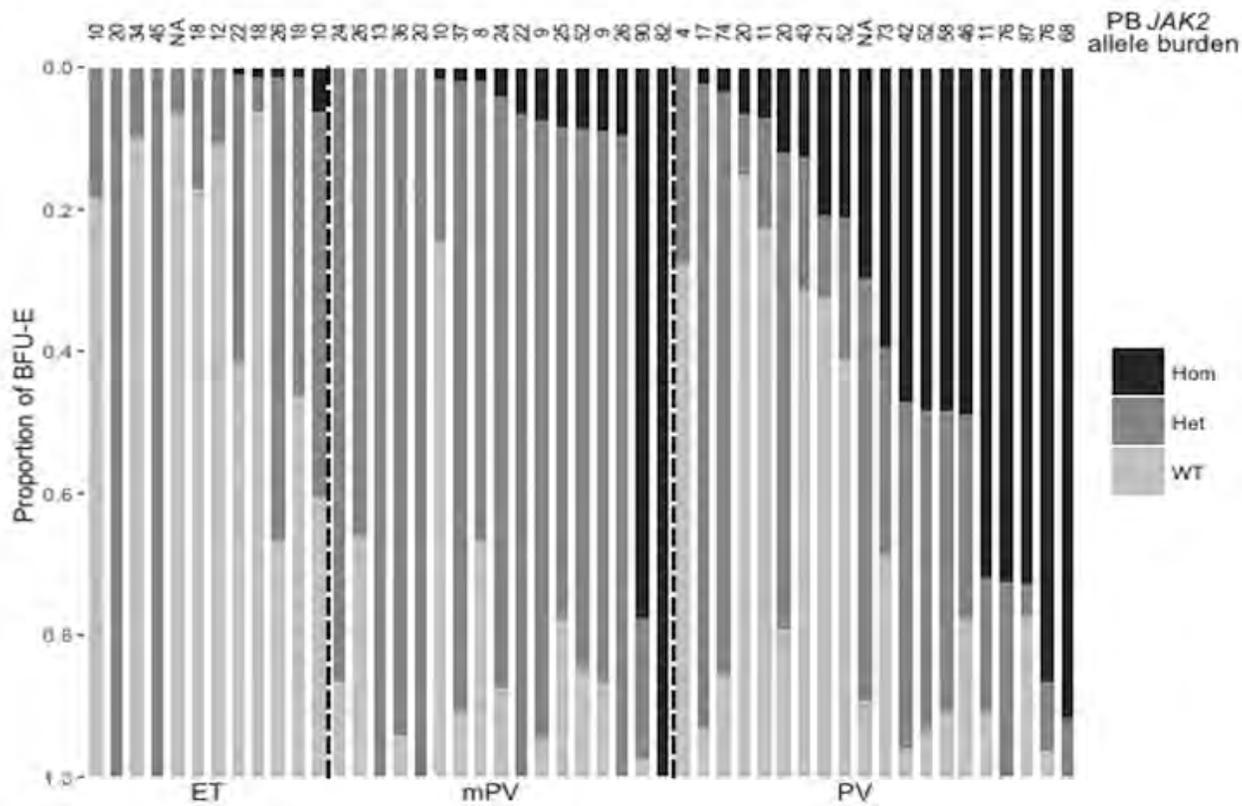
Result

The proportion of *JAK2* homozygous colonies increased from ET to mPV to overt PV; this correlated positively with erythrocytosis and negatively with platelet counts. Significant differences were observed in serum ferritin and uric acid concentrations, and a similar trend was seen for leukocyte counts, lactate dehydrogenase activity, and for PB *JAK2V617F* allele burden. An expanded ($\geq 20\%$) *JAK2* homozygous clone in mPV was relatively uncommon; only 2/17 mPV patients would have been re-classified based on this criterion. More common was dominance of a *JAK2* heterozygous clone.

Conclusion

These findings indicate that mPV is genetically as well as phenotypically intermediate between ET and PV, emphasizing the overlap between *JAK2*-positive MPN. Expanded *JAK2* homozygous clone has limited potential to clarify diagnostic ambiguity in mPV.

Figure: Distribution of *JAK2* genotypes in BFU-Es from patients with *JAK2*-mutated ET, mPV and overt PV. Each vertical bar represents a patient. PB *JAK2*V617F allele burden is shown above each bar. The mean number of colonies genotyped per patient was: ET, 60.4; mPV, 51.7; and overt PV, 69.



P130. Early Bortezomib failure predicts shorter PFS: a retrospective analysis from 4 Victorian centres

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Aim

To compare patients with NDMM bortezomib responsive and resistant MM (defined as <PR after 2 cycles) and assess the impact on clinical outcome particularly progression-free survival (PFS) and overall survival (OS).

Method

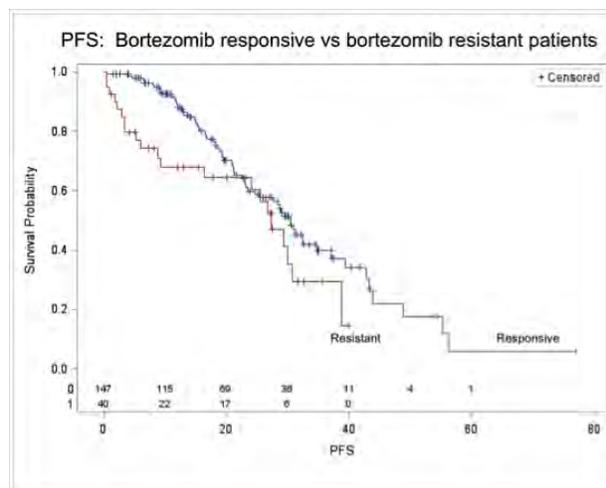
Retrospective review of all patients identified with NDMM treated with upfront bortezomib in four tertiary Victorian centres 01/01/2009-01/03/2016. Bortezomib responsiveness was defined as achievement of \geq PR after completing 2 cycles of any bortezomib containing therapy (total eight doses of bortezomib). Prior radiotherapy was allowed. Data were analysed via log rank tests for PFS and OS using SAS software. Predictors of bortezomib resistance were assessed by chi-square analysis.

Result

A total of 187 eligible patients were included (147 bortezomib responsive and 40 bortezomib resistant patients). Bortezomib resistance was associated with shorter median PFS, 27.4 (95%CI: 16.3, 30.8) vs 30.4 (95%CI: 23.4, 37.1) months (log-rank $p=0.05$) with a median potential follow up of 27.4 vs 29.7 months in the resistant and responsive groups respectively. Estimated PFS at 12 months was 68.0% (95%CI: 50.3%, 80.5%) vs 88.3% (95%CI: 81.2%, 92.8%) and, at 24 months, 60.4% (95%CI: 41.7%, 74.7%) vs 60.0% (95%CI: 49.9%, 68.6%) in the resistant and responsive groups respectively, indicating non-constant hazard in the resistant group (see figure). Bortezomib resistance was not associated with higher ISS (ISS3 23% vs 34%, $p=0.17$), high risk cytogenetics (17p del or t(4;14)) (16% vs 16%, $p=0.99$), MM subtype ($p=0.63$) or length of bortezomib cycle (21 vs 35 day $p=0.74$). Maintenance therapy appears to overcome the adverse prognostic risk of bortezomib resistance ($p=0.06$). OS data is not mature (median OS not reached in either group).

Conclusion

Bortezomib resistance is a poor prognostic factor and is associated with a shorter PFS. Consideration of early therapy change, maintenance therapy and/or referral for assessment for allograft in eligible patients appears to be warranted.



P131. Health-Related Quality of Life (HRQoL) Over Time in Patients With Newly Diagnosed Multiple Myeloma (NDMM) Who Were Not Eligible for Transplant and Treated With Lenalidomide and Dexamethasone (Rd) Until Progression

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Aim

This analysis aimed to examine the effect of Rd until progression on HRQoL beyond 18 months by identifying predictors of change in HRQoL scores using data from the FIRST trial. The FIRST trial established a PFS and OS benefit of Rd until progression vs melphalan, prednisone, and thalidomide in transplant-ineligible patients with NDMM (Benboubker et al, 2014) as well as improved HRQoL over the first 18 months of Rd treatment (Delforge et al, 2015).

Methods

Univariate linear mixed-effects regression analyses (n=535) identified variables collected until progression as predictors of 7 predefined HRQoL domains: EORTC QLQ-MY20 (disease symptoms [DS], side effects of treatment [SE]); EORTC QLQ-C30 (global QoL [GQ], physical function [PF], fatigue [F], pain [P]); and EQ-5D (health utility [HU]). Variables with univariate $P < .1$ were combined into multivariate models for each domain; variables with $P < .1$ were retained for final models. Modeled values were validated against observed values prior to 18 months and extended to extrapolate HRQoL changes from baseline based on observed predictor values beyond 18 months for patients still on Rd treatment at 24, 30, 36, 42, and 48 months.

Results

Key time-varying determinants of final mixed models included ECOG PS (all domains), serum albumin (GQ, PF, F, P, SE, HU), RBC transfusion within 30 days (F, P, DS), adverse events (GQ), grade ≥ 3 pain (PF, F, P, DS, SE, HU), grade ≥ 2 fatigue (F), grade 3/4 anemia (PF, SE), and hospitalization (GQ, F, SE, HU). Nearly all changes from baseline fell within the 95% CI of the final predictive models.

Conclusions

The gains in HRQoL over the first 18 months of Rd therapy were maintained through 48 months of treatment. Further research is necessary to determine the impact of Rd on HRQoL compared with a fixed duration of therapy followed by a period of observation.

Financial support for study form Celgene Corporation

P132. Minimal residual disease (MRD) monitoring by multicolour flow cytometry and post-treatment reconstitution of normal plasma cells in upfront and relapsed/refractory myeloma patients

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Background

MRD monitoring of myeloma by multicolour flow cytometry (FC) is an important prognostic factor in assessing upfront and subsequent treatment responses.¹ We retrospectively audited the degree of immunophenotypically normal plasma cell (PC) reconstitution, in addition to MRD, in two myeloma studies encompassing upfront and relapsed/refractory cohorts.

Methods

Patients were identified from:

The LITVACC study (frontline lenalidomide/dexamethasone, autograft, lenalidomide maintenance, adjuvant dendritic cell vaccination)

A phase 1b study of venetoclax/bortezomib in relapsed/refractory patients, following a median of 3 prior therapies [range 1-10].

Data were collected from FC analysis of trial-mandated bone marrow samples at timepoints specified below, using a validated assay (10⁻⁴ sensitivity).²

Results

After follow-up months 16 (44%) in

LITVACC (n=36)		
	Patients with normal PCs	Median % normal PCs
Baseline	3 (8%)	15 (all 3)
Post-induction	3 (8%)	55 [27-70]
1 month post-autograft	19 (53%)	70 [7-100]
Post-cycle 4 maintenance	22 (61%)	93 [15-100]

median of 68 [55-94], remained remission

(13 CR, 2 VGPR, 1 PR) – 8 MRD-negative and 9 demonstrating normal PC reconstitution (p=0.73).

VENETOCLAX/BORTEZOMIB (n=24)		
	Normal PCs at baseline (n=12)	No normal PCs at baseline (n=12)
% normal PCs – baseline	Median = 26.5 [1-100]	-
Patients with normal PCs post-cycle 4	7 (58%)	4 (33%)
% normal PCs post-cycle 4	Median = 100 [71-100]	Median = 30.5 [1-100]
Ongoing responders (median follow-up 12 months [5-30])	7 (58%) – all had further normal PC reconstitution after 4 cycles	6 (50%) – including 3 with normal PC reconstitution
Depth of ongoing responses	4 sCR, 2 VGPR, 1 PR	1 CR, 4 VGPR, 1 PR

Conclusion

High rates of normal PC reconstitution were seen in the frontline LITVACC cohort. Surprisingly, this was also observed in the relapsed/refractory venetoclax/bortezomib cohort, possibly reflecting depth of response and this regimen's biological activity. Larger studies are needed to clarify the significance of this phenomenon.

P133. Autophagy: Double-Edged Sword in Multiple Myeloma

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Two contemporaneously derived human myeloma cell lines (HMCL) [TK-1 (bone marrow), TK-2 (peripheral blood)] from a patient with primary plasma cell leukaemia provide a model of disease progression. Preliminary characterisation suggests upregulation of autophagy, a pro-survival pathway implicated in tumour growth and chemoresistance, in TK-2 compared to TK-1.

Aim

To further characterise differences in autophagy in a model of disease progression. To explore the role of autophagy induced resistance to bortezomib in MM.

Method

HMCLs (TK-1, TK-2) were monitored for growth for 2 weeks followed by p62 and LC3 immunoblots at baseline and hydroxychloroquine treated. Gene expression levels of p62 was validated using qRT-PCR and cells were imaged using confocal fluorescence microscopy [DAPI (nuclear stain), p62 and LC3]. Cell viability in response to bortezomib treatment (50nM to 1000nM), with and without addition of hydroxychloroquine (40µM), was examined by flow cytometry (propidium iodide) at 24, 48 and 72hrs.

Results

TK-2 proliferated at a greater rate than TK-1. Immunoblotting demonstrated 7-fold greater baseline p62 expression in TK-2 compared to TK-1 ($p=0.014$). Turnover of LC3, measured by immunoblot in the presence and absence of hydroxychloroquine, was almost 3-fold greater in TK-2 compared to TK-1 ($p=0.045$). Confocal microscopy demonstrated greater co-localisation of LC3 and p62 in TK-2 compared to TK-1. Autophagic flux was 3-fold greater in another model, KMS-12PE compared to KMS-12BM. Despite differences in baseline autophagy, TK-1 and TK-2 demonstrated similar sensitivity to bortezomib. Pharmacological autophagy inhibition with hydroxychloroquine resulted in an additive but not synergistic effect on cell death.

Conclusion

Upregulation of autophagy in extramedullary derived HMCLs compared with marrow derived HMCLs may represent an important event in MM disease progression. We found that inhibition of autophagy did not synergise with bortezomib, suggesting that autophagy may not be implicated in mediating bortezomib resistance, in contrast to published data. This warrants further investigation.

P134. The impact of up-front treatment with the proteasome inhibitor bortezomib in newly diagnosed patients with multiple myeloma: an update

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Background

The introduction of the proteasome inhibitor bortezomib has led to significant improvements in survival outcomes in both primary and relapsed multiple myeloma in several clinical trials.

Aims

To investigate in a 'real-world analysis' how the introduction of bortezomib in induction therapy has impacted upon overall response rates, tolerability and toxicity in patients with multiple myeloma since its inclusion on the PBS for newly diagnosed patients, as compared to the previous 'standard of care' thalidomide.

Methods

A retrospective analysis was undertaken of newly diagnosed patients with multiple myeloma treated with either thalidomide (pre-October 2012) or bortezomib (post-October 2012) containing induction regimens at Monash Health.

Results

One hundred and fifty-two patients aged 36-89 with newly diagnosed multiple myeloma were included in the study, including 59 patients who received thalidomide-based induction (mean age 70.4 years, 35.6% female) and 93 patients who received bortezomib-based induction (mean age 66.9 years, 46.2% female). Our preliminary data demonstrates that induction regimens containing bortezomib resulted in a greater than two-fold increase in the number of patients who achieved a very good partial response or better, 41/93 (44.1%) of patients post bortezomib vs 12/59 (20.3%) of patients post thalidomide ($P=0.003$) (Fig 1). This was found to be significant independent of transplant eligibility. However, no significant differences in overall or progression free survival have been seen to date, with a median follow-up of 19 months and 45 months for patients receiving thalidomide and bortezomib respectively.

Conclusion

Bortezomib based regimens produce deeper responses to thalidomide based regimens in induction therapy for multiple myeloma irrespective of transplant eligibility. However currently, with a median follow-up of 19 and 45 months respectively, this has not translated into statistically significant differences in survival outcomes.

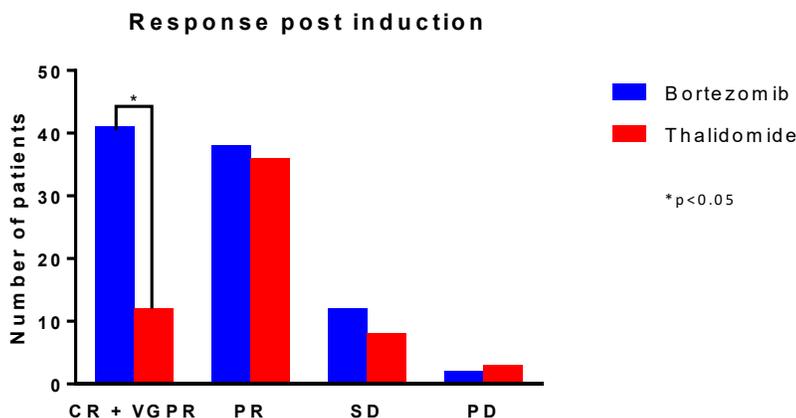


Figure 1: CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease.

P135. Prognostic significance of serum free light chain and paraprotein levels in patients with multiple myeloma receiving Bortezomib

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Background

The introduction of the serum-free light-chain (sFLC) assay in 2001 has enhanced the monitoring of myeloma. sFLC assay is well validated as a serum biomarker for real-time monitoring of response to treatment as well as disease progression. This is possible owing to the short physiological half-life of sFLC in blood (2-6 hours). However the role of sFLC assay in prognosis and risk stratification is not well defined.

Aims

The aim of this study was to examine the prognostic significance of baseline sFLC levels and rate of reduction of sFLC levels, as well as baseline paraprotein levels and rate of fall of paraprotein during therapy in multiple myeloma.

Methods

In this retrospective study, patients diagnosed with multiple myeloma between January 2014 to January 2016 at Fiona Stanley Hospital, Royal Perth Hospital and Fremantle Hospital were collated using medical record coding criteria. To minimize treatment bias, we narrowed the group to newly diagnosed multiple myeloma receiving Bortezomib based induction chemotherapy, leaving a total of 60 patients.

Paraprotein and sFLC levels at baseline and post each cycle of treatments were collected. The longitudinal data were analysed against progression-free survival (PFS) on cox regression analysis model.

Results

We found that neither baseline sFLC nor rate of reduction of sFLC levels correlated with PFS.

We observed that paraprotein is significantly associated with PFS. The level at diagnosis is not statistically significant ($p=0.132$). There is a statistically significant association between the level of paraprotein over time and PFS with a hazard ratio <1 ($HR=0.998$, $p=0.042$). There is also a fall in risk of progression with increasing cycle number ($HR=0.320$, $p=0.031$) which may also reflect the fall in paraprotein level over time.

Conclusion

Based on our study, sFLC levels do not appear to have prognostic significance, however the low number of light chain only myeloma patients may under-power this analysis. We could however demonstrate that reduction in paraprotein during treatment is associated with improved PFS.

P136. Case study: Heavy chain paraproteinemia

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Aim

1. To present a case study of a patient with heavy chain disease from an Immunology laboratory perspective.
2. To describe how the paraprotein was monitored using multiple methodologies.
3. To give examples of the gels and other methods used

Method

The patient was initially diagnosed with cold haemagglutinin disease (CHAD) in 2001. Serum protein electrophoresis and immunoelectrophoresis was requested as part of the work-up.

Within the laboratory, a heavy chain paraprotein was identified, quantified and then monitored by three methodologies.

The first method was performed by an automated capillary electrophoresis system. This method allows detection and quantitation of abnormal bands.

The second used an instrument called the 'SPIFE' which is an electrophoresis quick-gel chamber. This method involves a two-stage procedure - proteins are first separated on a high-resolution agarose gel by electrophoresis. Antisera is then added allowing an immunoprecipitate to form. Staining then visualises the band.

The third methodology employed is the technique of Isoelectric focusing. This also involves electrophoresis, but instead of the separation being carried out at a constant pH, the separation is carried out in a pH gradient. The proteins migrate until they align themselves at their isoelectric point.

Results

The first abnormal result was detected in 2004 which was an IgG heavy chain band. The patient was diagnosed with heavy chain disease in 2005. The above methods were employed at various times over the years to monitor the paraprotein size. The patient had a moderate increase in the paraprotein quantitation and additional abnormal monoclonal bands were found and typed. This is illustrated by gel scans and printouts from the instruments.

Conclusion

Our laboratory enabled the clinician a better overview of the patient's Gamma heavy chain disease process, enabling improved patient management.

P137. A proposed pharmacogenetic mechanism for cauda equina syndrome due to intrathecal chemotherapy: a case report in CNS myeloma

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Central nervous system (CNS) involvement with myeloma is a rare complication of plasma cell dyscrasias and is managed with multi-dosing intrathecal chemotherapy in combination with systemic anti-myeloma chemotherapy. Although intrathecal chemotherapy has previously been associated with cauda equina syndrome in acute leukaemia, this has not been reported in the setting of multiple myeloma. We report here a case of a 62-year-old gentleman with progressive CNS myeloma who received intrathecal chemotherapy with cytarabine, methotrexate and hydrocortisone in conjunction with systemic chemotherapy. After receiving five intrathecal administrations, he developed irreversible cauda equina syndrome. A diagnosis of a toxic axonopathy was established on nerve conduction studies and confirmed by nerve root biopsy. Testing for methylene tetrahydrofolate reductase (MTHFR) mutations revealed a heterozygote polymorphism at C677T that has previously been associated with increased toxicity to systemic methotrexate but not to intrathecal methotrexate*. We thus propose a potential pharmacogenetic mechanism for this rare complication of intrathecal chemotherapy.

P138. Targeting the ras-MAPK in multiple myeloma with small molecule inhibitors

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Introduction

Multiple myeloma is a molecularly heterogeneous disease. Activating mutations in the Ras-Mitogen Activate Protein Kinase pathway (Ras-MAPK) are abundant with a frequency in symptomatic disease over 50%. These mutations operate as secondary drivers and are thought to play a significant role in disease progression. This provided the rationale to investigate inhibition of the Ras-MAPK pathway with two small molecule inhibitors: (i) Trametinib, a MEK inhibitor and (ii) Rigosertib, a Ras binding domain inhibitor.

Methods

We tested Trametinib and Rigosertib on a panel of molecularly distinct human myeloma cell lines (HMCLs) and mouse E μ -Myc lymphomas harbouring Ras mutations (Ras mutated), FGFR3 over-expressers without Ras mutation (FGFR3 +ve, leading to signalling through the Ras-MAPK) or having neither a Ras mutation nor FGFR3 over-expression (wt). Analysis of proliferation, apoptosis, and the effects on down-stream ERK and phospho-ERK signalling was undertaken.

Results

Treatment of HMCLs and E μ -Myc lymphomas with Trametinib inhibited proliferation in Ras mutated lines and down-regulated phospho-ERK without causing apoptosis. No effect on proliferation or apoptosis was observed in the FGFR3 +ve or in wt HMCLs. In contrast, treatment with Rigosertib resulted in significant cell death in all three HMCL groups, with the FGFR3 +ve showing the greatest cell death followed by Ras mut then wt. Both Ras wt and mutant E μ -Myc lymphomas were sensitive to apoptosis induction by Rigosertib, which was abrogated by Bcl2 overexpression, but independent of p53 loss. Treatment of Ras mutated HMCLs with Trametinib in combination with Dexamethasone resulted in marked synergistic apoptosis, suggesting Ras-activation mediates dexamethasone resistance.

Conclusion

Recent therapeutic advances permit targeting of canonical Ras signalling directly (e.g. Rigosertib) or via downstream effectors (e.g. Trametinib). The relative cytotoxicity of direct anti-Ras inhibition with Rigosertib suggests this may be more effective than downstream MEK inhibition in Ras mutated or FGFR3+ve diseases.

P139. MGUS and Congo red do not equal systemic AL amyloidosis – the importance of excluding TTR with mass spectrometry in the over 75s

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Introduction

Amyloidosis is a disease whereby misfolded proteins deposit in extracellular spaces, resulting in end-organ dysfunction. Treatment is dictated by the protein that has misfolded. We present three cases of transthyretin amyloidosis (TTR) that were misdiagnosed as AL amyloidosis (AL) and highlight bone scintigraphy and mass spectrometry in older patients.

Case 1: Mr RS, 77, presented with dyspnoea. His troponin I was elevated (0.1). Echocardiography suggested myocardial infarcts. Five months later, he re-presented with proximal myopathy and autonomic dysfunction. A muscle biopsy showed eosinophilic thickened vessel walls with Congo red positivity. Kappa light chains were 1014 mg/L. NT proBNP was 627 pmol/L. He was treated with CTD for 6 months to PR. However, mass spectrometry later confirmed TTR and treatment changed to Diflunisal.

Case 2: Mr MB, 82, presented with two years of dyspnoea, weight loss, abnormal liver function. NTproBNP was 2271 pmol/L, echocardiography showed left ventricular hypertrophy and pericardial effusions. He had an IgA lambda 5g/L, lambda light chains of 108mg/mL, thus suggesting AL. However, technetium bone scan showed marked cardiac uptake, suggesting TTR. Chemotherapy was withheld and cardiac biopsy arranged. Mass spectrometry confirmed TTR. He now receives Diflunisal.

Case 3: Mr SD, 77, presented with four years progressive dyspnoea. He was treated as ischaemic heart disease, but red flags were anorexia, early satiety and bilateral carpal tunnel releases thus amyloidosis was considered. NTproBNP was 380 pmol/L, IgG Kappa paraprotein 6g/L. Thought to be AL, he was treated with CVD. This was ceased when mass spectrometry revealed TTR and treatment changed to EGCG.

Discussion

These cases illustrate the importance of confirming the amyloid subtype. Carpal tunnel syndrome, male sex and advanced age is suggestive of TTR, even with MGUS. Bone scintigraphy can help distinguish AL and TTR. International guidelines suggest mass spectrometry for patients over 75 years to guide appropriate therapy.

P140. Carfilzomib, Lenalidomide, and Dexamethasone (KRd) vs Lenalidomide and Dexamethasone (Rd) in Patients with Relapsed Multiple Myeloma (RMM) and Early Progression During Prior Therapy: Secondary Analysis from the Phase 3 Study ASPIRE (NCT01080391)

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Background

The ASPIRE trial demonstrated an improvement in median PFS for KRd compared with Rd in patients with RMM (26.3 vs 17.6 months, $P=0.0001$) (Stewart AK et al. *N Engl J Med.* 2015;372:142–52). In this post-hoc analysis of ASPIRE, we studied KRd vs Rd in patients with early disease progression in the first prior line.

Methods

The study design has been described previously. The following subgroups were analyzed: patients who relapsed ≤ 1 year from starting the first line of prior therapy (early relapsers) and patients who relapsed ≤ 1 year from the first prior transplant (post-transplant early relapsers).

Results

The subgroup of patients who relapsed ≤ 1 year from starting the first prior regimen included 87/396 patients in the KRd arm and 72/396 patients in the Rd arm. Median PFS for early relapsers was 24.1 months for KRd vs 12.5 months for Rd (hazard ratio [HR]: 0.75; 95% confidence interval [CI]: 0.50–1.13), and overall response rate (ORR) was 79.3% for KRd vs 61.1% for Rd (\geq complete response[CR], 21.8% vs 4.2%). The subgroup of patients who relapsed ≤ 1 year from first prior transplant included 48 KRd and 49 Rd patients. Median PFS for post-transplant early relapsers was 17.3 months for KRd vs 11.1 months for Rd (HR: 0.87; 95% CI: 0.54–1.41), and ORR was 83.3% for KRd vs 61.2% for Rd (\geq CR, 12.5% vs 4.1%). Grade ≥ 3 adverse events that occurred $\geq 5\%$ more frequently in KRd than Rd (in either subgroup) were hypokalaemia, neutropenia, febrile neutropenia, hypophosphatemia, and respiratory tract infection.

Conclusions

In this post hoc subgroup analysis, addition of K to Rd led to clinically meaningful improvements in PFS and ORR in RMM patients who had early progression following prior therapy (including transplant) and may have more aggressive disease.

This research was supported by Amgen Inc. The company assisted with Conception and design of Study; Analysis and interpretation of data and preparation of the abstract

P141. Carfilzomib and Dexamethasone (Kd) versus Bortezomib (BTZ) and Dexamethasone (Vd): Subgroup Analysis of the Phase 3 ENDEAVOR Study to Evaluate the Impact of Prior Treatment on Patients with Relapsed Multiple Myeloma (RMM)

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Background

In the randomized phase 3 ENDEAVOR study, Kd significantly improved median PFS versus bortezomib (BTZ) and dexamethasone (Vd) (18.7 vs 9.4 months; HR 0.53; 95% CI: 0.44, 0.65; p<0.0001) in 929 patients with RMM.

Methods

The study design has been previously described. This subgroup analysis evaluated treatment with Kd versus Vd in RMM patients after first relapse versus ≥ 2 prior lines of therapy as well as the effect of previous exposure to BTZ or lenalidomide (LEN).

Results

Outcomes by prior lines of therapy and by prior use of BTZ and LEN are shown in Tables 1 and 2, respectively. Grade ≥ 3 adverse events (AEs) were reported in 69.8% (Kd) and 63.9% (Vd) of patients with 1 prior line and 76.6% (Kd) and 69.9% (Vd) of patients with ≥ 2 prior lines. Grade ≥ 3 hypertension, dyspnoea, and cardiac failure were more common with Kd vs Vd. The rate of grade ≥ 2 PN was lower with Kd vs Vd in patients with 1 prior line (6.5% vs 30.0%; OR: 0.16; 95% CI: 0.09, 0.29) and ≥ 2 prior lines (5.6% vs 34.1%; OR: 0.12; 95% CI: 0.06, 0.22).

Conclusion

A clinically meaningful improvement in PFS was seen for patients with RMM who were treated with Kd compared with Vd, regardless of the number of prior lines of treatment. The improvement was greatest for those who had received 1 prior line, where median PFS was over 1 year longer in patients treated with Kd vs Vd. PFS benefit with Kd vs Vd was also maintained regardless of prior exposure to BTZ and LEN. A higher ORR was also observed with Kd vs Vd across these subgroups. Kd had an acceptable benefit-risk profile in this study.

This research was supported by Amgen Inc. The company assisted with Conception and design of Study; Analysis and interpretation of data and preparation of the abstract

Table 1:

Outcome	1 prior line		≥2 prior lines	
	Kd (n=232)	Vd (n=233)	Kd (n=232)	Vd (n=232)
Median PFS, months	22.2	10.1	14.9	8.4
HR Kd vs Vd (95% CI)	0.447 (0.330-0.606)		0.604 (0.466-0.783)	
Best overall response, n (%)				
Stringent complete response	6 (2.6)	6 (2.6)	2 (0.9)	3 (1.3)
Complete response	21 (9.1)	12 (5.2)	29 (12.5)	8 (3.4)
Very good partial response	117 (50.4)	53 (22.8)	77 (33.2)	51 (21.9)
Partial response	46 (19.8)	80 (34.5)	58 (25.0)	77 (33.0)
Minimal response	11 (4.7)	21 (9.1)	13 (5.6)	32 (13.7)
Stable disease	16 (6.9)	24 (10.3)	24 (10.3)	29 (12.4)
Progressive disease	6 (2.6)	15 (6.5)	19 (8.2)	16 (6.9)
Not evaluable	9 (3.9)	21 (9.1)	10 (4.3)	17 (7.3)
ORR, % (95% CI)	81.9 (76.3-86.6)	65.5 (59.0-71.6)	72.0 (65.7-77.7)	59.7 (53.1-66.0)
Median DOR, months	21.3	14.1	NE	10.3
Grade ≥3 AEs of interest, n (%) ^a				
Hypertension ^b	24 (10.3)	8 (3.5)	17 (7.4)	4 (1.7)
Dyspnoea ^b	12 (5.2)	5 (2.2)	13 (5.6)	5 (2.2)
Cardiac failure ^b	5 (2.2)	2 (0.9)	5 (2.2)	1 (0.4)
Acute renal failure ^b	2 (0.9)	3 (1.3)	7 (3.0)	2 (0.9)

^aIn the 1 prior line group, 232 (Kd) and 227 (Vd) patients were evaluable for safety; in the 2 prior lines group, 231 (Kd) and 229 (Vd) patients were evaluable for safety
^bPreferred term

Table 2:

Outcome	Prior BTZ (Kd vs Vd)		Prior LEN (Kd vs Vd)	
	Yes	No	Yes	No
Median PFS, months	15.6 vs 8.1	NE vs 11.2	12.9 vs 7.3	22.2 vs 10.2
HR Kd vs Vd (95% CI)	0.56 (0.44-0.73)	0.48 (0.36-0.66)	0.69 (0.52, 0.92)	0.43 (0.32, 0.56)
ORR, %	71.2 vs 60.3	83.6 vs 65.3	70.1 vs 59.3	81.2 vs 64.6
OR Kd vs Vd (95% CI)	1.63 (1.12-2.36)	2.72 (1.72, 4.31)	1.60 (1.03-2.49)	2.37 (1.62-3.47)

OR, odds ratio

P142. Carfilzomib and Dexamethasone (Kd) vs Subcutaneous (SC) Bortezomib (BTZ) and Dexamethasone (Vd) in Patients With Relapsed or Refractory Multiple Myeloma (RRMM): Secondary Analysis From the Phase 3 Study ENDEAVOR (NCT01568866)

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Background

SC administration of BTZ is non-inferior to IV in terms of efficacy while offering less peripheral neuropathy (PN) (Moreau, et al. Lancet Oncol 2011). Currently, most BTZ use in MM is SC. The phase 3 ENDEAVOR study which allowed use of either SC or IV BTZ demonstrated a significant improvement in PFS for Kd vs Vd in patients with RRMM.

Methods

A subset analysis of the efficacy and safety of Kd vs SC Vd in the ENDEAVOR study was performed in patients with RRMM (1-3 lines of therapy); the effect of prior exposure to BTZ was also investigated. Full study details have been previously published. Choice of BTZ route of administration (if randomized to Vd) was a stratification factor. This analysis compared Kd against Vd amongst patients for whom SC BTZ was selected pre-randomization.

Results

929 patients were randomized to Kd (n=464) or Vd (n=465); 360 Vd patients received SC BTZ. Among 464 Kd patients, 356 patients selected SC route of BTZ administration if randomized to Vd arm. Results are shown in the table. Median OS: not reached for Kd, 24.3 months for SC Vd (HR: 0.75; CI, 0.53–1.08).

Conclusion

Prolonged PFS was observed with Kd vs SC Vd. Higher response rates, a trend for prolonged OS, and lower rate of grade ≥2 PN were observed with Kd vs SC Vd. A similar pattern was observed in patients previously treated with BTZ. These results suggest that Kd has a favourable benefit-risk profile and delivers improved efficacy and clinical outcomes compared with SC Vd for RRMM.

	Kd (n=356)	SC Vd (n=360)	Prior BTZ	
			Kd (n=196)	SC Vd (n=203)
Median PFS, mo	Not reached	9.5	13.4	8.4
HR for Kd vs SC Vd (95% CI)	0.58 (0.46–0.72)		0.66 (0.50–0.87)	
ORR, % (95% CI)	76.1 (71.3– 80.4)	64.4 (59.3–69.4)	70.4 (63.5– 76.7)	62.1 (55.0–68.8)
Grade ≥2 PN rate, %	6.5	33.3	6.2	29.1
Odds ratio (95% CI)	0.139 (0.09–0.22)		0.160 (0.08–0.31)	
Grade ≥3 AEs ^a , %	74.4	67.5	71.8	64.5

^aSafety population was 355 (Kd) and 360 (SC Vd), and 195 (Kd) and 203 (SC Vd) in the prior BTZ group

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P143. Identification of long non-coding RNA and fusion transcripts in blood plasma-derived circulating cell-free RNA may facilitate non-invasive therapeutic monitoring in multiple myeloma patients

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Aim

Whole transcriptome RNA-sequencing (RNA-Seq) has emerged as a powerful tool for biomarker discovery. Utilising RNA-Seq for the interrogation of circulating cell-free RNA (cfRNA) may provide a novel non-invasive approach for molecular diagnostics and therapeutic monitoring. In this study, we evaluated whether RNA-seq of cfRNA could be used to identify patient-specific biomarkers for non-invasive therapeutic monitoring in multiple myeloma (MM).

Methods

Peripheral blood plasma (PL) was collected from 13 MM and 5 normal volunteers (NV) for cfRNA isolation, quantification and 100bp paired-end RNA-Seq. For 6 of 13 patients contemporaneously acquired bone marrow (BM) samples underwent CD138 enrichment and were also subject to RNA-Seq. Bioinformatic analyses included sequence alignment and transcript assembly using the human genome version 19 for long non-coding RNA (lncRNA) identification. Fusion transcripts were determined using the JAFFAv1.6 pipeline.

Results

Bioinformatic analyses revealed that a total of 7094 (of 13,870 genes) annotated as lncRNA were expressed. Multidimensional scaling plots showed that MM and NV samples clustered distinctly. Differential expression analyses comparing MM vs NV indicated that 433 lncRNA as downregulated (FDR \leq 0.05; fold change \geq 2) and 1,229 as upregulated (FDR=0.05; fold change \geq 2). Fusion transcript analyses for 6 matched BM and PL samples showed that only 1 NV had a fusion transcript detected, while a total of 12 fusion transcripts were detected in 4/6 MM samples. Paired analyses of BM MM and cfRNA in 4 MM patients with fusion transcripts revealed that one patient had 4/7 transcripts detected in both BM and cfRNA confirming that the fusion transcripts were derived from the tumour.

Conclusion

The identification of lncRNA and fusion transcripts in PL-cfRNA validates the use of this RNA-Seq approach for detecting blood based biomarkers in MM and supports the hypothesis that sequential quantification of patient specific transcripts may enable the non-invasive monitoring of disease kinetics.

P144. An Open-label, Randomised, Phase 3 Study of Daratumumab, Lenalidomide, and Dexamethasone (DRd) Versus Lenalidomide and Dexamethasone (Rd) in Relapsed or Refractory Multiple Myeloma (RRMM): POLLUX

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Aim

We compared the efficacy and safety of daratumumab (D) in combination with Rd vs Rd alone in patients with RRMM in a randomised, open-label, multicenter, phase 3 study (POLLUX; NCT02076009).

Method

Patients with ≥ 1 prior line of therapy for myeloma were randomised (1:1) to R 25 mg orally on Days 1-21 of each 28-day cycle and d 40 mg weekly, with or without D (16 mg/kg qw for 8 weeks, q2w for 16 weeks, then q4w until progression). The primary endpoint is progression-free survival (PFS).

Result

569 patients were randomised. Patients received a median of 1 prior line of therapy (range 1-11), 19% received ≥ 3 prior lines of therapy. 86% received prior PI, 55% received prior IMiD (including 18% prior R), and 44% received both PI and IMiD; 27% were refractory to last line of prior therapy, 18% were PI refractory, and none were R refractory. After median follow-up of 13.5 months, D significantly improved median PFS (63% reduction in the risk of progression/death) and TTP for DRd vs Rd (Table). D significantly increased ORR (93% vs 76%) and rates of VGPR or better (76% vs 44%) and CR or better (43% vs 19%) for DRd vs Rd, respectively (all $P < 0.0001$). The most common ($>30\%$) TEAEs (DRd/Rd) were neutropenia (59%/43%), diarrhoea (43%/25%), fatigue (35%/28%), upper respiratory tract infection (32%/21%), and anaemia (31%/35%). Most common grade 3/4 TEAEs ($>10\%$) were neutropenia (52%/37%), thrombocytopenia (13%/14%), and anaemia (12%/20%). The rate of grade 3/4 infections was 28% and 23%, respectively. Discontinuation rates due to TEAEs were similar (7%/8%). D-associated infusion-related reactions (IRR; 48% of patients) mostly were grade 1/2 (grade 3/4, 5%/0%); most (92% of IRRs) occurred during the first infusion.

Conclusion

The combination of D and Rd potentially represents a new standard of care for patients with ≥ 1 prior treatment.

	DRd	Rd
PFS		
Median, mo	NR	18.4
HR (95% CI)	0.37 (0.27, 0.52)	
<i>P</i>	<0.0001	
TTP		
Median, mo	NR	18.4
HR (95% CI)	0.34 (0.23, 0.48)	
<i>P</i>	<0.0001	

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P145. Plasma Exchange may be a Cost Effective Strategy to Reduce Initial Free Light Chain Burden in Patients with Myeloma Cast Nephropathy with Bortezomib based Induction Therapy to Improve Renal Recovery Times

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Introduction

In newly diagnosed myeloma patients, 10% have dialysis dependent myeloma cast nephropathy. The majority will require long term renal replacement therapy. Rapid reduction in light chain burden correlates with improvement in renal recovery times. The efficacy of plasma exchange has not been demonstrated in the pre-bortezomib era. Extended haemodialysis with high cut off membrane dialysers are promising but its usage hampered by high costs and inaccessibility.

We report a case of kappa light chain myeloma presenting with acute renal injury requiring haemodialysis. Initial plasma exchange over three days prior to administration of bortezomib based induction therapy given twice weekly after haemodialysis resulted in effective reduction in nephrotoxic light chains. The patient was dialysis independent in less than three months.

Case Discussion

77 year old female presented with progressive acute renal failure requiring haemodialysis. Investigations detected monoclonal light chain burden with kappa LC > 3000mg/L and kappa to lambda ratio of 308. Bone marrow aspirate showed 35% plasma cells consistent with light chain myeloma.

Rapid reduction in light chain burden was initiated with daily plasma exchange over three days prior to commencement of bortezomib based therapy administered twice weekly after haemodialysis. The initial kappa light chain burden was reduced by 67% after three days of plasma exchange. Further effective sustained reduction in light chain burden was achieved shown by a kappa to lambda ratio of 308 at initiation of treatment down to 4.95 after 2 cycles Bortezomib, cyclophosphamide and dexamethasone.

Renal recovery mirrored normalisation in light chain ratio and patient was successfully weaned off haemodialysis after 2 cycles bortezomib based therapy.

Conclusion

This case illustrates that plasma exchange may be a potential cost effective strategy to reduce nephrotoxic light chain burden prior to bortezomib therapy, improving renal recovery times and prognosis in patients with dialysis dependent myeloma cast nephropathy.

P146. Neurotoxicity in patients with Multiple Myeloma treated with Bortezomib – case of variant Guillain-Barre' syndrome and unilateral lumbosacral plexopathy

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Background

Bortezomib is a proteasome inhibitor in the treatment of multiple myeloma in frontline and relapsed settings. Neurotoxicity in the form of painful peripheral and autonomic neuropathy is common adverse event. There are rare reports of severe motor involvement with distal weakness due to mixed axonal-demyelinated neuropathy. Here we report two cases variant Guillain Barre' syndrome and unilateral lumbosacral plexopathy in multiple myeloma treated with bortezomib

Case report

First case, 72 year-old man diagnosed with myeloma in January 2016. Treatment with cyclophosphamide, bortezomib (1.3mg/m² weekly) and dexamethasone resulted in a good response after two cycles of therapy. However he developed unilateral distal right leg weakness. The nerve conduction study showed chronic right lumbosacral plexopathy without evidence of active denervation. He had an MRI of spine and lumbosacral plexus showing lytic lesions in lumbar spine. CSF was unremarkable, with no malignant cells. The symptoms were non-progressive after cessation of bortezomib, and he has proceeded to high dose melphalan conditioned autologous progenitor cell transplant.

Second case, 70 year-old female, diagnosed with myeloma in 2009. Bortezomib based therapy was delivered in 2009, and 2012. The most recent treatment was venetoclax and bortezomib and dexamethasone on a clinical trial. Bortezomib dosing was 1.3mg/m² at twice weekly schedule. She had a preexisting mild sensory peripheral neuropathy which did not impair function. During cycle 8 of treatment she developed a respiratory tract infection, and two weeks following this presented with acute neurological symptoms of weakness and became ventilator dependent due to respiratory failure. CSF showed elevated protein. Nerve conduction study showed acute motor and sensory axonal neuropathy (AMSAN), suggestive of variant Guillain Barre' syndrome. The myeloma treatment was withheld. She had plasmapheresis and 5 days IVIG 1gm/kg with minimal improvement.

Conclusion

The two cases demonstrate neurotoxicity from bortezomib may be associated with an atypical presentation.

P148. Efficacy and Safety by Cytogenetic Risk Status: Phase 3 Study (ASPIRE) of Carfilzomib, Lenalidomide and Dexamethasone (KRd) versus Lenalidomide and Dexamethasone (Rd) in Patients with Relapsed Multiple Myeloma (RMM)

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Background

Single-agent carfilzomib has demonstrated activity in patients with relapsed and refractory multiple myeloma with high-risk cytogenetic abnormalities. In the phase 3 study (N=792 patients) KRd significantly improved progression-free survival (PFS) versus Rd in patients with RMM. A pre-planned subgroup analysis of the efficacy and safety of KRd versus Rd according to patients' baseline cytogenetic risk status was performed.

Methods

The study design has been described previously. The high-risk group was defined as patients with the genetic subtype t(4;14) or t(14;16) or deletion 17p in ≥60% of plasma cells (as assessed using fluorescence in situ hybridization). The standard-risk group consisted of all other patients with known baseline cytogenetics.

Results

A total of 396 patients were randomized to each arm; 100 patients were included in the high risk group and 317 in the standard risk group. For patients with known baseline cytogenetics, risk status was similar across treatment arms (high-risk: KRd, 24.6%; Rd, 23.4%; standard-risk: KRd, 75.4%; Rd, 76.6%). Efficacy outcomes and adverse events (AEs) of interest by cytogenetic risk status are presented in the Table.

Conclusion

In patients with high-risk cytogenetics, treatment with KRd improved median PFS by 9 months versus Rd (median PFS of nearly 2 years vs 13.9 months). KRd was also associated with a 10-month improvement in median PFS vs Rd in patients with standard-risk cytogenetics. Treatment with KRd resulted in higher response rates, greater depth of response and a longer DOR versus Rd, regardless of patients' baseline cytogenetic risk status. The triplet regimen of KRd had a favourable benefit–risk profile in patients with RMM and improved

outcomes in patients with high-risk disease.

Table. Efficacy outcomes and AEs of interest by baseline cytogenetic risk status

Outcome	High-Risk		Standard-Risk	
	KRd (n=48)	Rd (n=52)	KRd (n=147)	Rd (n=170)
Median PFS, months	23.1	13.9	29.6	19.5
HR for KRd vs Rd (95% CI)	0.639 (0.369–1.106)		0.657 (0.480–0.901)	
Best overall response, n (%)				
Stringent complete response	8 (16.7)	2 (3.8)	22 (15.0)	6 (3.5)
Complete response	6 (12.5)	1 (1.9)	34 (23.1)	5 (2.9)
Very good partial response	15 (31.3)	11 (21.2)	55 (37.4)	66 (38.8)
Partial response	9 (18.8)	17 (32.7)	23 (15.6)	48 (28.2)
Minimal response	3 (6.3)	4 (7.7)	4 (2.7)	15 (8.8)
Stable disease	0	6 (11.5)	3 (2.0)	15 (8.8)
Progressive disease	2 (4.2)	6 (11.5)	2 (1.4)	2 (1.2)
Not evaluable	5 (10.4)	5 (9.6)	4 (2.7)	13 (7.6)
ORR, % (95% CI)	79.2 (65.0–89.5)	59.6 (45.1–73.0)	91.2 (85.4–95.2)	73.5 (66.2–80.0)
Median DOR, months	22.2	14.9	30.4	20.4
Grade ≥3 AEs of interest, n (%) ^a				
Dyspnea ^b	2 (4.3)	0	5 (3.4)	5 (3.0)
Hypertension ^b	1 (2.2)	0	9 (6.2)	3 (1.8)
Acute renal failure ^c	3 (6.5)	1 (2.0)	6 (4.1)	3 (1.8)
Cardiac failure ^c	0	0	8 (5.5)	4 (2.4)
Ischemic heart disease ^c	0	1 (2.0)	7 (4.8)	2 (1.2)
Peripheral neuropathy ^c	0	1 (2.0)	6 (4.1)	4 (2.4)

^aIn the high-risk group, 46 (KRd) and 51 (Rd) patients were evaluable for safety; in the standard-risk group, 146 (KRd) and 168 (Rd) patients were evaluable for safety.

^bPreferred term.

^cGrouped term.

P149. The burden of success: Increasing myeloma prevalence through improved survival

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Myeloma survival has improved through better drugs, better use of drugs and improved support care; turning myeloma from a fatal to a chronic disease. An implication is numbers of patients surviving with myeloma has increased.

Aims

We wanted to explore the magnitude of this increase and to understand whether new treatments have changed the demographics of the surviving myeloma population.

Methods

We extracted data on new cases of myeloma (ICD-10 code C90.0) from the New Zealand Cancer Registry from 1994 to 2013. Deaths were extracted from the national death registry, and population figures from national census data.

Results

The median overall survival increased in each 5 year era, from 21 to 47 months ($p < 0.001$) Benefit was seen both in those ≤ 65 years, and those > 65 years.

Era	1994-2000	2001-2005	2006-2010	2011-2013	
OS all*	21 (19-23)m	29 (26-32)m	38 (34-42)m	47 m	in months 95%
OS < 65yrs*	44 (35-53)m	67 (57-77)m	79(68-88)m	Not reached	
OS >65 yrs*	15(13-17)m	19(17-21)m	23(20 -36)m	31(26-36)M	
4yr OS	21 \pm 2%	26 \pm 2%	32 \pm 2%	36 \pm 2%	
4yr OS<65 yrs	49 \pm 2%	59 \pm 2%	65 \pm 2%	72 \pm 2%	
4yr OS >65yrs	22 \pm 2%	26 \pm 2%	33 \pm 2%	36 \pm 2%	

confidence interval)

The incidence of new diagnoses increased over time by 37% from 2000 to 2013 (increasing from 5.65 to 7.74 persons per 100 000,). Point prevalence more than doubled from 2000 to 2013 (from 13.8 to 32.3 per 100,000, 134% increase). The proportion of patients within 5 years of diagnosis (more likely to require active intervention) was 13.3 per 100,000 in 2000, increasing by 56% to 20.8 per 100,000 by 2013, The proportion of patients living with myeloma who were > 65 was 63% in both 2000 and 2013. Over the same time period, the country's population increased by 15%, resulting in a net increase in those living with myeloma in 2013 to 269% of 2000 levels.

Conclusion

There has been an increase in survival, incidence and prevalence of myeloma patients in New Zealand, dramatically increasing the burden of managing the disease. This increase is set to continue as new treatments improve outcomes further. The burden of success is also applicable to other diseases where treatment outcomes have improved without curing patients.

P150. Impact of the New IMWG Criteria in MGUS and Smouldering Myeloma at the Royal Hobart Hospital

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Aim

To retrospectively review the proportion of patients diagnosed at the RHH with MGUS and Smouldering Myeloma (SM) who met criteria to commence early Anti-Myeloma Treatment.

Background

Evidence shows irrespective of end organ damage (CRAB features), patients with > 60% plasma cells on BMAT are likely to progress to MM within 2 years. In October 2014 the IMWG published updated diagnostic criteria for MM. Any patients with BMAT of >60% plasma cells, a SFLC ratio of >100, or MRI showing > 1 focal bone lesion, are now classified as having MM and should be commenced on anti-myeloma treatment.

Methods

Bone marrow biopsy reports from 01/01/2010-30/06/2015 at the RHH were reviewed and patients classified as MGUS, SM, or MM. BMAT, SFLC ratio and skeletal surveys were reviewed to determine if they met the updated IMWG criteria for MM. Clinical data was also collected on patients who were commenced on anti-myeloma treatment but did not meet the new criteria nor had end organ damage.

Results

Of 1638 bone marrow biopsies, 93 had MM, 5 SM, 48 MGUS and 1 an isolated plasmacytoma. No patients with MGUS or SM met the new IMWG MM criteria. Of the 93 patients treated for MM during the 5.5 year period, 7 (8%) did not have evidence of end organ damage, nor did they meet the new MM criteria. Indications to commence treatment in these patients were rapidly increasing para-protein and symptomatic peripheral neuropathy.

Conclusions

There were no patients treated at the RHH from 01/01/2010-30/06/2015 with MGUS or SM who met or would have met the IMWG MM criteria to commence early MM treatment. A small number of patients were commenced on treatment for clinical and biochemical indications not captured by the new IMWG criteria. This audit recommends investigation of these indications as other possible justifications to commence early MM treatment.

P151. Phase 3 randomised controlled study of daratumumab, bortezomib and dexamethasone (DVd) vs bortezomib and dexamethasone (Vd) in patients with relapsed or refractory multiple myeloma (RRMM): CASTOR

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Aim

We report a pre-specified interim analysis of the first randomised controlled study of daratumumab (D; CASTOR [NCT02136134]).

Method

Patients with ≥ 1 prior line of therapy were randomised (1:1) to 8 cycles of bortezomib (V)/dexamethasone (d) \pm D (16 mg/kg iv qw in Cycles 1-3, Day 1 of Cycles 4-8, then q4w until progression). Primary endpoint was PFS.

Result

498 patients (DVd, 251; Vd, 247) were randomised. Baseline demographics and disease characteristics were well balanced. Patients received a median of 2 prior lines of therapy (range 1-10). 66% received prior V; 76% received prior IMiD; 48% received prior PI and IMiD; 33% were IMiD-refractory; 32% were refractory to last line of prior therapy. With median follow-up of 7.4 months, D significantly improved median PFS (61% reduction in risk of progression) and TTP for DVd vs Vd (Table). D significantly increased ORR (83% vs 63%, $P < 0.0001$), and doubled rates of \geq VGPR (59% vs 29%, $P < 0.0001$), and \geq CR (19% vs 9%, $P = 0.0012$) for DVd vs Vd, respectively. Most common ($>25\%$) AEs (DVd/Vd) were thrombocytopenia (59%/44%), peripheral sensory neuropathy (47%/38%), diarrhoea (32%/22%) and anaemia (26%/31%). Most common grade 3/4 AEs ($>10\%$) were thrombocytopenia (45%/33%), anaemia (14%/16%), neutropenia (13%/4%). 7%/9% of patients discontinued due to a TEAE. D-associated infusion-related reactions (45% of patients) mostly occurred during the first infusion; most were grade 1/2 (grade 3/4, 9%/0%).

Conclusion

D significantly improved PFS, TTP, and ORR in combination with Vd vs Vd alone. DVd doubled both VGPR and sCR/CR rates vs Vd alone. Safety of DVd is consistent with the known safety profile of D and Vd. The addition of D to Vd should be considered a new standard of care for RRMM patients currently receiving Vd alone.

	DVd	Vd
PFS		
Median, mo	NR	7.2
HR (95% CI)	0.39 (0.28, 0.53)	
P	<0.0001	
TTP		
Median, mo	NR	7.3
HR (95% CI)	0.30 (0.21, 0.43)	
P	<0.0001	

This research was supported by Janssen Research & Development. The company was responsible for the study design and statistical analysis. Under the guidance of authors, professional medical writers prepared the abstract and were funded by the company.

P152. Heavy/light chain assay enables accurate quantitation of IgA paraproteins co-migrating in the beta-globulin region

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Aim

Many myeloma patients have an IgA paraprotein detected in the β -globulin region by serum protein electrophoresis (SPE). Difficulties in IgA paraprotein quantitation impair assessment of disease progression and treatment response. This study evaluated the role of Hevylite, an assay measuring both heavy and light chains of intact immunoglobulins, compared with current standard assays to determine if it provided additional clinical information.

Method

93 sera from 28 myeloma and 4 MGUS patients with IgA paraprotein detected by immunofixation electrophoresis (IFE) over a 6 month period were tested using the HevyLite assay (Binding Site) on Siemens BNTMII platform. IgAK, IgAL and IgAK/IgAL (heavy/light chain ratio – HLCR) were compared with total IgA, SPE, IFE, estimated paraprotein (total β -globulins subtracted 10.5g/L, the mean normal serum β -globulin in the population), β 2-microglobulin, albumin, and time to progression (TTP) by Pearson's correlation coefficient.

Results

48 sera from 16 patients had quantifiable paraprotein (10 IgAK, 6 IgAL) and 40 sera from 15 patients had unquantifiable paraprotein (8 IgAK, 7 IgAL) co-migrating in the β -globulin region. Total involved heavy/light chain levels correlated very closely with SPE-quantifiable paraproteins (IgAK $r=0.91$; $p<0.0001$; IgAL $r=0.93$; $p<0.0001$), but not as closely with estimated paraprotein (IgAK $r=0.70$; $p=0.0005$; IgAL $r=0.88$; $p<0.0001$). HLCR did not correlate with quantified paraprotein (IgAK $r=0.65$, IgAL $r=0.18$). 21/88 IFE-positive samples had normal HLCR, a discrepancy of 24%. There was no correlation between HLCR and ISS (used as measure of tumour load). 5/16 patients with abnormal HLCR progressed, whilst 0/3 patients with normal HLCR progressed; median follow-up 9 (5-11) months.

Conclusion

Measurement of involved heavy/light chain accurately quantitates the IgA paraprotein and can be utilised for monitoring patients with paraproteins co-migrating in the β -globulin region. The discrepancy between normal HLCR and IFE-positivity may relate to recovery of polyclonal immunoglobulins. Its role in predicting disease progression and prognosis requires further evaluation.

This research was supported by Binding Site, who supplied the IgA Kappa and IgA Lambda reagent kits. The company had no role in analysing the data or preparing the abstract.

P153. PCAB is An Effective, Tolerable Treatment Regimen in Patients with Double Refractory Multiple Myeloma (MM)

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Patients with double-refractory MM (as defined by disease progression following a proteasome inhibitor and IMiD) have an extremely poor prognosis, with median progression-free and overall survival (OS) of 5 and 9 months, respectively.¹ The emergence of multiple treatment-resistant and proliferative subclones following novel agent use suggests a potential role for traditional, multi-agent cytotoxic DNA-damaging chemotherapy in this setting. PCAB (prednisone 60 mg/m² days 1-5, cyclophosphamide 600 mg/m² day 1, BCNU 30 mg/m² day 1 and doxorubicin 30 mg/m² day 1) was widely deployed and well tolerated before the introduction of novel agents, yielding an overall response rate of 48% in newly diagnosed MM patients.² PCAB may be a therapeutic option for double-refractory MM patients lacking clinical trial options (particularly elderly patients, those with comorbidities and geographically isolated patients).

Aim

To assess the tolerability and efficacy of PCAB in double-refractory MM patients lacking alternative treatment options.

Method

We undertook a retrospective review of all double-refractory MM patients receiving PCAB at University Hospital Geelong between January 2007 and June 2016. Baseline characteristics, clinical information and outcomes were retrieved from patient medical records and the hospital's electronic chemotherapy dispensing database (CHARM). All responses were evaluated as per the IMWG's Uniform Response Criteria.³

Result

Nine patients with double-refractory MM were treated with PCAB during the review period. Median age was 73 (53 – 87). Response was noted in four of nine patients (ORR 44%). One of the four responders achieved a VGPR, while three others achieved a PR. 2 patients able to maintain stable disease on PCAB. The mean duration of treatment with PCAB in non-responders was 1.3 cycles. 3 Patients who completed at least 6 cycles of PCAB tolerated it well, with no significant toxicities reported. Further data on median follow up, toxicity, treatment scheduling and duration and OS will be presented at the meeting

Conclusion

Double-refractory MM patients may, due to advanced age, comorbidities, or geographical status, lack access to other novel therapies (e.g., pomalidomide, carfilzomib, clinical trial therapy). These patients have a poor prognosis. In our experience, treatment with PCAB is shown to be efficacious and well tolerated.

P154. Developing safe haematological monitoring guidelines for patients with myeloma on bortezomib based regimens

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Introduction

Bortezomib is an effective treatment for myeloma but can cause significant haematological toxicity, particularly thrombocytopenia. There is little data on the frequency of haematological monitoring required for bortezomib based regimens.

Methods

A retrospective cohort study of patients treated with bortezomib based regimens was performed at the Princess Alexandra Hospital in Brisbane, between October 2009 and June 2014. Platelet count (Plt) and absolute neutrophil count (ANC) were recorded and incidences of thrombocytopenia and neutropenia were calculated.

Results

383 cycles of bortezomib regimens were given, 60 as initial therapy and 27 at relapse. Regimens included CVD (62%), PAD (11%), VD (10%), VMP (9%), others (7%). 47 received bortezomib twice weekly, 36 weekly and 4 fortnightly maintenance. 5% of first cycles were complicated by severe thrombocytopenia (Plt $\leq 20 \times 10^9/L$) which was more common in those whose day 1 Plt was $< 100 \times 10^9/L$ (25% vs 1%, $p=0.01$) but not associated with phase of therapy, dosing or regimen. Only two patients had an ANC nadir $< 0.5 \times 10^9/L$ during cycle #1. In subsequent cycles all but one patient with Plt $\geq 100 \times 10^9/L$ on day 1 had Plt nadir of $\geq 20 \times 10^9/L$. All patients with a day 1 ANC count of $\geq 1 \times 10^9/L$ had mid-cycle ANC nadirs $> 0.5 \times 10^9/L$. Patients continuing on bortezomib regimens tended to have higher counts on day 1 of each cycle and a lesser drop in their Plt count and ANC with each treatment.

Conclusions

Cytopenias are relatively predictable across varied bortezomib based regimens in the up front and relapsed settings. We suggest monitoring pathology on each day of bortezomib for the first cycle. Beyond cycle #1, if the day 1 Plt is $> 100 \times 10^9/L$ and the prior cycle Plt nadir was $> 50 \times 10^9/L$, interim pathology monitoring is not required. Reducing frequency of blood count monitoring safely may save time, improve efficiency and facilitate bortezomib home injection programs.

P155. Implementing a bortezomib home injection service

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Introduction

Bortezomib has a proven role in the treatment of newly diagnosed and relapsed myeloma, both in the transplant eligible and non-transplant eligible setting. For patients at the Princess Alexandra Hospital, bortezomib was only available in the day oncology unit and the Division of Cancer Services (DOCS) explored the feasibility of introducing a home based program to administer subcutaneous bortezomib.

Method

Initiation and support for the program was approved by the DOCS team and the Acute Care @ Home (AC@H) program. Policy and procedures were developed and all relevant AC@H nursing staff were educated in the administration of this drug and attained chemo competence. Consultation with relevant team members, in particular pharmacy, ensured appropriate pathways for drug supply and dispensing for home use were developed. Haematologists in the Myeloma unit were educated regarding referral and patient's recruited according to ethics. A Clinical Nurse Consultant (CNC) led bortezomib toxicity telephone clinic was established.

Results

The program commenced in November 2015 and 12 patients have been enrolled to date. All patients completed at least one cycle of bortezomib in the hospital prior to commencement and were reviewed in clinic with pathology on Day 1 of each cycle. 84 doses of bortezomib have been administered in the home. 5 as part of initial therapy, 6 relapsed and 1 post transplant. 11 patients have received weekly bortezomib and 1 fortnightly. To date no hospital admissions, delays or missed doses have occurred. However, 2 doses were not delivered at home due to adverse effects reported during telephone toxicity check.

Conclusion

This program benefits patients by reducing unnecessary time spent travelling to the hospital for chemotherapy delivery. The program is well established and continues to deliver safe effective treatment. Careful selection of patients and weekly assessment of side effects minimize the risk to the home bortezomib program.

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

P157. Assessment of the Red cell Exchange Program for Sickle cell Disease at a Paediatric Centre

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Aim & Background

Sickle cell Disease (SCD) is a chronic multisystem disorder requiring intermittent or chronic blood transfusion for management of acute and chronic complications such as chest crisis, stroke and vaso-occlusive pain¹. A Red Cell Exchange Program was commenced in 2015 at The Royal Children's Hospital (RCH) for management of children with severe Sickle cell Disease who had significant complications from transfusion therapy^{2,3,4}. An ongoing Audit has been commenced to monitor the quality of the procedure and inform ongoing development of the service.

Methods

Audit of Red cell Exchange at RCH to determine efficiency of the procedure (measured by target haematological parameters Hb S %, Hb (g/dL), haematocrit and ferritin) and to monitor for clinical outcomes and complications.

Results

.58 RCE procedures have been undertaken to date in 6 children with SCD (4 on chronic program and 2 for acute indications). Efficiency of reduction in Hb S was observed. The target end haematocrit post RCE was achieved in all encounters (average difference 0.13, post-exchange not available for three procedures). A reduction in serum ferritin has been observed across the whole cohort. Minor complications have included difficulties with IV access, mild hypotensive episodes, iron depletion and line infection. A reduction in presentations to the Emergency department and admissions to hospital has been observed for the cohort.

Conclusion

The established program of Red Cell Exchange for SCD at RCH has been effective in achieving a reduction in Hb S levels and has contributed to management of iron overload. Clinical outcomes including reduced admissions/presentations have also been achieved. Ongoing audit will form part of the quality management system for Red Cell Exchange and Apheresis at RCH.

P158. Impact of the presence of triplicated alpha globin gene mutation on observed alpha thalassaemia phenotypes – a case series

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Introduction

Co-inheritance of triplicated alpha-globin genes is known to alter the clinical and haematological phenotypes of beta-thalassaemias, exacerbating the phenotypic severity by causing additional globin-chain imbalance. There is a paucity of data regarding the impact of triplicated alpha-globin on alpha-thalassaemia phenotypes.

We report two cases of amelioration of the expected alpha-thalassaemia haemoglobin H (HbH) phenotype referred to our molecular reference laboratory and clinical service by co-inheritance of a triple-alpha gene variant.

Case Reports

Patients identified were referred to our centre for molecular testing between 2002-2016. Patients were asymptomatic of haemoglobinopathy with no evidence of a clinically significant thalassaemia phenotype as commonly seen in HbH disease.

Molecular testing for alpha thalassaemia mutations was performed via multiplex polymerase chain reaction (PCR) with subsequent confirmatory PCR. Triple alpha variants were detected on multiplex PCR.

Case 1: 26 year-old female with asymptomatic microcytic anaemia; Hb 117g/L, (120-160g/L), MCV 68fL(78-98fL), MCH 22.4pg(27-34pg), MCHC 326g/L(310-355g/L), ferritin 21ug/L(15-180ug/L), iron saturation 21%(15-45%). HbA2 2.5%(2-3.6%), HbF 0.4%(<1%), HbH inclusions not seen.

Molecular testing showed South-East Asian two-gene deletion (--SEA) and single-gene deletion (- $\alpha^{3.7}$) alpha-thalassaemia mutation. Additional DNA studies demonstrated triple alpha-anti4.2 variant ($\alpha\alpha\alpha^{4.2}$).

Case 2: 26 year-old female with low red cell indices; Hb 132g/L, MCV 71fL, MCH 21pg, ferritin 71ug/L, iron saturation 24%. HbA2 2.6%, HbF <1%, occasional HbH inclusions. No HbH band was seen on HPLC performed by the referring pathologist.

Molecular testing showed --SEA and - $\alpha^{3.7}$ alpha-thalassaemia mutation. Further DNA studies demonstrated $\alpha\alpha\alpha^{4.2}$.

Conclusion

This series demonstrates that the presence of triplicated alpha-globin genes appears to ameliorate the clinical and haematological severity of the phenotype in alpha-thalassaemias associated with 3 alpha-globin gene deletions, which ordinarily may be associated with deletional HbH disease. Furthermore, these cases highlight the need to look for alpha-globin gene triplications in patients whose molecular testing results appear discordant with their phenotypic and haemoglobin HPLC findings.

P160. Experience with Immune-Mediated Thrombotic Thrombocytopenic Purpura in the Northern Territory

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Introduction

There is a high prevalence of autoimmune disease in the Indigenous population of the Northern Territory. Immune-mediated thrombotic thrombocytopenic purpura (TTP) is characterised by pathogenic antibodies to A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13 (ADAMTS-13). We have observed a high incidence of TTP at Royal Darwin Hospital (RDH).

Aim

To study the clinical and demographic characteristics of patients with TTP at RDH.

Methods

Patient information was collected from medical records and the laboratory database. Remission was defined as a platelet count $> 150 \times 10^9/L$ for more than two weeks. Refractory TTP was considered after failure of haematological response by seven days of treatment.

Results

Between 2005-2015, 13 patients were diagnosed with TTP. Levels of ADAMTS-13 were recorded in 11 patients. Two patients with ADAMTS-13 $> 10\%$ were excluded. There were eight Indigenous patients (three males and five females) from the remote Top End. At presentation, median haemoglobin was 83.5g/L (range, 77–131g/L), and median platelet count was $10.5 \times 10^9/L$ (range, 3×10^9 – $20 \times 10^9/L$). All Indigenous patients had abnormal ANA and ENA titres. All Indigenous patients were resistant to initial management with plasma exchange and corticosteroids. Rituximab was introduced at a median of 14 days from admission, with a median time to response in platelet count of 23 days (range, 6–36 days). One patient died within 72 hours of admission from pulmonary embolism despite timely initiation of plasma exchange and corticosteroids. Another patient relapsed after two years of presentation with normal ADAMTS levels and was managed as atypical HUS with eculizumab without further plasma exchange and corticosteroids.

Conclusion

Immune-mediated TTP in Indigenous Australians appears refractory to plasma exchange and corticosteroids. Early introduction of rituximab in the treatment course may reduce morbidity and mortality in this cohort. The disease appears mostly monophasic and highly responsive to rituximab.

P161. Anabolic Androgenic Steroid Induced Polycythemia in the Australian Context - 3 Case Studies

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Aim

Medical and recreational testosterone use in men has increased over the last decade(1). In randomised trials, erythrocytosis is an adverse event associated with testosterone therapy (2). Haemoglobin (Hb) and haematocrit (Hct) increase in a linear, dose dependent way being more pronounced in older men (3). The exact mechanisms and prevalence of anabolic androgen steroid (AAS) induced polycythemia are unclear (4). We aim to highlight this phenomenon in the Australian context.

Method

A retrospective study of 3 cases referred to a major Australian Haematology outpatient department.

Result

Case 1: A 39 year old body builder with no relevant medical history utilised human and veterinary grade testosterone. At presentation, his Hb was 200g/L, and Hct 0.63. His testosterone level was 33.8nmol/L (8-30nmol/L) with normal iron studies, white cell and platelet counts. His Hb and Hct improved with a single therapeutic venesection, and reduction in testosterone supplementation.

Case 2: A 57 year old man with vascular risk factors and clinical history of hypogonadism treated with testosterone undecanoate presented with Hb 188g/L and Hct 0.56. White cell, platelet, erythropoietin and total testosterone levels were normal. JAK2 mutation was negative. A single therapeutic venesection and testosterone cessation normalised his Hb and Hct.

Case 3: A 57 year old man with cardiovascular risk factors and a purported history of testosterone replacement for hypogonadism presented with Hb 192 g/L and Hct 0.58, White cells, platelet, JAK2 mutation, iron studies, erythropoietin and bone marrow biopsy were unremarkable. Subsequent reviews revealed activity highly suspicious of androgen abuse. With medical and pharmaceutical vigilance, venesection requirements ceased in correlation with normalisation of Hb/Hct.

Conclusion

AAS use is an uncommon but noted cause of polycythaemia. The true prevalence in Australia is unknown, and may be more common than expected. AAS induced polycythemia should be considered in unexplained polycythemia in otherwise healthy men.

P163. Enzyme replacement therapy as long-term treatment for congenital haemolytic anaemia, as shown in a murine model of TPI deficiency

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Rationale/Aim

TPI deficiency is a rare red cell disease caused by a mutation in the enzyme *TPI-1*, resulting in haemolytic anaemia, splenomegaly, and ultimately death in early childhood. No treatments exist for TPI deficiency, but animal models have shown that enzyme replacement therapy is a viable long-term solution. Using a mutagenised murine model of TPI deficiency, called RBC19, we hope to demonstrate the first mammalian bone marrow-derived enzyme replacement therapy as a plausible clinically applicable treatment for human TPI deficiency.

Methods

The RBC19 mutant mouse line was generated on an SJL/J background using ENU mutagenesis. RBC19 mice were subject to whole exome sequencing (WES), which isolated a phenylalanine to serine base-pair mutation in amino acid 57 of the *tpi-1* gene. Enzyme-replacement therapy was conducted using 1×10^6 bone marrow cells harvested from adult SJL/L wildtype femurs, which were intravenously transplanted into lethally irradiated RBC19 homozygous recipients.

Results

RBC19 homozygous mice were found to have an elevated MCV compared to SJL/J wildtypes, as well as splenomegaly, reticulocytosis, and a reduced red cell half-life. The F57S mutation in the *tpi-1* gene was shown to cause TPI protein instability and reduced enzyme activity. Following 6 weeks recovery from bone marrow transplantation, RBC19 recipients showed normalised MC, reduced reticulocytes, and a normalised red cell half-life.

Conclusions

ENU mutagenesis has generated a viable homozygous murine model that accurately recapitulates TPI deficiency with genetic, enzymatic, and haematological parallels to the human disease. This is the first instance in which enzyme replacement therapy has been adapted to a mammalian model of TPI deficiency, and has demonstrated that this red cell disease can be effectively treated long-term using bone marrow transplantation.

P164. Necrobiotic xanthogranuloma with associated MGUS successfully treated with bortezomib

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Introduction

NXG is a non-Langerhans histiocytosis characterised by xanthogranulomatous infiltrates of foamy multinucleated histiocytes, palisading around areas of necrobiosis extending from the dermis into the subcutis. Clinically it most commonly presents as violaceous, xanthomatous, atrophic cutaneous plaques, with a predilection for the periorbital area. We present a unique case of necrobiotic xanthogranuloma (NXG) successfully treated with the proteasome inhibitor bortezomib.

Case Presentation

A 45-year-old lady was referred to our service in 2003 with a new diagnosis of NXG lesions of the buttock, left forearm and bilateral periorbital regions, together with an IgG-kappa paraprotein of 15.0 g/L. Bone marrow examination revealed <10% plasma cells and the patient was diagnosed with monoclonal gammopathy of uncertain significance (MGUS). She was initially treated with melphalan and prednisone for approximately 12 months when this was ceased due to drug intolerance. Her lesions showed good response, however relapsed two years later. Treatments with cyclophosphamide, thalidomide, cladribine and radiotherapy to the left forearm were ineffective. Four cycles of bortezomib were administered with virtual complete remission of the cutaneous lesions, a response that has been sustained over time without further intervention. Serum paraprotein has been faintly detectable but not quantifiable by EPG/IEPG since bortezomib treatment.

Discussion

NXG is a rare condition, with roughly 120 cases reported in the literature. Although it can occur as a solitary pathological finding, 80% of cases are associated with a monoclonal gammopathy, typically an IgG-kappa MGUS, with multiple myeloma occurring less commonly. Various other associated haematological conditions have also been reported. There is little consensus as to the optimal treatment modality. In patients with MGUS, reported treatments, with varied success, have revolved around melphalan, thalidomide and lenalidomide (each with or without corticosteroids). Other treatments have included chlorambucil, cyclophosphamide, interferon-2b-alpha, IVIG, dapsone, and even autologous stem cell transplant. To our knowledge, this is the first reported case of NXG to be successfully and durably treated with bortezomib.

P165. An Unusual Case of Haemolysis

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Aim

Thrombotic microangiopathy (TMA) can be caused by a number of pathological processes including thrombotic thrombocytopenic purpura (TTP) and atypical haemolytic uraemic syndrome (aHUS). Severe autoimmune disease can also produce this picture and clinical features may overlap. This case elucidates important aspects of this differential diagnosis.

Method and results

A 66 year old female presented with symptomatic anaemia, hypertension and congestive heart failure. Her full blood count showed a normochromic, normocytic anaemia with Hb 94 g/L, platelets 44/nL (160-420/nL) and normal white cell count. She also had renal failure with creatinine 143 umol/L (45-90) and a high NT-proBNP of 5853 ng/L (<125) and D-dimer 2,89 mg/L (< 0.5). A ventilation/perfusion scan showed no evidence of pulmonary embolism and an echocardiogram was normal. Coagulation profile was normal.

She had high LDH 317 IU/L (120-250), negative Coombs test and normal haptoglobin.

The initial blood films showed occasional fragments but subsequently, their numbers became more prominent and her haptoglobin progressively reduced.

Urgent plasmapheresis was arranged due to possible Thrombotic Thrombocytopenic Purpura (1). This was discontinued after the result of ADAMTS13 became available (153%).

The provisional diagnosis was atypical Haemolytic Uraemic Syndrome (aHUS) and she commenced treatment with Eculizumab (anti-C5 monoclonal antibody)(2).

The patient's hypertension was treated aggressively and her heart failure improved. Further investigation due to an active urine sediment showed positive ANA and Anti-SSA/Ro as well as low C3 and C4 levels.

Consequently a renal biopsy was performed. On electron microscopy this showed features strongly suggestive of lupus nephritis (abundant sub endothelial and mesangial dense deposits). Eculizumab treatment was discontinued and treatment with cyclophosphamide and prednisone was commenced. The patient's thrombocytopenia and haemolytic process resolved initially but she subsequently relapsed and is currently being treated with a second course of cyclophosphamide and steroids.

Conclusion

Accurate diagnosis of TMA is important to allow appropriate management. Severe autoimmune disease such as systemic lupus erythematosus may be difficult to distinguish from TTP or aHUS and a number of investigations may be needed to establish the diagnosis.

P166. The case of an unusual thrombosis

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Primary venous sarcomas are exceedingly rare. Often mistaken for a DVT there is a delay in diagnosis, and they carry a very poor prognosis. We report a case of a large internal jugular vein (IJV) tumour which was found on post mortem to be a sarcoma. There have been two case reports published about a sarcomas arising from the IJV, and both had associated extra-vascular masses.

An 82 year-old female presented on New Year's Eve with peri-orbital swelling, throat tightness and visibly distended veins. A CT venogram demonstrated an extensive internal jugular vein thrombosis extending from C3 to the brachiocephalic vein. She was initially started on clexane 1mg/kg and then warfarinised. Subsequent outpatient imaging demonstrated extension of the thrombus despite therapeutic INRs. She was changed to clexane 1.5mg/kg/day as this was thought to be a malignancy associated thrombus.

CT imaging also demonstrated a retro-sternal goitre. It was postulated that the thrombus could be extension of a malignant thyroid nodule through the middle thyroid vein. Surgical options to bypass the thrombus were explored including an endovascular stent to open a channel through the thrombus, or a subclavian–femoral bypass. The patient and family declined biopsy and surgery due to the risk of complications from either interventions. The patient was treated with anticoagulation and diuretics. She suffered from significant limb oedema and dyspnoea when supine causing significant deterioration in her quality of life.

The patient presented 11 months after her initial presentation with circulatory collapse due to extension of the "thrombus" into the right ventricle and pulmonary embolism. The family requested a limited autopsy. A biopsy of the IJV demonstrated a poorly differentiated sarcoma. This case demonstrates how soft tissue sarcomas and venous sarcomas can be mistaken for DVTs leading to a delay in diagnosis and be associated with a poor prognosis.

P167. Evaluation of D dimer levels as a predictive tool in pregnancy related venous thromboembolism

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Background

Venous thromboembolism (VTE) is uncommon in pregnancy but remains a leading cause of maternal mortality in developed countries. The likelihood of VTE using combinations of clinical predictive tools, D-dimer levels and imaging tests are well studied in the non-pregnant population, with D-Dimer functioning as a pre-test probability modulator. However, it is not validated in the pregnant population. Normal D-dimer level cut points in the non-pregnant population cannot be extrapolated to the pregnant population as D-dimer levels increases physiologically during pregnancy.

Aim

To identify a suitable D-dimer cut point using a local population cohort

Methods

A cohort of pregnant and postpartum women who were assessed for VTE was identified retrospectively between January 2011 and April 2016 from 2 tertiary hospitals (Guy's & St Thomas' Trust, London, UK and Monash Medical Centre, Clayton, Victoria, Australia). Patients who had valid D-dimer measurements (ACL TOP, IL Test D-dimer HS Kit #20007700), documentation of presence or absence of VTE (excluding distal deep vein thrombosis(DVT)) and stages of pregnancy were included for analysis.

Accuracy indices using the the nonpregnant cut point (0.2mg/L) and a new cut point determined by Receiver Operator Curves (ROC) analysis were calculated.

Results

107 women were identified of whom 15 had confirmed VTE of whom 7 were antepartum with 4 DVT and 3 pulmonary embolism. Using a non-pregnant D dimer level cut point of 0.2mg/L, the sensitivity and specificity for VTE was 88.2%. and 5.6% respectively.

ROC analysis suggested a cut point of 0.71mg/L with a sensitivity of 86% and specificity of 57%.

Conclusion

Our data confirm that a pregnancy specific cut point for VTE has improved specificity. It also illustrates the difficulty of conducting research in this area. Large multicentre multinational studies with simple trial protocols to enhance participation are required to solve this problem.

P168. Intravenous ferric carboxymaltose versus standard care in the management of postoperative anaemia: a prospective randomised controlled trial

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Background

Despite increasing efforts in perioperative management, postoperative iron deficiency anaemia (PIDA) persists. There is a lack of data on management of PIDA. This study aims to determine whether postoperative intravenous ferric carboxymaltose (FCM) improves iron stores, Hb and outcome postoperatively.

Methods and Participants

A prospective, open-label, randomized, controlled study of patients undergoing elective surgery between 17th December 2014 and 7th May 2015 with functional-IDA (haemoglobin 70-120 g/L and ferritin \leq 100 μ g/L or iron saturation \leq 20%), measured at day one postoperatively. Consecutive routine elective surgical patients (n=201) were recruited with major orthopaedic surgery (156), abdominal (19) and genitourinary surgery (18), and others (8). Patients underwent computer generated randomisation between a single-dose of intravenous 1000 mg FCM (103 patients) and standard care (98 patients), consisting of observation. The primary endpoints were improvement in haemoglobin (Hb) and iron stores at four weeks postoperatively, in addition to numbers of blood transfusion units. Secondary outcomes include length of hospital stay (LOS), rate of infection and quality of life assessment. Analyses were conducted on an intention-to-treat basis.

Findings

Baseline mean haemoglobin was 105.5 g/L in the standard care group versus 106.2 g/L in the FCM group, improving by four weeks to 121.5 g/L and 130.1 g/L respectively (95% CI 3.79-11.9; $p < 0.001$). Significant improvement in ferritin levels were found in the FCM group at four weeks (95% CI 3.79-11.9; $p < 0.001$). There was a reduction in transfused blood units in the FCM group (IRR 0.1; 95%CI 0.01 to 0.85; $P = 0.035$). LOS was significantly less at 7.8 days in the FCM group versus 11.6 days (95% CI -7.5 to -0.02; $P = 0.049$) with a decreased rate of infection (IRR 0.14; 95% CI 0.03 to 0.63; $P = 0.010$). No adverse events were observed with FCM treatment.

Interpretation

Postoperative FCM is a feasible and pragmatic management approach in surgical patients with functional-IDA. Our study suggests that patient blood management guidelines should be updated, incorporating the use of postoperative intravenous iron infusion to optimise patient outcomes. Further trials to assess our approach are warranted.

P169. A single intravenous ferric carboxymaltose versus oral iron sulphate for the management of preoperative anaemia: a randomised controlled trial

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Background

Prevalence of anaemia in orthopaedic surgery ranges between 10-20% with the main causes of anaemia identified as nutritional deficiency and anaemia of chronic disease. Perioperative anaemia, blood loss and allogeneic blood transfusion are associated with increased postoperative morbidity and mortality, and prolonged length of hospital stay (LOS).

Methods and Participants

We recruited 125 patients preoperatively who were randomized to a single intravenous ferric carboxymaltose (FCM) infusion (1000 mg standard dose) versus oral daily iron sulphate (325mg) until the time of the surgery. The median age was 71 years (range, 38-94) with a male to female ratio of 67:58. The vast majority of patients were Caucasian. There were 70 patients who underwent major orthopaedic surgery, 23 abdominal surgery, 27 genitourinary surgery and 5 patients underwent general surgery.

Findings

Median baseline Hb level at recruitment was 113 g/L in the FCM intervention group versus 120 g/L in the oral iron group that increased respectively to an average of 122 g/L versus 124 g/L immediately prior to surgery and was maintained at 126 g/L versus 123 g/L at 3 months after treatment respectively. The median baseline ferritin in the FCM group was 32 mcg/L versus 76 mcg/L that increased immediately prior to surgery to 544 mcg/L in the FCM versus 107 mcg/L in the oral iron group and was maintained for 3 months at 176 mcg/L versus 90 mcg/L respectively ($p < 0.001$). Mean LOS was reduced by 1 day in the FCM group as compared to the oral iron group. FCM was well tolerated. The requirement for blood transfusion was reduced in FCM group by a median 1.5 packed red cell unit per patient. Quality of Life (QoL) assessment using SF36 showed improvement in physical function, energy and general health in favour of the FCM group.

Interpretation

Our data suggest that IV FCM was associated with improvement of preoperative Hb and ferritin levels compared to oral iron. Accordingly, there was a reduction of LOS and improvement of QoL after surgery. Further trials that aim to improve and optimize preoperative and postoperative Hb are warranted.

P170. Haemoglobin Terrant in a Chinese family as a cause of familial erythrocytosis

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Introduction

Haemoglobin (Hb) Terrant is a rare high oxygen affinity haemoglobin that causes erythrocytosis. Here we report a Chinese family with this Hb variant presenting with unexplained familial erythrocytosis. Molecular study has an important role in confirmation of the diagnosis.

Case Report

A 52-year old Chinese female referred for investigations of erythrocytosis, which was incidentally identified at the age of 20. She was lifelong non-smoker with no features of obstructive sleep apnea (OSA). Of note, family history revealed two (51 and 54 years old) out of four siblings also suffered from erythrocytosis requiring regular venesection. Both of them were presumed to have secondary erythrocytosis due to OSA. Clinical examination of the index patient was unremarkable.

Investigations revealed raised Hb, haematocrit (Hct) and red blood cells (RBC) at 17.3 g/dL, 0.52 and 5.41 x 10¹²/L respectively. Her leucocytes and platelets were within reference limits. Bone marrow examination showed normocellular marrow with trilineage haematopoiesis and was negative for JAK2 V617F gene mutation. Hb analysis was performed because of the suspicion of a high oxygen affinity Hb variant. Alkaline Hb electrophoresis revealed an abnormal band moving to a position between Hb-S and Hb-F. It migrated to Hb-A position on acid Hb electrophoresis. The findings suggested the presence of an alpha chain Hb variant with high oxygen affinity.

Genetic identification of Hb variant was performed. Globin genotyping identified a heterozygous status for a substitution of nucleotide G to A (GAC>AAC) at codon 126 in alpha 2 globin gene, resulting in substitution of amino acid asparagine for aspartic acid in position 126 (Asp>Asn) and corresponding to Hb Tarrant. She was treated with regular venesection to prevent thrombotic complications.

Conclusion

Proper identification of this rare but clinically significant Hb variant is helpful for family counselling and will help to guide appropriate management of absolute erythrocytosis.

P171. The effect of habitual dietary salt intake on circulating microvesicle levels in type two diabetes mellitus

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Background

Microvesicles (MV) are membrane vesicles released during cellular activation, death and damage. Endothelial MV are surrogate markers of endothelial dysfunction, while CD36 expressing MV are novel biomarkers of atherosclerotic plaque instability. Low dietary salt intake has been associated with higher mortality risk in individuals with Type Two Diabetes Mellitus (T2DM).

Aim

To investigate the effect of habitual dietary salt intake on MV levels in individuals with T2DM. We hypothesised lower habitual dietary salt intake to be associated with higher endothelial MV levels compared to higher habitual salt intake.

Method

Eighty-one patients attending diabetes clinics at an Australian tertiary hospital were recruited in a prospective cross-sectional study. Flow cytometry characterised and quantified MV isolated from peripheral blood samples. Habitual dietary salt intake was estimated using corrected 24-hour urinary sodium excretion measurements. Seven participants were excluded due to pre-analytical processing differences. Thus seventy-four participants were included for final analysis. Corrected 24-hour urinary sodium excretion was correlated continuously with MV levels using Spearman rank correlation analyses. A P-value <0.05 was considered statistically significant.

Result

An inverse correlation was found between corrected 24-hour urinary sodium excretion and CD36+/CD235a+ MV levels ($\rho = -0.23$, $P = 0.05$). An inverse correlation was also observed between corrected 24-hour urinary sodium excretion and endothelial MV levels (total CD31+/CD42b-: $\rho = -0.17$, $P = 0.14$; CD31+/CD42b-/Annexin V+: $\rho = -0.14$, $P = 0.24$; CD54+/CD105+: $\rho = -0.08$, $P = 0.50$; CD105+/CD62e+: $\rho = -0.07$, $P = 0.54$; total CD105+: $\rho = -0.17$, $P = 0.15$).

Conclusion

We found an association between low salt intake and higher CD36+/CD235a+ MV levels. Thus our findings may provide a mechanistic link underlying the association between low salt intake and increased mortality risk in T2DM. Accordingly, population-wide salt restriction guidelines may require revision in consideration of high-risk populations such as individuals with T2DM.

P172. Epstein Barr virus-driven haemophagocytic lymphohistiocytosis in an adult with Crohn's disease treated with Rituximab

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Aim

We describe the diagnostic features and treatment of Haemophagocytic Lymphohistiocytosis (HLH) in a patient with Crohn's disease. The case is the first to describe successful treatment with Rituximab alone in adults.

Case History

A 24 year old male was diagnosed with Crohn's 2 years prior to presentation, and treated with 6-mercaptopurine (6-MP) and adalimumab. He presented with a 10 day history of fever, cervical lymphadenopathy and hepatosplenomegaly. Tests revealed pancytopenia. TPMT activity and 6-MP levels were normal. No infective organism was identified, so he was referred for further investigation.

Results

At presentation Hb 82 g/L neutrophils $0.39 \times 10^9/L$ and platelets $101 \times 10^9/L$. Hepatitis, hypertriglyceridaemia and hypofibrinogenaemia were noted. Ferritin was 11300 $\mu\text{g/L}$. Soluble CD25 was elevated, and NK cells were absent on PB flow cytometry. Diagnostic criteria ('HLH 2004') were met despite a marrow biopsy not demonstrating conspicuous haemophagocytosis. An EBV viral load of $3.02 \times 10^4/mL$ was discovered. Initial treatment with dexamethasone and immunoglobulins failed to produce a durable response. Rituximab $375\text{mg}/\text{m}^2$ was given resulting in improvement in clinical and laboratory parameters within 48 hours. Following a second dose, EBV viral load became undetectable. Serial testing of NK cell number and function showed improvement and eventual normalisation. Genetic analysis did not discover mutations known to be associated with HLH, although polymorphisms of unknown significance were noted in PFR1.

Conclusion

This HLH case is one of a small number of reports in Crohn's disease occurring mainly in adolescents. EBV is the most commonly described trigger and thiopurines appear to be a predisposing factor. A significant mortality rate is reported despite the use of cytotoxics suggested in HLH protocols. In our case, treatment with Rituximab led to a successful outcome without the need for more intensive therapy.

P173. Breakthrough Invasive Fungal Infections in High-Risk Haematology Patients Receiving Antifungal prophylaxis: Experience in a Single WA Tertiary Centre

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Aim

We evaluate the rates and epidemiology of breakthrough IFD in haematology patients on prophylactic anti-fungals in our centre.

Method

We retrospectively reviewed a cohort of patients (n=101) on prophylactic anti-fungal agents in 2014 – early 2015 and assessed the rates of proven, probable or possible breakthrough IFDs as defined by EORTC/MSG consensus group. Patients receiving antifungal prophylaxis were identified via the Pharmacy database and include those on chemotherapy for Acute myeloid leukemia, Myelodysplastic syndrome, Acute lymphoblastic leukemia, intensive chemotherapy for Non-Hodgkin's Lymphoma, Autologous and Allogenic stem cell transplant. Breakthrough infections were identified through imaging and laboratory reports.

Results

Of the 101 patients receiving antifungal prophylaxis, overall incidence of proven and probable IFDs was 11% (3 proven, 8 probable) with 18% possible IFDs (n=18). The 3 proven fungal infections were pulmonary aspergillosis, invasive maxillary aspergillosis and candidaemia. Six out of 8 probable IFDs were due to pulmonary aspergillosis. Two of the three patients with proven IFD died within 1 month of diagnosis and 1 was successfully treated. Thirty three allogenic transplants were carried out in our centre that year and in this cohort, the rates of IFDs were 1 proven, 1 probable and 2 possible cases within 6-months post-transplant. Overall mortality in patients with proven and probable IFD was 54% (6/11), with 2 deaths directly attributed to IFD.

Conclusion

Incidence of breakthrough fungal infections in our study was comparable to most available cohort studies from other centres. All-cause mortality in patients with proven/probable fungal infection is high. The rate of proven or probable IFD in our limited sample of allogenic transplant patients was low despite the lack of mould-active prophylaxis.

P174. RCPAQAP bone marrow morphology external quality assurance program using virtual microscopy

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RCPAQAP Haematology

Aim

A 2015 feasibility survey for bone marrow (BM) morphology using virtual microscopy had a low response rate but no significant barrier to introducing a regular RCPAQAP program was evident. The results of the first survey in the new program were reviewed to detect any evidence that this technology affects performance.

Method

The first case in the regular BM morphology program was available in April 2016. It showed BM granulomas (due to *Mycobacterium genevense* post BM transplant). Links to virtual microscopy scans of a blood film, BM aspirate (high, low power and iron stain) and trephine were provided. A DVD was available if preferred. Participants were required to submit a BM differential count and a diagnosis (from a list) - both assessed. An online BM reporting proforma was provided for reporting qualitative changes and additional investigations. The last two were not formally assessed but participants can compare their findings with overall responses.

Results

92 laboratories enrolled for 2016 and 78 (85%) submitted responses for this case. One participant preferred a DVD rather than online links. All 78 provided a diagnosis, 77/78 (99%) performed a differential count and 74/78 (95%) submitted other investigations. The various descriptive sections were completed by 77-78 (99 - 100%) and 75 (96%) participants for the aspirate and trephine respectively. 70/78 (90%) of participants identified the granulomas in the trephine and 62/78 (79%) submitted acceptable diagnoses. The morphological descriptions generally showed good consistency, with most variation seen in assessment of cellularity and quantitative and qualitative assessment of erythropoiesis.

Conclusion

Responses showed good consistency and 90% of participants correctly identified the key abnormality (granulomas) indicating that participants have the necessary technology and skills to use virtual images. This confirms that virtual images were appropriate for use in proficiency testing for these participants. However the reasons for non-participation by 14 (15%) of enrolees will be investigated further.

P175. An interesting case of superwarfarin poisoning

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We report a case of severe coagulopathy from long-acting anticoagulant rodenticide (superwarfarin) poisoning. A 53 year old man with psychiatric comorbidities presented after routine blood tests demonstrated profound and persisting coagulation abnormalities. The internationalised normalised ratio (INR) was >10 with a prothrombin time (PT) >100seconds and activated partial thromboplastin time (aPTT) at 122seconds, with both the PT and aPTT correcting with mixing studies (to 50% normal plasma).

His full blood count profile, liver function tests and d-dimer were unremarkable and there were no clinically significant bleeding manifestations at time of initial review. Further laboratory analysis confirmed deficiencies in all vitamin-K dependent clotting factors. Subsequent testing for serum levels of brodifacoum and warfarin were positive, confirming superwarfarin poisoning.

Therapy was instituted with high doses of vitamin K₁. An initial loading dose of 100mg vitamin K₁ administered intravenously was followed with a total daily dose of 200mg oral vitamin K₁. Close observation for bleeding, adherence to treatment and assessment of coagulation studies was instituted through regular clinic reviews. Only after 10 weeks of vitamin K₁ therapy did the PT normalise. A referral was also made to the local psychiatric service.

This case demonstrates the diagnostic challenge associated with superwarfarin poisoning. Appropriate laboratory interpretation in conjunction with persisting coagulation abnormalities is central to establishing the diagnosis. Further, it is imperative to appreciate the greatly increased potency and duration of action of such agents compared to warfarin as a vitamin K antagonist. This necessitates the use of high doses of vitamin K for a protracted period of time alongside close monitoring of laboratory parameters. Psychiatric evaluation is considered an important part of the management given that the majority of adult cases of superwarfarin ingestion are deliberate or obscure, and often in the context of psychiatric comorbidity.

P178. Acquired coagulopathy in paraproteinaemia: two cases with different bleeding phenotypes

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Introduction

Whilst reports of abnormal coagulation tests in plasma cell dyscrasias date back as early as 1930s, clinically significant bleeding related to these are uncommon. Literature regarding management and outcomes of these patients is limited to case reports and guidelines for acquired FVIII inhibitors. We describe two cases of paraproteinaemia with direct thrombin and FVIII inhibitors.

Report

An 85-year-old man presenting with back pain and confusion was diagnosed with myeloma, IgG kappa 36g/L. Incidentally, prolonged PT 23.2 seconds (normal range(NR) 10.6-15.3), APTT 47.6 seconds (NR 26.0-38.0), TCT 84.1 seconds(NR 14.0-24.0) were found. Fibrinogen, reptilase time, platelet count, FII, FV, FX, lupus inhibitor screens were unremarkable. TCT shortening upon serial dilution with normal pooled plasma(NPP) suggested presence of a thrombin inhibitor in patient plasma. TCT prolongation was more prominent with human than bovine thrombin reagent. Despite possible malaena, no source of haemorrhage was found. No bleeding occurred following endoscopic and bone marrow biopsies. Myeloma treatment partially improved the paraprotein and TCT.

In contrast, a 72-year-old man presenting with spontaneous lower and upper limb intramuscular haematomas demonstrated prolonged APTT 99.4 seconds(NR 26.0-38.0 seconds) which failed to correct on 1:1 mix with NPP. PT, fibrinogen, platelet count were normal. We discovered marked FVIII reduction 1.2%(NR 50-150%), inhibitor titre 29.4BU/mL(NR 0-0.5 BU/mL). He was initially managed with recombinant FVIIa and immunosuppression. Subsequently he was diagnosed with MGUS(IgG kappa 9g/L) and at inhibitor relapse, received Bortezomib/dexamethasone(Vd) resulting in partial reduction of paraprotein and inhibitor titres. Concurrent immunosuppression escalation made the relationship between chemoimmunotherapy and inhibitor eradication unclear. Cessation of Vd led to relapse requiring cyclophosphamide salvage.

Conclusion

Coagulation test abnormalities in patients with paraproteinaemia may be associated with variable bleeding phenotypes. Treating the underlying condition may be adequate to reverse haemostatic abnormalities. However, in the setting of bleeding, immunosuppression for rapid inhibitor eradication and bypassing agents may also be warranted.

P180. Screening of Paroxysmal Nocturnal Hemoglobinuria (PNH) Clone by Fluorescent Aerolysin (FLAER) Based Flow cytometry – A Single centre experience of 624 cases from India

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Objective

To observe the frequency of paroxysmal nocturnal hemoglobinuria (PNH) positive clones in patients screened for various indications and to compare the laboratory parameters of PNH positive cases between these groups.

Method

This was a retrospective analysis of 624 patients screened for PNH over a period of 30 months (Dec 2013 - May 2016). FLAER based screening of leucocytes was done by flow cytometric. Various laboratory data well retrieved from medical records and comparison was done among the PNH positive cases of different groups.

Results

There were 445 adults and 179 pediatric patients. Indications for screening included AA(n=433), myelodysplastic syndrome (MDS)(n= 34), Hemolytic anemia(n=84) and thrombophilia workup group(n=63). PNH clones were found in 39.03%, 5.88%, 26.19% and 1.59% cases respectively. No significant difference among adult or pediatric population was noted. The bone marrow failure (BMF) group[AA and MDS] with PNH clone had a significantly lower PNH clone size(Median- 2.7%) as compared to classic PNH group(Median- 77.2%). Most of the classic PNH cases(78.26%) and a small proportion of AA(9.9%) showed a large clone size(>50%). In spite of having large clone size, there was a significant difference between the median LDH values of these two group(2511.5 vs 593 U/L).

Conclusion

FLAER based screening detects presence of PNH clone in a high proportion of AA patients and some MDS patients. These patients usually have a small clone size. Even if they have a large clone, it does not get translated into a high LDH or severe clinical.

Table1: Distribution of cases according to Indications for PNH screening

All cases		Male			Female			Total		
		Done	Positive	%ge	Done	Positive	%ge	Done	Positive	%ge
Aplastic anaemia	Total Screened	299	112	37.46	135	57	42.22	433	169	39.03
	Adults	187	75	40.11	91	43	47.25	278	118	42.45
	Pediatrics	111	37	33.33	44	14	31.82	155	51	32.90
Myelodysplastic syndrome	Total Screened	16	0	0.00	18	2	11.11	34	2	5.88
	Adults	16	0	0.00	18	2	11.11	34	2	5.88
	Pediatrics	0	0	0.00	0	0	0.00	0	0	
Megaloblastic anaemia	Total Screened	9	0	0.00	1	0	0.00	10	0	0.00
	Adults	9	0	0.00	1	0	0.00	10	0	0.00
	Pediatrics	0	0	0.00		0	0.00	0	0	0.00
Hemolysis work	Total	54	16	29.63	30	6	20.00	84	22	26.19

up	Screened									
	Adults	46	15	32.61	25	5	20.00	71	20	28.17
	Pediatrics	8	1	12.50	5	1	20.00	13	2	15.38
Thrombosis work up	Total Screened	43	1	2.33	20	0	0.00	63	1	1.59
	Adults	35	1	2.86	18	0	0.00	53	1	1.89
	Pediatrics	8	0	0.00	2	0	0.00	10	0	0.00

P181. Complications of heparin infusions in hospital inpatients

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Aim

To assess problems associated with the use of a ward based heparin infusion protocol as evidenced by instances of supratherapeutic activated partial thromboplastin time (APTT) levels. The study aims to improve the quality and safety of the heparin infusion protocol

Method

A retrospective file audit of eight inpatients at a tertiary referral hospital was performed. The patients received ward protocol heparin infusions in either January or February 2016 and had at least one APTT result of more than 150 seconds. Data was collected on patient demographics, adherence to the heparin protocol and events leading up to and after the raised APTT

Result

In all eight patients, the ward based heparin infusion protocol was not followed correctly on at least one occasion. Documentation on the heparin infusion chart was incomplete for seven of the eight patients. A weight was recorded on the heparin infusion chart for only one patient. A significant bleeding event occurred in two patients following an APTT result of more than 150 seconds

Conclusion

Heparin is a high risk medication. A standardised heparin infusion protocol is useful in guiding the safe administration of this medication in a hospital ward based setting. However, the protocol is often not followed correctly. This may be due to both individual and systemic factors

P182. An audit on bone marrow iron assessment and reporting at Waikato Hospital, Hamilton, New Zealand

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Aims

To audit local practice of assessing and reporting bone marrow iron stores compared to ICSH guidelines.[1] In addition, assess the utility of performing a routine PERLS stain on all trephine biopsies.

Method

Three hundred consecutive bone marrow samples collected at Waikato Hospital from January 2015 – October 2015 were retrospectively reviewed. The bone marrow aspirate iron stain was assessed microscopically and findings were compared with those reported. Particular attention was paid to:

The adequacy of the iron stain:[2] How many particles were available for assessment?

How the result was reported.

Did the iron stain on the trephine sample provide additional information to the aspirate assessment?

In addition, inter observer variation was assessed. We asked 7 different hematologists at our institution to grade 10 iron-stained aspirate films.

Results

Of the 300 iron-stained bone marrow aspirates reviewed, 143 were graded despite having < 7 marrow particles on the slide. Only 22 of these were reported as suboptimal for assessment due to inadequate particles on the slide. Methods for grading iron stores included 2 different semi-quantitative grades and a descriptive grade. There was increased concordance between hematologists at our laboratory when a grade from 0-4 or a descriptive grade was utilized.

The majority of bone marrow trephine reports 245 (81%) had no mention of the Perls stain findings. Of those reported, the majority (75%) did not add to the iron assessment from the aspirate.

Conclusion

This audit highlights how our institution is not following the published ICSH guidelines regarding bone marrow iron stores reporting. This led to a change in local laboratory practice. Seven particles must be assessed before an assessment of reduced or absent iron can be made. All iron stains are graded using a descriptive grade. We no longer perform a Perls stain routinely on trephine samples.

P183. An audit on heritable thrombophilia testing in Waikato, New Zealand

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Aim

Review patterns of testing for heritable thrombophilia in the Waikato region and audit these against the British Committee for Standards in Haematology (BCSH) guidelines 2010¹.

Method

All patients who had a heritable thrombophilia screen performed at Waikato hospital laboratory, New Zealand, during the month of August 2015 were included. Laboratory software and electronic clinical notes were used to collate data. This included patient age, gender, indication for testing, requestor information (hospital department or GP practice), test results and compliance with BCSH guidelines.

Results

A total of 94 patients were tested. The majority were females (n=70) with a median age of 38yrs (range 13-86 yrs). Overall the most common indications for testing were personal history of DVT (15), family history of thrombosis (15), miscarriage (13) and CVA/TIA (8). Most requests from within the hospital came from the obstetric and general medicine departments.

Only 1 request for heritable thrombophilia complied with the BCSH guidelines.

24 patients had a positive heritable thrombophilia test, however the majority were of limited clinical utility. There were only 2 patients with a positive test that may have changed clinical management. The clinical utility of 6 positive results were uncertain due to testing at the time of miscarriage and the incongruence of a positive genotype with a non thrombotic phenotype.

Conclusion

Current patterns of inherited thrombophilia testing in the Waikato region are not meeting best practice standards. There are significant psychological costs of unnecessary testing to both patients and their families in addition to unnecessary financial costs. As a result of this audit, systems have been implemented within our hospital, the regional hospitals and community GP practices to improve compliance with national and international guidelines for heritable thrombophilia testing (June 2016).

P184. Paroxysmal nocturnal haemoglobinuria with Budd-Chiari Syndrome: a true medical emergency

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Aim

To detail the case of a young woman with paroxysmal nocturnal haemoglobinuria (PNH) who developed Budd-Chiari syndrome (BCS). She was successfully treated with eculizumab and anticoagulation, but remains transfusion dependent with evidence of ongoing low-level extravascular haemolysis.

Method

The patient's medical records were reviewed and laboratory and radiological results were assessed. A literature review was undertaken to investigate the occurrence of Budd-Chiari syndrome in PNH and its response to eculizumab as well as the persistence of anaemia in PNH despite eculizumab treatment.

Result

A 24 year old woman presented with anaemia and was diagnosed with PNH. She developed progressive abdominal pain and ascites and was found to have BCS. Anticoagulation and eculizumab were commenced and the patient had rapid resolution of her ascites. Within six weeks, repeat imaging demonstrated partial hepatic vein and complete IVC recanalization. Despite this she had persistent anaemia requiring ongoing intermittent red blood cell transfusion. A positive DAT for C3d supported the theory that she had ongoing low-level extravascular haemolysis.

Randomised trials have demonstrated the efficacy of eculizumab in reducing thromboembolism and transfusion requirements in PNH. Long term follow-up of these patients has shown an improvement in survival. There is one other case study showing the success of the combination of eculizumab and anticoagulation in BCS, preventing the need for invasive treatment. Studies investigating the mechanism for persistent anaemia in patients with PNH on eculizumab suggest C3 accumulates on PNH red cell clones due to C5 blockade resulting in low-level extravascular haemolysis.

Conclusion

BCS is a well-known thrombotic complication of PNH and in this case led to decompensated liver disease with ascites. Eculizumab can prevent the need for invasive therapy in BCS caused by PNH, significantly reduces the rate of new thromboembolism but may unmask low-level extravascular haemolysis. This may delay independence from transfusion.

P185. Image-guided core biopsy of the spleen: The Central Coast experience

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Background and Aims

Although image-guided core biopsy of the spleen was initially described in 1980 it is not widely available in Australia predominantly due to concerns by both radiologists and haematologists regarding the risk of haemorrhagic complications. Since they are regularly performed at our centre we evaluated the safety and diagnostic efficacy of this procedure in an outpatient setting.

Method We performed a retrospective audit of all image-guided core biopsies of the spleen performed from May 2009 until March 2016 on the Central Coast of NSW. Cases were identified via the radiology database and information on the indication, complications, diagnosis and outcome were obtained from the referring clinician.

Results

Twenty-five image-guided (24 CT-guided, 1 ultrasound-guided) core biopsies of the spleen were performed. Pre-biopsy evaluation consisted of cessation of antiplatelet and anticoagulant therapy, and confirmation of satisfactory platelet counts ($>80 \times 10^9/L$) and coagulation studies. Biopsies were performed with both an 18-gauge needle for the core biopsy and a 22-gauge for a flow cytometry sample. The diagnostic yield depended upon the indication for the biopsy; those performed to evaluate focal splenic lesions (5 cases) or to enable subclassification of a lymphoproliferative disorder (8 cases) established the histological diagnosis in all cases whereas in those performed for unexplained splenomegaly (12 cases) the histological diagnosis was only established in 5 cases. Three patients had complications; 2 had post-biopsy haemorrhage but neither required blood transfusion or splenectomy and 1 had mild pain and nausea which settled spontaneously. Four patients subsequently proceeded to a therapeutic splenectomy and histopathology confirmed the core biopsy diagnosis in all cases.

Conclusions

Image-guided core biopsy of the spleen is a safe and well-tolerated procedure. Complications can be minimised by pre-biopsy screening and using an 18-gauge needle. Careful patient selection is required to optimise the diagnostic yield which is highest for focal splenic lesions and subclassification of lymphoproliferative disorders and lower for unexplained splenomegaly.

P186. Soluble CD25 testing in Haemophagocytic Lymphohistiocytosis

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Aim

Soluble CD25 (sCD25) of >2400 U/ml forms part of the diagnostic criteria for haemophagocytic lymphocytic histiocytosis (HLH). Here, we aim to investigate its usefulness and to determine an optimal sCD25 threshold in patients with HLH.

Method

Serum samples were collected for sCD25 testing from adult patients in whom HLH is likely to be present versus those who do not have HLH. It measures the human soluble interleukin-2 receptor (sIL-2R). Testing was performed utilising an enzyme-linked immunosorbent assay (ELISA) method (Thermo Scientific Human sIL-2R ELISA kit). The upper limit of detection is 6500 U/ml. Data forming part of the HLH 2004 diagnostic criteria as well as other accompanying features were collected from every patient if available.

Result

Total of 52 patient samples were analysed. 13 (25%) patients were identified as having HLH based on the HLH 2004 criteria and other clinical findings. All patients with HLH were due to secondary causes. The mean sCD25 level for patients with and without HLH is 6385 U/ml (+/-343 U/ml) and 2311 U/ml (+/- 2030 U/ml) respectively (assuming upper limit of detection is 6500 U/ml). The median ferritin in patients with HLH was 12641ug/L (interquartile range 15674ug/L). A sCD25 of >2400U/ml were seen in 26 patients (50%) and is associated with 100% sensitivity, however specificity was only 67% (LR+ 3.0, LR- 0.0). All patients with HLH had a sCD25 level of >5200 U/ml, which was associated with improved specificity (85%) and a sensitivity of 100% (LR+ 6.5 and LR- 0.0). Longitudinal data from 1 patient suggested a correlation between sCD25 levels and disease activity.

Conclusion

sCD25 is a useful marker in the diagnosis of HLH. A cut off of >5200 IU/L improves the specificity and maintains sensitivity in the diagnosis of HLH. These results should be further validated in other high risk populations. Further studies are warranted to examine the potential role of sCD25 in monitoring disease activity.

P187. Reduced PU.1 expression underlies aberrant neutrophil maturation and function in β -thalassaemia

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Aim/background

β -Thalassaemia is associated with several abnormalities of the innate immune system. Neutrophils in particular are defective, predisposing patients to life-threatening bacterial infections. The molecular and cellular mechanisms involved in impaired neutrophil function remain incompletely defined.

Method

We explored aspects of neutrophil biology that may contribute to impaired immune function using a clinically relevant mouse model of β -thalassaemia (Hbb^{th3/+}). We explored susceptibility to *S. pneumoniae* infection, neutrophil chemotaxis, opsonophagocytosis and ROS production. To extend our observations beyond mice, we evaluated neutrophil function in non-splenectomised and splenectomised HbE/ β -thalassaemia patients.

Results

We first demonstrate that Hbb^{th3/+} mice rapidly succumbed to infection with *S. pneumoniae*. Additionally, neutrophils from Hbb^{th3/+} mice displayed significant deficits in chemotaxis, opsonophagocytosis and ROS production. Expression of CXCR2, CD11b and several components of the NOX2 enzyme complex were significantly reduced. In addition, we noted a striking expansion of immature neutrophils, comprising mainly of band form and granulocytic myeloid-derived suppressor cells (G-MDSCs) in Hbb^{th3/+} mice. Importantly, expression of the ets-family transcription factor PU.1, required for terminal myeloid differentiation and effector function, was significantly lower in Hbb^{th3/+} mice compared to normal controls. Mirroring those of Hbb^{th3/+} mice, neutrophils isolated from non-splenectomised (n=10) and splenectomised (n=5) HbE/ β -thalassaemia patients were hyposegmented and displayed reduced PU.1 expression compared to healthy controls (n=7), indicating an aberrant or arrested state of neutrophil maturation.

Conclusion

Here, we show that Hbb^{th3/+} mice closely mimic the immune dysfunctions observed in β -thalassaemia patients. Our findings provide mechanistic insights into the compromised neutrophil function that results in reduced resistance to bacterial infection in the β -haemoglobinopathies.

P188. Review of Platelet Autoantibody Testing in Adults - A Single Centre Retrospective Review

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Aim

Platelet autoantibody testing is frequently requested to assist in investigation of the cause of thrombocytopenia or as part of an autoimmune screen. However, it is generally not recommended as the diagnostic test for immune thrombocytopenia (ITP). Flow cytometry based platelet immunofluorescence testing (PIFT) performed to determine the presence of IgG and IgM antibodies both circulating and bound to platelets. This retrospective audit aims to review the clinical indications for the platelet autoantibody testing and if the result had any clinical impact on clinical management.

Method

Retrospective audit data from 210 adult patients from single tertiary hospital for whom platelet immunofluorescence testing (PIFT) was requested between 2010 and 2015. Categorical analysis between groups with positive and negative PIFT.

Results

Of the 210 patients 79 (38%) had positive PIFT with a mean platelet count of 116 (range 1-497) $\times 10^9/L$. Requests were from haematologists (42%) gastroenterologists (23%), immunologists (19%) and obstetricians (8%). In patients with ITP, 54% had a positive PIFT and a calculated sensitivity and specificity of 61% and 54% respectively. Utilising quantitative immunofluorescence data, receiver operator curve yielded area under curve of 0.55-0.57. PIFT was found commonly in other conditions some of which are known secondary causes of ITP including in 57% of patients with liver disease, 55% with hepatitis C and 50% in hepatitis B. PIFT was positive in 31% of patients with gestational thrombocytopenia all with subsequent platelet count recovery. There were no altered management or bleeding events in the presence of PIFT results.

Conclusion

PIFT testing assay has poor sensitivity and specificity and is commonly found in patients with secondary causes of immune thrombocytopenia. There has been no alterations in management of patients based on PIFT results. The utility of PIFT assay in ITP should be reconsidered.

P189. Presentation and Mortality Associated with Severe Thrombocytopenia in a Single Australian Centre

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Aim

Severe thrombocytopenia, especially less than $10-30 \times 10^9/L$ is a clear risk factor for spontaneous or serious bleeding. Severe thrombocytopenia has also been shown to be independently associated with death in ICU patients. Similar evidence in the general patient population has not been well described. We aim to retrospectively describe the patient characteristics and outcomes of patients with platelet count $<10 \times 10^9/L$ at our institution.

Method

Inpatient and outpatients of our institution (which cares for paediatric, obstetric, cancer and ICU patients) with at least one episode of thrombocytopenia $<10 \times 10^9/L$ between 1 Jan 2013 to 31 Oct 2015 were identified by data extraction from the pathology service. Clinical records were reviewed and information including cause of thrombocytopenia, bleeding, mortality and therapy were obtained.

Results

There were 149 identified patients during the study period. Fifteen (9%) were spurious results and not included in the analysis. Characteristics of other patients are displayed in table 1 (some had more than 1 cause identified).

Cause for thrombocytopenia	No patients (% of all)	Any bleeding per cause (% per cause)	Death within 30 days of platelet nadir (% per cause)
ITP	48 (32)	40 (83)	3 (6)
Sepsis+/- DIC	19 (11)	2 (12)	15(79)
Chemotherapy	27 (18)	10 (37)	10 (37)
MDS/AML	16 (11)	4 (25)	7 (44)
Other cancer	20(13)	7 (37)	7 (35)
TTP	3 (2)	0	1 (33)
Aplastic anaemia	2 (1)	0	0
Other	5 (3)	2 (40)	0

Within the patients diagnosed with ITP, 34(71%) had minor bleeding (bruising, petechiae, minor epistaxis); 6(12.5%) had severe bleeding leading to death, red cell transfusion or necessitating surgical procedure. Seven (15%) patients died, of which 3(6%) were directly as a result of severe bleeding occurring in patients with relapsed/refractory ITP. De-novo ITP cases were 33(69%) of patients and relapsed/refractory were 15 (31%). Two (4%) patients were initially managed as ITP but subsequently diagnosed with another condition (aplastic anaemia, marrow infiltration by cancer).

Within the group with sepsis, 8 (42%) had concurrent malignancy and 5 (26%) were complicated by DIC. Of the 5 patients with DIC, 4 died within 30 days of the platelet nadir.

Conclusion

In our institution, severe thrombocytopenia $<10 \times 10^9/L$ was largely associated with ITP, malignancy and sepsis. Thirty day mortality was observed to be markedly higher in the group presenting with sepsis+/- DIC despite low bleeding rates, and is concordant with previous published evidence that severe thrombocytopenia is independently associated with mortality in critically ill patients.

P190. Idiopathic Cyclic Thrombocytopenia: Case Series of 5 Patients

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Background

Cyclic thrombocytopenia (CTP) is an uncommon disorder characterised by large cyclic platelet fluctuations (usually $>100 \times 10^9/L$), alternating between thrombocytopenia and periods of normal or high platelets. Importantly, most patients are initially diagnosed with idiopathic thrombocytopenic purpura (ITP), but response to typical ITP therapy is typically poor.

Methods

We describe 5 patients who have been diagnosed with idiopathic cyclic thrombocytopenia.

Results

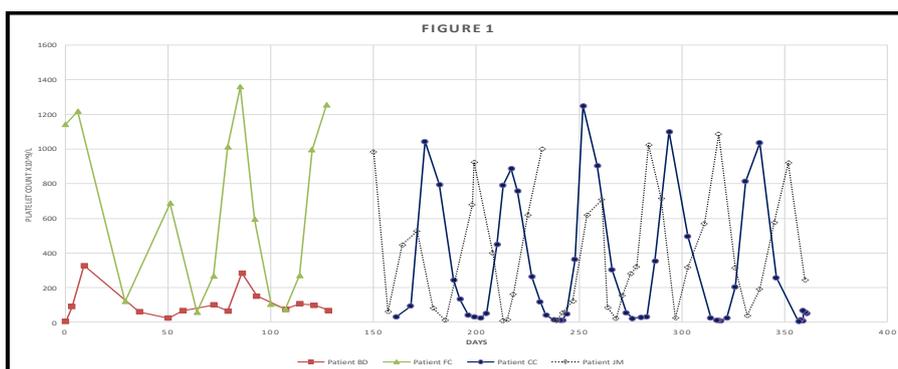
The characteristics of the 5 patients are displayed in the table below.

	BD	FC	CC	JM	HN
Age	23	81	80	35	37
Sex	F	F	F	M	F
Lowest platelet nadir $\times 10^9/L$	4	58	4	2	7
Highest platelet peak $\times 10^9/L$	327	1361	1306	1856	429
Cycle length (days)	28-29	35-43	35-43	27-34	Approx 30
Lines of therapy	Prednisolone, IVIG	Nil	Prednisolone, IVIG, azathioprine, danazol	Prednisolone, IVIG, azathioprine, splenectomy, danazol	Prednisolone, IVIG, rituximab
Time until CTP diagnosis (months)	3	3	12	36	24

Four patients (80%) were asymptomatic and diagnosed incidentally. One displayed petechiae and bruising at diagnosis. One patient presented during 1st trimester pregnancy. Four patients were diagnosed with ITP at presentation. The median time from ITP to CTP diagnosis was 18 months (range 3-36 months). One patient was also subsequently diagnosed with chronic myelomonocytic leukaemia (CMML).

Platelet cycles for 4 patients are displayed in Figure 1. Of the 2 pre-menopausal women, one (HN) had platelet nadirs coinciding with menstruation.

At the time of last follow-up, all 5 patients were alive. All have persistent cycling of platelets – 3 with nadirs $>50 \times 10^9/L$ and 2 with nadirs $<50 \times 10^9/L$. All patients have ceased immunosuppressive therapy, while 1 patient is on danazol. Bleeding symptoms were mild (bruising, mild epistaxis) and none required red cell transfusion as a result of blood loss.



Conclusion

CTP is a mostly clinically benign platelet disorder, often initially mistaken for ITP. It is likely under-recognised and should be an important differential diagnosis, in order to minimise lengthy and unnecessary over-treatment.

P191. Platelets and platelet-derived microparticles express P2X7 receptors in normals and patients with multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system characterized by acute relapses followed by remissions, which become incomplete and lead to accumulation of disability. Recent studies have shown an increase in circulating microparticles and exosomes derived from activated platelets in MS [1], but it is not known how this relates to the pathophysiology of acute relapse or disability progression. In a case-control genetic association study of MS we examined twelve functional polymorphisms of P2X7. One variant, rs28360457, coding for the loss of function Arg307Gln was associated with twofold protection against MS (OR 0.57, $p=0.0000024$) while the gain of function variant of P2X7, Ala348Thr, was associated with an increased risk of MS (OR 1.10, $p=0.0006$) [2].

Our aim is to find if P2X7 is expressed on circulating platelets and whether it plays a role in the generation of circulating platelet-derived microparticles found in MS. Flow cytometric analysis of both resting and activated human platelets showed binding of the L4 mAb specific for the extracellular domain of the P2X7 receptor. Microvesicles derived from activated platelets were also shown to express P2X7 receptors. Western analysis of platelet lysates from both MS patients and normal controls showed the presence of a 65-70kD protein staining with a sheep polyclonal antibody against residues 64-81 of human P2X7. The function of platelet P2X7 was studied in gel-filtered platelets loaded with the Ca-sensitive dye Fluo-4, and suspended in K-Tyroses medium with 3mM Ca^{2+} . Addition of ATP (5mM) gave a weak increase platelet Ca^{2+} while thrombin (1U/ml) gave a rapid Ca^{2+} increase.

In conclusion, platelets express several purinergic receptors (P2X1, P2Y1, P2Y12), and the presence of P2X7 has not been previously reported. However, the function of P2X7 in platelets remains to be established.

P193. X-linked Inhibitor of Apoptosis (XIAP) Deficiency leading to recurrent Haemophagocytic Lymphohistiocytosis (HLH) driven by Epstein Barr Virus (EBV): A Case Study of Treatment with Single Agent Rituximab

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Introduction

XIAP deficiency was first described in conjunction with X-linked lymphoproliferative (XLP) disease. In contrast to XLP which is associated with EBV-driven lymphoproliferative disorders, XIAP deficiency is associated with recurrent HLH from EBV in 30-67% and CMV in 20%. Treatment comprises dampening inflammation and eliminating possible triggers such as viral infection.

In patients with XIAP deficiency and EBV-HLH, treatment is difficult due to viral latency in sites such as B cells. To date, success in eliminating EBV with rituximab has been previously reported in only 2 cases of XIAP deficiency.

Case

A 17 year old male presented with ongoing fatigue and splenomegaly post EBV infection with persistent viraemia. He was suspected to have HLH. XIAP deficiency was considered due to his family history of Crohn's disease and similar presentations in a twin brother (Table 1). This was subsequently confirmed by genotyping.

Due to suspected EBV-HLH, we aimed to clear the viraemia by administering four doses of rituximab 375mg/m² at two to three weekly intervals. Treatment was required on two occasions with successful clearance (Figure 1), resulting in resolution of symptoms and splenomegaly.

Hence we describe rituximab clearing EBV leading to remission from XIAP deficiency related HLH.

Table 1. Family History of Case

	Case	Twin Brother	Older Brother
<i>Age at Presentation</i>	17 years	14 years	10 years
<i>HLH</i>	+ (17 years)	+ (19 years)	-
<i>Recurrent HLH</i>	+	-	-
<i>Splenomegaly</i>	+ 19cm	+ 20cm	+14cm
<i>Colitis</i>	+ (19 years)	+ (14 years)	+ (10 years)
<i>Hypogammaglobulinaemia</i>	+	-	-
<i>Hidradenitis Suppurativa</i>	+	+	+

Figure 1. Rituximab (R) and EBV Viral Load



P194. Establishment of a pathology north remote blood film morphology review to assist laboratory scientists in the Hunter region

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Aim

The Haematology unit in the Hunter is based at CMN. Laboratory staff working after hours and/or at other sites are sometimes faced with abnormal blood films that require urgent haematological review. Until recently, these reviews would have to wait until the blood film could be manually delivered to the attending registrar by Courier. In order to expediate patient review, the aim of this project was to implement easy to use photography and real time viewing of blood films across four hunter region hospitals.

Method

A dedicated, experienced staff member was assigned to the project and visited the 4 major hospitals (JHH, Maitland, Mater and Belmont) during Dec 2015. Existing microscope equipment was located and set up to be compatible with camera software and ensure systems were fully operational. Education sessions were conducted with staff both individually and in groups to guide them through method of operation, microscope adjustments at various magnifications, archiving images, and how to share information electronically across sites. Standard operating procedures were developed and tested.

Results

Full cooperation and enthusiasm was received from all staff at all sites. Protocols were developed for both Nikon and Olympus microscopes. Still images were the method of choice since real time streaming was not always efficient due to slow internet connections. Since its establishment, the system has been used on 19 occasions and enabled earlier confirmation and diagnosis of 2 new leukaemias and 1 Thrombotic thrombocytopenic purpura. The remaining occasions served to increase lab technician confidence in result reporting.

Conclusion

The new system has been embraced by staff and proven to be beneficial to facilitating timely patient care. Future plans include expansion of the network to all sites within Pathology North and capitalising on the new NBN internet to allow live streaming.

P195. Differences in quality of life in long-term stem cell transplant survivors meeting and not meeting recommended exercise guidelines

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Aim

Exercise has received increased attention as a supportive therapy for cancer survivors with both physical and psychosocial benefits reported. The majority of cancer survivors, however, do not achieve the minimal amounts of exercise required for health benefits. The aim of this study was to examine differences in quality of life (QoL) between long-term haemopoietic stem cell transplant (SCT) survivors meeting and not meeting recommended exercise guidelines.

Method

Consecutive adult participants ≥ 2 years post-transplant in ongoing remission were recruited from an established post-SCT long-term follow-up clinic at the Alfred Hospital, Melbourne, between July 2012 and June 2016. Physical activity over the previous week was assessed using the four-item Godin questionnaire. A Godin score ≥ 24 units was considered consistent with post-cancer treatment exercise recommendations of low-moderate intensity sessions 3-5 times/week for at least 20 minutes. Concurrently 5 QoL domains (physical, social, emotional, functional, transplantation specific) were assessed using the Functional Assessment of Cancer Therapy with transplantation subscale (FACT-BMT). Demographic and clinical variables including SCT type and time since transplantation were obtained by medical record review.

Results

163 participants (51% male) were recruited with a median age of 47.0 (range, 18.4–74.1) years. Median time since either autologous (23%) or allogeneic (77%) transplantation was 5.3 (range, 2-25.9) years. Only 25% (41/163) of participants met exercise guidelines. These survivors had better self-reported physical and emotional wellbeing, overall and transplant-specific QoL but not social wellbeing compared with those not meeting recommendations (Table 1). QoL score differences between the two groups meet proposed standards for clinically important differences for overall and transplant-specific QOL. These analyses were unchanged after controlling for age, gender, type of transplant or time since transplant.

Conclusion

These data corroborate research examining exercise behaviour in other cancer survivor groups and provide preliminary data to support a randomised controlled trial on exercise and QoL in this population.

Table 1: QoL differences in long-term SCT survivors meeting and not meeting exercise guidelines (n=163)

QoL variable (possible score range)	Not meeting guidelines (n=122) Mean (SD)	Meeting guidelines (n=41) Mean (SD)	Difference (95% CI)	Effect size (Cohen's d)	p value
FACT-G (0-108)	84.0 (16.8)	90.5 (13.2)	6.5 (0.8, 12.2)	0.43	0.03
FACT-BMT (0-148)	112.5 (21.4)	122.2 (17.1)	9.7 (2.4, 17.0)	0.49	0.009
Physical wellbeing (0-28)	22.4 (5.3)	24.3 (3.8)	1.9 (0.4, 3.4)	0.40	0.02
Social wellbeing (0-28)	21.9 (5.0)	23.1 (4.2)	1.2 (-0.5, 2.9)	-	0.18
Emotional wellbeing (0-24)	19.0 (4.2)	20.5 (2.6)	1.5 (0.1, 2.9)	0.42	0.01
Functional wellbeing (0-28)	20.6 (5.7)	22.6 (5.7)	2.0 (-0.1, 4.0)	-	0.06
Transplant specific (0-40)	28.7 (5.5)	31.7 (5.2)	3.0 (1.0, 4.9)	0.55	0.003

P196. Methoxyflurane is a suitable replacement for intravenous sedation during bone marrow biopsy in the Haematology Day Ward setting

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Aim

Bone marrow biopsy (BMB) procedures under local anaesthetic (LA) are a source of patient concern and discomfort. The use of intravenous sedation (IVS) with midazolam+/-fentanyl in addition to LA, whilst effective, changes the requirements for nursing care, anaesthetic team involvement and procedural time. Pentrox (Methoxyflurane, Medical Developments International, unit cost \$37) is a short-acting inhaled volatile anaesthetic agent available in a patient controlled device which seemed a suitable IVS replacement for BMB in the haematology Day Ward.

Method

The impact of Pentrox introduction for BMB was assessed prospectively by patient-completed questionnaire. Patient eligibility for Pentrox was in accordance with guidelines published by Spruyt *et al* 2013. Data collection points were BMB pain scores, IVS use, total procedural time and patient/ staff satisfaction.

Results

Data was collected on 92 BMB procedures. Baseline (pre-Pentrox) data was collected for 26 BMB, 10 of who received IVS plus LA. Baseline BMB pain scores were lower in IVS patients (mean 3.4 vs 4.6) at the cost of 30 minutes longer BMB. During the Pentrox assessment period a further 66 BMB were assessed, 51 of these (77.9%) occurring with Pentrox. Reported pain scores were 4.4 with mean BMB time of 58.9 minutes, 42.7 minutes in post BMB period for Pentrox BMB. Two patients required unanticipated IVS in the context of difficult procedures with IVS failing to reduce pain scores (9). Pentrox BMB patients included 27 who had prior BMB experience (13 of 27 with IVS). Pentrox BMB pain scores were lower than recalled for their non Pentrox BMB (4.3 vs. 6.8, n=21).

BMB systemic therapy (number)	Pentrox (51)	No Pentrox	
		None (28)	IVS (13)
Department BMB time (min)	58.9	65.5	113.9
Department post BMB time (min)	42.7	40.7	75.6
BMB pain rating	4.3	4.4	3.0
Pain rating of prior BMB (number)	6.8 (27)	5.4 (14)	6.0 (8)

Conclusion

BMB procedural time was not prolonged on account of Pentrox use. Pentrox BMB procedure times were comparable to LA only BMB and were rated favourably by patients who had undergone prior BMB. Pentrox has virtually eliminated the use of IVS for BMB with no negative impacts observed.

P197. The burden of febrile neutropaenia: a single centre review of protocol based management outcomes and analysis of the MASCC risk index as a tool for identifying low-risk patients suitable for outpatient management

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Aim

A review of the febrile neutropaenic (FN) patients admitted to our service and identification of the low-risk cases that could have been considered for safe outpatient management.

Background

FN is a common complication of chemotherapy, causing significant morbidity/mortality as well as placing considerable clinical and economic burden on services, with a median cost of \$AUD 764.08 and \$AUD 5640.87 per ED episode and admission respectively¹⁻³. Guidelines mandate prompt empirical IV antibiotics and hospitalisation.²⁻⁵ The MASCC Risk Index has been widely studied, with evidence that a score of ≥ 21 reliably predicts for a low risk of complications^{4,6-8}

Patients and Methods

All cases of FN admitted to Fiona Stanley Hospital (FSH) from 04/02/15–18/05/15 were reviewed. Inclusion: *current cytotoxic chemotherapy, objective OR subjective fever (recorded at home) and admission*. Patients with other causes for admission were excluded. Data was analysed with respect to a MASCC score ≥ 21 , absolute neutrophil count, antibiotics within 30min, LOS and mortality. Statistical analysis was performed using SPSS v.18.0.

Results

70 cases were identified, mean age of 61.7 years, 74% oncology and 26% haematology. The mean MASCC Score was 22.31 with a higher score noted for haematology patients (23.17 vs. 22.01). 76% patients had a MASCC Score ≥ 21 . Only 40% of patients diagnosed as FN had an ANC < 1.0 . The mean LOS was 5.36 days with an all cause mortality of 34.3%. No significant relationship was observed between MASCC scores and LOS or OS.

Discussion

Our study highlights the limitations of current protocol driven approaches to FN management. Compliance with protocols is clearly impeded by ED time and resource pressures. The majority of febrile 'cancer' patients are admitted when potentially, providing the availability of appropriate resources, a significant proportion could be re-stratified for outpatient management without compromising their overall care, thus reducing the inpatient burden.

Conclusion

Hospitals should consider incorporation of a risk stratification tool such as the MASCC Risk Index into their FN protocols to better identify low-risk patients potentially suitable for outpatient management.

P198. Risk Assessment for Thromboprophylaxis and Hospital Acquired Venous Thromboembolism at the Royal Hobart Hospital

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Background

Venous thromboembolism (VTE) is a common and life-threatening condition that affects thousands of Australians annually¹. VTE is 100 times more common in hospitalised patients² and active cancer is an independent risk factor for VTE^{3,4}.

Aim

To establish the documentation of VTE risk assessment and provision of appropriate thromboprophylaxis in cancer patients who suffered a hospital-associated VTE at the Royal Hobart Hospital.

Methods

Cases of hospital associated VTE - deep venous thrombosis (DVT) or pulmonary embolism (PE) – admitted under Cancer Services were identified using Royal Hobart Hospital Clinical Coding data. Electronic medical records were retrospectively examined for evidence of documented VTE risk assessment and to determine whether appropriate thromboprophylaxis was prescribed.

Results

Nine hospital-associated events were identified. Only 1 of 9 (11%) underwent VTE risk assessment however 6 of 7 (85.7%) of eligible cases received appropriate prophylaxis. The majority of events (88.9%) occurred despite prophylaxis or therapeutic anticoagulation.

Conclusions

VTE risk assessment is rarely documented in oncology and haematology patients who go on to have hospital-associated VTE. The majority of patients who had a hospital-associated VTE event received appropriate prophylaxis and the majority of events occurred despite appropriate prophylaxis, supporting the need for additional trials of alternative prophylaxis doses or methods in this high-risk cohort.

No conflict of interest to disclose

P199. Audit of erythropoietin use in haematology disorders at a tertiary referral hospital

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Introduction

Erythropoietin (EPO) is frequently used in the haematology clinic to promote transfusion independence in patients with anaemia from various causes. International guidelines are available for patients with malignancies, and for specific disorders (e.g. myelodysplasia - MDS). These describe patient selection, dosing and response criteria for EPO. Non-adherence to these guidelines could lead to inappropriate use of this costly agent.

Aim

We audited current practice in haematology at a large tertiary referral hospital (Fiona Stanley Hospital) against published US and European guidelines.

Method

We reviewed haematology patients prescribed EPO from 1/4/2015 until 1/4/2016. 33 patients were identified, with 2 patients excluded due to death prior to 8 weeks on treatment. Electronic record systems were used to review indication, dose and response.

Results

Indications for EPO were MDS (11/31) and multiple myeloma (MM) (5/31) amongst 6 other indications. 29/31 were over 65 years. Mean haemoglobin at initiation was 78 g/L (Range 54-97). 5 patients were transfusion dependant. 29/31 patients had eGFR <60, nil on dialysis. 14/31 used EPO alpha and 17 used Darbepoetin. 7/31 had EPO levels measured prior to commencing.

At 8 weeks, 11/31 had their EPO dose adjusted and three patients were ceased as had successfully met Hb targets. At 16 weeks, 77% had achieved a response (10/31 CR and 14/31 PR). Those with CR included patients with MDS (5), Myeloma (1), and anaemia chronic disease (2). Only 6/31 had ceased including three treatment failures. Four (13%) patients did not meet criteria for response but continued EPO, 3 remaining transfusion dependent.

Conclusion

The majority of patients were appropriately prescribed EPO according to guidelines which resulted in 77% achieving at least partial response. In those that failed EPO was generally discontinued, but in a minority therapy was continued without evidence of benefit.

P200. Systemic AA amyloidosis associated with malignant carotid paragangliomae – the importance of supportive care

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Introduction

Paragangliomas are neoplasms within the paravertebral sympathetic and parasympathetic chains.

The carotid body is a common site and these can grow extremely slowly.

There are only a few cases worldwide causing systemic AA amyloidosis (AA). We present a case of remarkable clinical improvement after peritoneal dialysis (PD), highlighting the need for supportive care.

Results

A 59 year old man presented with nephrotic syndrome, stage V CKD, and hypoalbuminaemia (14 g/L), 8kgs weight loss over 12 months, chronic diarrhoea, reduced left ventricular function and symptomatic postural hypotension.

Renal biopsy confirmed AA.

Past history included 30 years of inoperable right carotid paraganglioma with L3/L4 vertebral metastases, planned to be treated with novel radio-oncological treatment, Lu-TATE, when AA was diagnosed.

Our patient was refused for PD by his local hospital, citing poor prognosis; however, after discussion, PD was commenced at the Victorian and Tasmanian Amyloidosis Service.

Difficult social issues, depression, malnutrition, hypotension and fatigue

made PD training challenging, as well as finding the optimal clearance regimen.

Dextrose from PD fluid was found to provide additional calories, while absorption of PD fluid helped raise blood pressure.

Oral nutritional supplements was commenced to balance the catabolic state, as well as an antidepressant.

Lu-TATE was commenced to treat paragangliomae.

18 months later, the patient had settled on PD, was emotionally stable, normotensive and free of peripheral oedema. Follow-up PET scanning revealed a decrease in paragangliomae bulk.

Discussion and Conclusions

Carotid paragangliomae are rare causes of AA. New treatments are available that slow the progression to AA.

This case reinforces the role of PD, even in advanced cases. Intensive supportive care and PD led to a improvement in symptoms and quality of life and allowed Lu-TATE treatment, resulting in reduction in paragangliomae load, further symptom relief and no doubt, improvement in overall survival

P201. Comparison of the Adverse Effects and Efficacy of Intravenous Iron Polymaltose Versus Ferric Carboxymaltose – a Retrospective Cohort Study

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Aim

To compare the adverse effects and efficacy of intravenous iron polymaltose (IP) infusions with ferric carboxymaltose (FCM) infusions in anaemic gynaecology and obstetric patients.

Method

A retrospective observational study examined 220 anaemic obstetric and gynaecology patients who received either IP (n=110) or FCM (n=110) within a 5-year period. Medical records were reviewed to collect patient demographics, dosage, adverse effect documentation and pathology results. Treatment safety was evaluated by comparing the incidence and nature of documented adverse effects. Efficacy was evaluated by comparing a subset of 65 patients from each cohort who had post-infusion haemoglobin (Hb) results available and had similar baseline Hb levels (70-115g/L).

Result

Adverse effects were documented for 3 of 110 (2.7%) FCM patients and 18 of 110 (16.4%) IP patients. The majority of the adverse effects experienced by patients were of a systemic nature but no incidence of anaphylaxis was documented.

The mean baseline Hb levels were 87.8g/L (± 10.4) for IP and 89.7g/L (± 9.7) for FCM ($p > 0.05$). Increased Hb levels were achieved in both the IP and FCM cohorts within 1-12 weeks post infusion. The mean post-infusion Hb levels were greater in the IP cohort than the FCM cohort [115.1g/L (± 14.0) vs 110.1g/L (± 13.0); $p < 0.05$]. The greater Hb increase in the IP cohort may be attributed to the significantly higher number of IP patients than FCM patients who received doses calculated as adequate for their Hb deficit.

Conclusion

Intravenous FCM infusions resulted in fewer incidences of adverse effects in comparison to IP for the treatment of anaemia in gynaecology and obstetric patients. Further studies may be required using patients who have received doses in compliance with clinical recommendations to evaluate comparative efficacy.

P202. The Utility Of Routine Chest Radiography In Patients With Febrile Neutropenia

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Background

Febrile neutropenia (FN) is a common complication of myelosuppressive chemotherapy. Due to their immunocompromised state, fever may be the only sign of serious infection. As such, there are recommendations to perform a chest radiograph (CXR) on all patients with FN, even in the absence of respiratory signs and symptoms. However, there is a paucity of strong evidence for the routine use of CXR in FN.

Aim

The goal of this study is to determine the utility of routine chest radiography, for the detection of pneumonia and its role in management decisions in patients with FN.

Methods

Clinical records of 428 admission episodes from 2011-2015 of haematology patients, >age 16 years on chemotherapy presenting to the emergency department or on the ward with FN were retrospectively reviewed. Only patients who have received treatment for a haematological malignancy within the past 2 months were included. Respiratory symptoms (dyspnoea, chest pain and cough) and signs (crepitations, wheeze, decreased breath sounds, increased respiratory rate and hypoxia) prior to initial chest radiograph for FN was recorded. CXR was then evaluated for the detection of pneumonia. Any pre-existing lung conditions were also noted. Univariate analysis was used to determine any significant associations with a clinically abnormal CXR.

Results

Of the 428 admission episodes, 323 patients had a CXR after being diagnosed with FN. Preliminary results show that only 2 out of 172 CXRs (1.5%) performed in asymptomatic patients had a result clinically significant for pneumonia. In contrast, in the patients with respiratory signs and/or symptoms 21 CXRs out 120 (17.5%) were clinically significant for pneumonia. Having at least a respiratory sign or symptom had an odds ratio of 17.13 (P<0.001, CI: 3.94 – 74.40) for having an abnormal CXR. Only 5 of the 323 (1.5%) CXRs resulted in a change in antibiotic management. These results question the usefulness of policy recommending CXR on all patients with febrile neutropenia.

Conclusions

CXR should not be performed routinely in patients with FN in the absence of respiratory signs and/or symptoms.

Eliza Hawkes has accepted travel expenses from Takeda and research funding from Merck Serono and Bristol Myer Squibb.

P203. Music therapy for the alleviation of pain during bone marrow biopsy

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Aim

To examine the effects of music intervention with standard care compared with standard care alone for analgesia during bone marrow biopsy.

Method

Standard analgesia at Canberra Hospital involves the use of subcutaneous lignocaine and intravenous midazolam. Patients were randomized to music with standard analgesia versus standard analgesia alone. Verbal pain score is obtained at time of aspiration. Baseline and post procedure questionnaires were completed by all patients.

All patients over the age of eighteen fluent in verbal and written English were consented. Blocked randomization by sequential enrolment following a computer generated random sequence was utilized. Ninety-six patients will be enrolled into the study by completion ($1-\beta=0.80$, $2\alpha=0.05$).

Baseline data collected includes: age, gender, presence of an accompanying family member, previous bone marrow, baseline and anticipated visual pain analogue scores, body mass index and operator experience. Verbal pain score was obtained at bone marrow aspiration during the procedure. Post procedure pain and satisfaction scores, as well as dose of midazolam used were obtained.

Result

Interim results of 30 patients are presented. No significant differences in baseline characteristics of gender, accompanying family member and previous bone marrow were observed ($\chi^2=0.20$, $p>0.50$; $\chi^2=0.02$, $p>0.50$, $\chi^2=0.74$, $p>0.10$ respectively). In addition, there were no significant differences in age, body mass index or midazolam doses used ($t=0.13$ $p>0.20$; $t=0.38$ $p>0.20$; $t=0.315$ $p>0.20$). These results suggest effective randomization.

Outcome variables seen in table one below have thus far been non-significant.

	Music	No Music	T statistic	P value
Verbal pain during aspirate	5.3	5	0.475	$p>0.20$
Post procedure pain score	4.75	4.96	0.368	$p>0.20$
Average post procedure satisfaction scores	9	9.5	0.136	$p>0.20$

Conclusion

Early interim analysis suggests there is no significant difference in pain or satisfaction with the use of patient preferred music in conjunction with standard analgesia.

P204. Dapsone-related oxidative haemolysis and methaemoglobinaemia: significance following treatment for haematological malignancy

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Aim

Dapsone is used as second-line prophylaxis for immunocompromised patients at-risk of *Pneumocystis jirovecii* pneumonia (PJP). Methaemoglobinaemia and oxidative haemolysis are known complications of dapsone therapy even with normal glucose-6-phosphate dehydrogenase (G6PD) activity. Risk factors predictive of dapsone intolerance and its frequency in different patient populations are unknown.

Method

All patients dispensed dapsone during 2014 at a large adult tertiary public hospital were identified through pharmacy records. Retrospective examination of pathology testing, medications and clinical history was performed. Oxidative haemolysis was identified by blood film examination and detection of biochemical haemolysis by high LDH/bilirubin/reticulocyte count, low haptoglobin and methaemoglobin (MetHb) by venous blood gas analysis. Two-tailed P-values were calculated using Fisher's exact test.

Results

79 adult patients received dapsone (median age 48 years, 65% male). Dapsone was given in 73 cases due to sulfamethoxazole intolerance (n=45 haematological malignancies (51% post haematopoietic stem cell transplant (HSCT), 49% acute leukaemia), n=7 solid organ transplants, n=19 HIV, n=2 other conditions), for dermatological conditions in 4 cases, two unknown. Ten patients (12.6%) had MetHb testing, 35 (44.3%) had haemolysis testing; overall 47% (42 patients) tested. Negative G6PD test results in 16 (20.2%) patients; no PJP cases identified.

Dapsone toxicity occurred in 29 patients (37% of total, 67% of those tested): 22 (27.8%) oxidative haemolysis alone, 2 (2.5%) methaemoglobinaemia alone, 5 (6.3%) both. Dapsone was continued in 4 (5.1%) patients long-term despite self-limiting haemolysis.

Higher rates of oxidative haemolysis/MetHb were found with concomitant administration of cytochrome P450 (CYP) inducers known to increase toxic dapsone metabolites (72% vs. 44% no CYP, p=0.02, RR 1.6), haematological malignancy (51% vs. 18% other conditions, p=0.025), dose >525mg/week (41% vs. 8% low dose, p=0.047). A trend to higher rates was seen with inpatients (43% vs. 23% outpatients, p=0.09) and those with bodyweight <50kg (17% vs. 4%; p=0.09).

Conclusion

High rates of oxidative haemolysis and MetHb limit the utility of dapsone in hospitalized patients with acute leukaemia or post HSCT, particularly at higher dose or with concurrent CYP inducers. Further analysis of a larger cohort, including trials of lower doses, may determine patients in whom continuation of dapsone is safe despite initial toxicity.

P205. Evaluating factors that influence Intensive Care Unit outcome in patients with haematological malignancy

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Aim

To evaluate the prognostic factors that influence mortality of patients admitted to the Intensive Care Unit (ICU) with a background of haematological malignancy.

Background

Haematological malignancies are usually life-limiting conditions. Limitations of care need to be decided early, based on acceptability to the patient, family, physician and community. Inappropriate ICU admission and subsequent treatment is likely to result in significant physical, psychological and economical burden. The goal was to identify factors that contribute to ICU mortality before admission to ICU.

Method

Electronic medical records, laboratory results and Intensive Care data for all patients (n=77) with haematological malignancy admitted to the Calvary Mater (Newcastle) ICU between 1 January 2013 and 30 June 2015 were retrospectively analysed. Age, gender, condition, Eastern Cooperative Oncology Group (ECOG) and Charlson Comorbidity scores were compared to ICU outcome. Predictions of prognosis based on recognised tools were compared to ICU outcome.

Results

A quarter of subjects (27.3%) did not survive ICU admission, while an additional 9.1% did not survive the hospital admission. Of the 55 survivors from ICU, 23 patients (41.8%) survived for up to and longer than 12 months. Age, gender, condition and ECOG scores were not significantly associated with ICU outcomes. Predicted survival of <12months at diagnosis, based on HM prognosis, was significantly associated with ICU mortality (p = 0.045). Based on overall comparisons of Charlson Scores to ICU mortality, a higher pre-ICU Charlson Comorbidity score may be associated with ICU mortality (p = 0.056).

Conclusion

Selection for ICU admission for patients with haematological malignancy should include consideration of haematological prognosis and Charlson Comorbidity scores. Prospective validation would be useful for advance care directives for such patients.

P206. Hypokalaemia In Haematology Patients Taking Posaconazole Extended Release Tablet Therapy

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Posaconazole is a broad-spectrum triazole anti-fungal therapy approved in Australia for prophylactic treatment in neutropaenic patients at high-risk of developing invasive fungal infections. The posaconazole tablet was introduced into clinical practice in 2015 to replace a suspension formulation, boasting more predictable absorption, tolerability and a similar side effect profile as its predecessor.

Aim

This study measured the incidence of hypokalaemia in haematological patients receiving posaconazole and the requirement for intravenous potassium replacement, with the aim of determining if this incidence has clinical significance for the management of patients receiving posaconazole prophylactic therapy.

Methods

The study is a retrospective case review of 42 patients receiving posaconazole tablet (22) or suspension (20) therapy for at least two consecutive weeks while under the management of the Haematology Department at Fiona Stanley Hospital in 2015.

Results

The results of this review highlighted a higher than expected incidence of hypokalaemia, with 59% of those receiving the tablet formulation experiencing hypokalaemia, with 84% of those patients requiring intravenous replacement of potassium and/or magnesium.

Conclusion

The phase three clinical trial data of the pharmacokinetics and safety of the posaconazole tablet in 210 Haematological patients reported only eight incidents of treatment related hypokalaemia (3%) and 46 (22%) incidences of all-cause events^{3,4}. These results were far different from the high incidence observed in this retrospective case review. The incidence of hypokalaemia in the case review required active management with intravenous replacement and was not clearly associated with gastrointestinal losses or drug interactions. The findings of this retrospective study have prompted further investigation of the incidence and mechanism of hypokalaemia in patients receiving posaconazole prophylaxis, with the aim of clarifying the relevance to clinical management of patients.

³ Merck & Co., Inc. Noxafil (posaconazole) package insert. Whitehouse Station, NJ; 2014

⁴ ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2016 January. Identifier NCT0177763, Pharmacokinetics and Safety of Posaconazole Tablet in Participants at High Risk for Invasive Fungal Infections (MK-5592-065/P05615) [cited 2016 March 12]; Available from: <https://clinicaltrials.gov/ct2/show/study/NCT0177763?term=posaconazole&rank=5§=X401256>

P208. Delayed haemolytic transfusion reactions - time for a national registry

Akers C, Bielby L, Atkinson H, Bates G, Crispin P, Cole-Sinclair M, Davis M, Wood E, Hennessy C, Glazebrook B, Magrin G, Maxwell E, McArdle S, Noutsos T, Rogers R, Kelsey G, Botting K, Yttrup A, Harper A, Hogan C

Blood Matters Serious Transfusion Incident Report Expert Group

Aim

To report the number and types of delayed haemolytic transfusion reactions (DHTR), reported to Blood Matters Serious Transfusion Incident reporting (STIR) system since 2011, including specificity of implicated antibodies. To consider how these reactions could be minimised.

Method

De-identified voluntarily reported data is validated and analysed by the STIR Expert group.

Results

Between 2011-2015, 34 reports received. Thirty validated as DHTR, 2 events excluded due to insufficient information, two attributed to other causes. Eleven patients had a known antibody prior to transfusion and went on to develop a new antibody. For six patients there was no available transfusion/antibody history at the health service in which they were transfused. All reported patients had received red cells, often multiple units, with a small number also receiving other products (FFP 3, platelets 2, cryoprecipitate 1).

Table 1: Implicated antibodies

Antibody	Number (%) (Some patients had multiple allo-antibodies reported)	Thirteen patients (40%) required a temporary increase in care, 11 (37%) no
Kidd	15 (50)	
Rhesus (excluding D)	9 (30)	
Duffy	6 (20)	
MNS	4 (13)	
Kell	3 (10)	
Unspecified	3 (10)	

increase in care and 5 (17%) had associated increased length of stay. One patient is recorded as being admitted to ICU, requiring haemodialysis post-reaction. Outcomes were not recorded for two patients.

Conclusion

Although a relatively rare complication of transfusion, DHTR could be minimised by sharing information about those patients with a past history of red cell antibodies. In many instances those clinically significant antibodies may not be detectable at subsequent pre-transfusion antibody screening, and hence the importance of the antibody history being available. The 2008-09 & 2011-13 STIR reports recommended the development of a regional or national database to enable laboratories to access established patient antibodies results, to prevent re-exposure to antigen-positive units, and possible DHTRs as a consequence.

P210. Blood Stewardship, Bringing It All Together...

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Aim

To develop a blood stewardship policy directive for SA Health.

Method

The 2010 Australian Health Ministers joint statement on stewardship expectations for blood and blood products was used as the basis for the policy directive. Specific SA activities and requirements were built into this through consultation with relevant blood sector stakeholders.

Result

A consolidated blood supply stewardship program and accompanying policy directive for SA Health was developed. Stewardship is more than just an overarching statement of expectations. Embedding the principles into everyday practice is the enabler of lasting change. These include:

- Implementing the relevant national patient blood management (PBM) guidelines applicable to each clinical unit's specialty/service type through the establishment of specific criteria that represent appropriate use of blood and blood products.
- Specific PBM initiatives, which currently include a focus on anaemia management, access to IV iron and cell salvage techniques.
- Maintaining optimal inventory holdings and rotation of blood products. SA has achieved the lowest red cell wastage rate in the country (1.3% compared to national 2.9% for year to date April 2016), and also a significant reduction in platelet wastage (from 21.4% in 2014 to 6.0% in 2016) through adoption of these activities.
- Regularly linking hospital and pathology data to provide detailed information on blood and blood product utilisation to public hospitals, their transfusion committees and individual clinicians.
- Activities such as haemovigilance, contingency plans and national registries.
- Strong governance arrangements, including a State-wide Blood Management Council.

Conclusion

While many of the above activities occur through good management practice and the goodwill and passion of local champions, the mandate given by the policy directive ensures that they are applied consistently across SA Health. The principles enshrined in the policy directive are also strongly recommended to all non-public health care providers who utilise government funded blood and blood products.

P211. The incidence of clinically significant antibodies detected in late pregnancy In Rhesus D positive pregnant women of the Northern Territory

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Aim

To assess the proportion of Rhesus-D positive pregnant women with negative antibody screens at booking who developed clinically significant antibodies in late pregnancy. Current ANZSBT guidelines do not recommend re-screening at 28 weeks if initial screen was negative. In contrast, the BSCH guidelines make a positive recommendation.

Method

We used TrakCare Lab (Laboratory Information System) to extract data on blood group and antibody screen over the calendar year 2013 of women who received antenatal care in the Northern Territory. Medical records of women with positive antibody screen were reviewed regarding neonatal outcomes.

Results

Eighty six (3.4%) of 2612 Rhesus-D positive women had positive antibody screens and 22 were excluded based on pre-specified exclusion criteria. Of the remaining 64 (2.5%) 51 were Aboriginal; 45 out of 64 had clinically non-significant antibodies detected and 19 (0.73% of total) had clinically significant antibodies (Table 1).

Table 1. Clinically significant antibodies detected in late pregnancy in RhD positive women with initial negative antibody screen

Antibody (N19)	Number of pregnant women	%
Anti-c	6	28.5
Anti-M	5	23.8
Anti-Kpa	1	4.8
Anti-e	1	4.8
Anti-E	5	23.8
Anti-s	1	4.8
Anti-S	2	9.5

Six out of 19 women with clinically significant antibodies had a negative antibody screen at booking. In the remaining 13 women, 6 had incomplete information and in 7 the antibody was previously identified. Two of the 6 women were Aboriginal. Three babies in this group developed mild neonatal jaundice requiring phototherapy. There was no severe haemolytic disease of the fetus/newborn (HDFN) recorded in our study population. No cases of HDFN resulted in association with clinically non-significant antibodies.

Conclusion

Given the small incidence of clinically significant antibodies (0.73%) and no demonstrable adverse clinical outcomes, the results of our study do not support a need for routine antibody screening in Rhesus-D positive pregnant women.

P212. Method Validation Project Column Agglutination Technology for Blood Group Allo-antibody Titrations in Antenatal setting

Arunachalam S

Aim

Antibody titrations are performed as a semi-quantitative screening tool in the antenatal setting for monitoring titres. A rise in titres gives reason for obstetrician referral and could indicate requirement for foetal monitoring. Currently in New Zealand (NZ), antenatal antibody titrations are performed using indirect antiglobulin test (IAT) by tube technique. Published data on the sensitivity and reliability of results produced by Column Agglutination Technique (CAT)¹, warrants this investigation of antenatal titration using this technique.

Methods

Validation of titrations being performed using CAT was examined by analysing data collected from previous studies and results from the Royal College of Pathologists of Australasia (RCPA) transfusion science quality assurance programme. The RCPA surveys were for Anti-D antibody titres only. Data from 2014 and 2015 was analysed of which 466 were tube titres and 187 were CAT titres². A review of reproducibility of titres was also carried out using commercially derived Anti-D reagent, titrated by tube and CAT.

Results

There was concordance in titres reported by tube and CAT from the RCPA surveys of 2014 and 2015, including titres which were within one dilution of each other. This is considered as an acceptable difference by ANZSBT, as well as the RCPA committee. Evaluation of reproducibility of titres by CAT using commercially derived anti-D showed no significant difference in titres between tube and CAT.

Conclusion

Results of this study provided evidence to recommend titrations can be performed by CAT when the identified antibody is Anti-D. CAT has been found to be the most reliable and consistent method for performing titrations. Because the data collected from RCPA surveys were based on anti-D antibody, recommendation can be only made for Anti-D antibody titrations. This recommendation can be extended to other antibodies when more data is collected.

P213. Storage-Related Morphological Changes in Red Blood Cells: Telling the Whole Story

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Introduction

It is widely accepted that red blood cells (RBC) in storage undergo a morphological transformation from biconcave discocytes to “spiky” echinocytes. To minimise haemolysis, storage solutions are hypertonic and this transformation is thought to be a consequence of hypertonic storage. In this study we investigated RBC morphology during routine storage in SAGM and the shape change when stored RBC were returned to plasma.

Methods

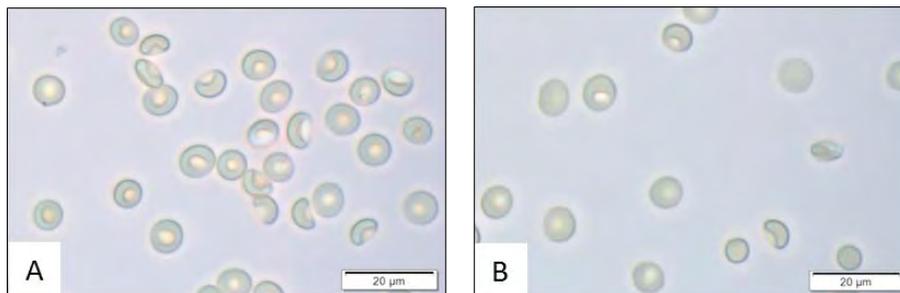
The morphology of packed RBC, manufactured using standard procedures, was evaluated by microscopic examination weekly during storage (42 days). RBC of differing morphology were counted following 2 hours equilibration, in either fresh SAGM or ABO compatible donor plasma, at 4°C or room temperature (RT). Size and shape were evaluated for more than 200 RBC for each condition.

Results

For the duration of storage the majority of RBC maintained a discocytic or curved stomatocytic morphology. Echinocytes were evident from day 23 of storage, however represented <10% of the RBC. There was little change in diameter for the sampled population during storage in SAGM. When reconstituted in plasma, from day 23 onwards >70% of RBC assumed an echinocyte morphology and cell diameter was reduced. These results were consistent for both 4°C and RT.

Conclusion

The majority of RBC did not develop an echinocytic morphology when stored in SAGM, but maintained a discocytic or stomatocytic morphology. Shape changes were observed when reintroducing the cells to a physiological environment. While RBC stored for <23 days retained their discocytic morphology when reconstituted in plasma, after 23 days of storage, the majority of RBC adopted an echinocytic morphology, a change likely to be a consequence of the “storage lesion”. Examination of RBC returned to a physiological environment provides a measure of RBC quality different from examination in the storage solution.



RBC in SAGM at 4°C A) 2 days; B) 42 days

P214. Review off Autologous Stem Cell Collection and Transplantation in Multiple Myeloma Patients in Waikato, NZ from 2012-2015

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Autologous Stem Cell Transplantation continues to play an important role in the management of patients with Multiple Myeloma. It is usually done in the 1st remission when the patient has responded to initial chemotherapy treatment. Younger and fit patients are often collected to achieve a double collect with one collection transplanted at a later date.

The review will look back on Multiple Myeloma patients collected in Waikato, NZ and review the outcomes of transplantation in these patients. This will also include the time period when these patients achieved remission and how many of these patients underwent the second transplant.

Results will be compiled and compared with the outcomes from published literature.

P215. Economic Aspects Of Using Thrombelastography (TEG) For Reducing Blood Product(S) Utilization In Cardiac Surgery

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Saud Al Babtain Cardiac Center

Aim

Retrospectively testing the economic impact of Thromboelastography (TEG) guided transfusion therapy regarding transfusion of allogeneic blood products in cases of bleeding after cardiac surgery. We hypothesized that the TEG- guided treatment reduces the transfusion of allogeneic blood products and consecutively decreasing hospital stay with a reduction of overall cost per patient.

Methods

Data of patients who suffered from post cardiac surgery bleeding were retrospectively collected for a period of 32 months. 100 patients were included in this study. Patients were divided into two equal groups; TEG group and Non-TEG group. All relevant data for patients were collected and compared in terms of number and cost.

Results

On comparing both groups, the number and cost of transfused fresh frozen plasma (FFP) to patients in TEG group were reduced with a very significant statistical difference (P.value=0.0016). Also, the number and cost of transfused platelets (Plts) to patients in TEG group were reduced with a very significant statistical difference (P.value = 0.0018).

No statistically significant difference (P.value=0.8943) was founded between two groups regarding the number and cost of transfused red cell concentrate (RCC).

Also, there was no statistically significant difference regarding the cost of hospital stay between both groups (P.value=0.5906).

The overall cost of hospital stay, blood and blood products transfusion and TEG test was lower in patients of TEG group by average of 1924 US\$ per patient.

Conclusion

TEG-guided transfusion reduces the number and cost of transfused FFP and Plts in patients with post cardiac surgery bleeding, but it does not affect the hospital stay. A transfusion therapy based on TEG results reduces transfusion requirements for complex cardiac surgery when compared to transfusion based on conventional coagulation assays. Furthermore, the TEG also helps in identifying coagulopathies in patients with massive bleeding.

P216. Introduction of electronic prescription for fresh blood products

Behan D

St Vincents

Aim

A tertiary hospital commenced electronic prescription for fresh blood products in March of this year, aligning blood prescription with medications in the same electronic prescribing system. This poster demonstrates implementation stages, and audit results

Methods

1. Liaison with pharmacy on prescription wording, formatting and restrictions. 2. National Blood Authority Patient Blood Management Guidelines indications for transfusions were used - recommended transfusion time and default single unit red blood cell transfusion, although this can be overridden. 3. Transfusion form altered to remove blood prescription. 4. Trial period of electronic prescription on haematology ward. 5. Update blood transfusion policy and procedure; notify medical and nursing staff about the change to electronic prescription. Hospital wide implementation of electronic prescription, except in operating theatres, Emergency Department and outpatient areas. 6. Audit process to assess compliance and any problems with the hospital implementation.

Results

An audit was conducted one month after the introduction of electronic prescription. With the introduction of electronic prescribing all prescriptions had indication for transfusion although almost 50% had different indication than what was written/ inferred from the medical records. Signatures were signed 100% of the time (being electronic) compared to 90% from a prior audit.

Conclusion

The introduction of electronic prescription for fresh blood products has been commenced. The changeover has been fairly smooth with no major problems. The advantages include legible prescription, prescription in the same location as medications, which will increase awareness of the prescription. The discrepancy between electronic indication for transfusion and written in notes is of concern, one suggestion is that medical staff are selecting any indication for transfusion for the electronic prescription. We are presently examining the possibility of forcing prescribers to type out indication for transfusion as part of the prescription process.

P217. Over-Utility of CMV-negative and Irradiated Blood? – Room for Clinical Practice Improvement

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SA Pathology Flinders Medical Centre

Aim

Cytomegalovirus (CMV)-seronegative and irradiated blood products are prescribed to prevent transfusion-transmitted CMV infection and transfusion-associated graft versus host disease respectively. These products significantly increase the costs of blood supply. An audit was performed to examine the appropriateness of transfusion of modified blood products in a tertiary haematology department.

Method

All red cell transfusions given to haematology patients between January and May 2016 were identified. CMV and irradiation status of blood products received, haematological diagnosis and treatment, and CMV serology status of patients were retrieved from electronic records. Indications for CMV negative and irradiated blood according to Australian and British guidelines were compared.

Result

773 units of red cells were transfused to 88 patients during the study period. Australian, but not British, guidelines recommend CMV negative blood for CMV negative recipients of highly immunosuppressive chemotherapy and/or haemopoietic stem cell transplants. CMV negative blood was requested for 20 patients, of whom 13 were CMV seropositive or untested for CMV serology (Table 1). Most patients receiving irradiated blood had a possible, or no, indication for its use as per Australian (ANZSBT) guidelines (Table 2).

Table 1. CMV-status of patients and blood requested (no. of patients)

ANZSBT indication	Blood requested for CMV negative patients (n=11)		Blood requested for CMV positive/unknown patients (n=76)	
	CMV negative	CMV untested	CMV negative	CMV untested
Yes	6	1	9	21
No	1	3	4	42

Table 2. Indications for irradiated blood

Diagnosis (no. of patients)	ANZSBT 2011 guidelines	British 2010 guidelines	Irradiated blood prescribed (no. of patients)	
			Yes (n=38)	No (n=50)
Autologous stem cell transplant (4)	Definite	Yes	4	0
Nucleoside analogue therapy (4)	Definite	Yes	2	2
Hodgkin lymphoma (2)	Definite	Yes	1	1
B or T-cell malignancies (24)	Possible	No	14	10
Acute leukaemia (16)	Possible	No	10	6
Myelodysplastic syndrome (24)	No	No	6	18
Myeloproliferative neoplasm (9)	No	No	1	8
Anaemia from other causes (5)	No	No	0	5

Conclusion

Many CMV negative blood products were prescribed for patients who were CMV seropositive, had no serology testing, or had no indication. Universal leucodepletion opens current indications for CMV seronegative blood for debate. Australian, compared with the British, guidelines includes many more patients with “possible” indications for irradiated blood, the benefit of which is unknown. Despite a trend of over-utility of modified blood products, some patients with definite indications were missed. Strategies for clinical practice improvement include (1) standardised and regularly updated documentation of indications for modified blood products, (2) reflex CMV testing for patients with unknown serological status prescribed CMV negative blood, and (3) vetting CMV negative blood issue to CMV positive patients.

P218. A retrospective review of the clinical relevance of alloimmune red blood cell alloantibodies in the Northern Territory

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Aim

To determine the prevalence of red blood cell (RBC) alloantibodies in the Northern Territory (NT) and their clinical relevance. Most studies report the prevalence of alloantibodies in the patient population is 2-3% with anti-E and anti-K the most prevalent.

Method

We used TrakCare Lab (Laboratory Information System) to collect data on antibody screens and consequent antibody identifications performed at Territory Pathology laboratories over the 2012 calendar year. Patients that had clinically significant (IgG) antibodies detected had further data collected, when available: sex, ethnicity, transfusion history and/or pregnancy history, the number and specificity of RBC alloantibodies, and whether RBC alloantibodies were detected at the initial group and hold or after at least one prior negative antibody screen.

Results

11563 antibody screens were performed on 7844 patients in 2012. 540 patients (6.9%) had a positive antibody screen. 101 patients (1.3%) had at least one clinically significant IgG alloantibody detected with 55 patients having previous red cell transfusion history. 24 of the 101 patients were men (23.7%) and 77 (76.2%) were women. 65 (64.4%) patients were Aboriginal. Anti-E was the most commonly detected antibody (48.5%) followed by anti-Fy(a) (10.9%) and anti-K (9.9%). 439 patients (5.6%) had clinically insignificant cold IgM alloantibodies or recent prophylactic Rh(D) Immunoglobulin administration. 63 were men (14.4%) and 376 (85.6%) were women. 52.6% of patients were Aboriginal. Prophylactic anti-D was the most common antibody detected (40.8%) followed by anti-Le(a) (17%) and anti-Le(b) (16%).

Conclusion

This study was an effort to characterise blood group alloantibody formation in the NT population. Our results show that the overall prevalence of RBC alloantibodies in the NT was 6.9%, which is significantly higher than the 2-3% that most studies report.

P219. Approach to the adaptation of the criteria for BloodSTAR

Cameron J, Heland R, Hetzel P, Roberts J

National Blood Authority

The evidence based [Criteria for the Clinical Use of Intravenous Immunoglobulin \(IVIg\) in Australia](#) (*the Criteria*) was first published 2007 to assist clinicians to identify the conditions and circumstances for which the use of Intravenous Immunoglobulin (IVIg) is appropriate and therefore able to be funded under the National Blood Agreement. The Criteria were reviewed in 2012 (Version 2).

In 2012 Australian governments commissioned the independent [Review of the Clinical Governance and Authorisation Process for Intravenous Immunoglobulin](#), a review of the adequacy of the current IVIg authorisation and governance arrangements. A key finding of the review was to implement an ordering and outcomes database to manage the use of IVIg.

BloodSTAR is currently being [implemented nationally in a staggered roll out between July and December 2016, and](#) is the new immunoglobulin management system developed by the National Blood Authority on behalf of all Australian Governments. BloodSTAR will support prescribers, nurses, dispensers and authorisers in managing their Ig Governance obligations as set out in the [National Policy: Access to Government Funded Immunoglobulin Products in Australia](#).

To maintain equity of access throughout the implementation period, at go-live the BloodSTAR system will be based on *the Criteria* Version 2 and the current request forms. Some adaptation has been required because there are certain fields in BloodSTAR that could not be populated directly from Version 2 as they were absent or ambiguous, or only referred to indirectly. Each qualifying criterion allows for supporting evidence to be provided.

The National Blood Authority Ig Governance Specialist Working Groups have commenced a review of the Criteria to Version 3. These strengthened criteria provide much more detail around qualifying requirements, and describe evidence required to support any claim against the criteria.

Once all jurisdictions have transitioned to BloodSTAR successfully, implementation of full Version 3 criteria will commence.

P220. Evidence-Based Patient Blood Management Guidelines for Neonatal and Paediatric Patients

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Introduction/Aim

The National Blood Authority (NBA), Australia has funded and managed the development of evidence-based Patient Blood Management (PBM) guidelines, comprising six modules: Critical Bleeding/Massive Transfusion (2011), Perioperative (2012), Medical (2012), Critical Care (2012), Obstetric and Maternity (2015) and Neonatal and Paediatrics (2016). To support the implementation of the guidelines, the NBA manages the development of materials to support implementation at a health provider level. This abstract presents the findings of the Neonatal and Paediatrics module.

Methods

The module was developed collaboratively by professional partners in systematic review and guideline authorship and a Clinical/Consumer Reference Group (CRG), comprising consumer and indigenous representatives and experts in the areas of fetal-maternal medicine, neonatology, paediatric haematology, paediatric anaesthesia, intensive care, cardiac surgery, oncology, haemoglobinopathies, nursing and PBM. Systematic review questions addressed the effect of red blood cell (RBC) and other blood component transfusion on patient outcomes, the effect of non-transfusion measures to increase haemoglobin on patient outcomes and the effect of strategies to minimize blood loss and/or reduce RBC transfusion. The Module's recommendations were approved by the National Health and Medical Research Council on 21 March 2016.

Results

Twelve recommendations for or against the use of a number of interventions were developed. These included RBC transfusion in critically ill patients and sickle cell disease (SCD), erythropoietin-stimulating agents in preterm infants, hydroxyurea in SCD, fresh frozen plasma based primes and recombinant factor VIIa in cardiac surgery, intravenous immunoglobulin for rhesus disease of the newborn, prevention of hypothermia and anti-fibrinolytics in surgical patients. Consensus-based practice points and expert opinion points were also developed in the absence of high quality evidence.

Conclusions

The Module will aid decisions on whether to transfuse in the context of specific patient circumstances, and the full range of other available treatments, balancing the evidence for efficacy and improved clinical outcome against the potential risk.

P221. Pre-hospital transfusion - Auckland New Zealand

Clark T

NZBS

Aim

This project is a joint initiative between Auckland Helicopter Emergency Medical Service (HEMS) and New Zealand Blood Service (NZBS).

NZBS provides whole blood units for transfusion in a pre-hospital setting, where patients are at risk of bleeding to death before reaching a hospital emergency department.

Method

When the project began at the end of 2014, NZBS was providing one emergency O negative whole blood unit for pre-hospital transfusion. As of 5th October 2015, NZBS moved to providing two units of whole blood. The units needed to be stored and transported in such a way that the cold chain would be preserved and wastage was kept to a minimum.

In order to achieve this, we are using a "Golden Hour Container" Credo Cube. (Series 4 model 272) This cooler was validated for storage of whole blood or red cell units for a period of up to 72 hours when held at ambient temperature.

Each whole blood unit is placed into a plastic transport bag with its own Temperature Datalogger. Each bag is sealed with a Tamper Label. The Credo Cooler carry bag is "locked" closed with a cable tie. The Credo Cooler containing the whole blood units is stored at the HEMS base in a refrigerator. At Auckland Blood Bank we store a conditioned Credo Cooler and a second set of O negative whole blood units in our cool room. The boxes are rotated every 48 hours or when required, if units have been transfused.

Results

Since introducing the 48 hour rotation we have wasted no whole blood units due to uncontrolled storage conditions. Robust Standard Operating Procedures are in place. Blood Bank staff have been trained to pack the Credo Cooler and are capable of downloading and interpreting the Datalogger information. HEMS clinical crews have been trained in the indications for and techniques of blood transfusion.

Conclusion

Since the introduction of this project, 14 (as of 17/06/16) patients have received safe pre-hospital whole blood transfusions.

P222. The impact of elearning and PBM

Clark T, Thomson A, Catherwood A, Verrall T, Peterson D

BloodSafe eLearning Australia

Introduction

BloodSafe eLearning Australia (BEA) develops and delivers online courses designed to support national patient blood management (PBM) and/or transfusion guidelines. Courses are developed by transfusion and elearning experts and undergo an extensive peer-review process prior to release. Courses are interactive and include case studies, clinical scenarios and videos. A certificate is provided upon successful completion of online assessment.

User feedback is sought through voluntary, online surveys upon completion of each course. The information provided is used to evaluate and improve the BEA program, to ensure that courses are meeting their objectives of enhancing knowledge of patient blood management and safe transfusion practice in order to improve patient outcomes.

Results

Analysis shows that:

- there are more than 375,000 registered learners with more than 500,000 course completions
- an average of 14,800 courses are completed each month
- approximately 1% of learners provide feedback (>2,700 users in the last 15 months)

Analysis of user feedback shows the courses:

- are easy to use - 67%
- are relevant to clinical practice - 60%
- improved their knowledge of transfusion and/or PBM - 89%
- assist them to identify a near miss or adverse event - 62%
- help to change clinical practice - 82%
- Improve patient outcome/safety - 86%

Survey respondents identified a number of areas where transfusion practice and processes could be improved in their clinical setting including:

- reviewing blood ordering practices in stable, non-bleeding patients to ensure one blood unit and then review
- implementing a massive transfusion protocol
- implementing a postpartum haemorrhage emergency box
- optimising patient's haemoglobin prior to elective surgery

Conclusion

Survey respondents indicated that online education courses provided by BEA improve transfusion practice and PBM knowledge enabling learners to identify practice changes that may improve patient outcomes.

P223. Does questioning donors about acute retroviral syndrome assist in maintaining HIV transfusion safety?

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Background

The risk of transfusion-transmitted HIV in Australia is mitigated by the Blood Service Donor Questionnaire (DQ) and donor selection guidelines. The DQ identifies donors reporting rash and lymphadenopathy during the previous year as indicators of possible acute retroviral syndrome (ARS), a variable clinical manifestation of recent HIV infection. Identified donors may be deferred for 12 months following symptom resolution.

Aim

To evaluate the relevance and impact of questioning and deferring donors for possible ARS.

Method

Data were extracted from Blood Service databases regarding donors who declared ARS on the DQ. The proportion of donors deferred for possible ARS and the proportion that later returned, including the time to their return, were calculated and compared with other 12 month deferrals. The infectious disease status on subsequent donation of all donors that declared possible ARS was assessed. Separately, donors that tested HIV-positive were assessed to determine the proportion with symptoms of ARS.

Results

Of donors who declared symptoms consistent with ARS, 65.6% were deferred. Only 34.5% of these donors later returned to donate. Compared to other 12 month deferrals, individuals deferred for possible ARS were significantly less likely to return. Of deferred donors who returned none tested HIV-positive on subsequent donation. None of the 56.9% of donors who tested HIV-positive and experienced symptoms of ARS reported rash and lymphadenopathy in combination.

Conclusion

The majority of donors deferred for symptoms consistent with ARS did not return to donate, and of those who returned, none subsequently tested HIV-positive. Deferral for possible ARS was associated with a lower return rate compared to other 12 month deferrals. Although the majority of donors who tested HIV-positive experienced symptoms of ARS, none experienced the exact symptoms questioned on the DQ. This study provides an evidence-based assessment, which may potentially trigger modification of the DQ to better reflect contemporary understanding of HIV.

P224. ORTHO VISION™ Analyser – Results of Field Application Trials Conducted At 2 Major Centres

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Background

The ORTHO VISION™ analyser is a recent addition to the list of fully automated immunohematology instruments available in Australia.

Currently, ORTHO AutoVue® Innova analysers are used for routine and specialised immunohaematological testing in the transfusion laboratories at two busy Melbourne paediatric and adult trauma centres. Field Application Trials of the ORTHO VISION™ analyser were undertaken by these laboratories as an opportunity to use the instruments in their respective settings and to provide feedback on the general ease of use and system efficiency of the analysers.

Study Design and Methods

Field Application Trials of the ORTHO VISION™ analysers were conducted in the laboratories of the Royal Children's Hospital (RCH) and the Royal Melbourne Hospital (RMH), Parkville VIC Australia between October 2015 and January 2016.

Two or more staff members at each site completed on-line and extensive key operator training on the ORTHO VISION™ analyser prior to commencement.

The Field Application Trial involved the completion of a series of structured test activities/cases using the ORTHO VISION™ analyser and completion of the associated questionnaire for each case, per site.

Test activities/cases were undertaken to evaluate:

- System Training
- System Configuration
- Quality Control (QC)
- Maintenance, Start-up and Shutdown
- System Efficiency
- Serial Dilution
- Remote Result Review

Results

Key findings from the Field Application Trials of the ORTHO VISION™ analyser were:

- System training was easy and intuitive and on-line training tools were easy to follow
- System configuration, QC setup and maintenance activities were easy to perform
- System efficiency of the ORTHO VISION™ was improved when compared to ORTHO AutoVue® Innova
- Serial Dilution functionality was easy to use and results for IgG antibodies tested were found to be comparable with tube antibody titre results.

Conclusion

The RCH and RMH laboratories found the ORTHO VISION™ analysers to be efficient, easy to operate and with improved functionality over the current ORTHO AutoVue® Innova analysers.

This research was supported by Ortho Clinical Diagnostics. The company had no role in preparing this abstract or influence in the results/feedback given.

P225. Making Changes To A Disjointed Process; How Outcomes Improve When the Appropriate Areas Are Targeted

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¹ *Fiona Stanley Hospital*, ² *Serco*

Aim

To improve the delivery of blood and blood products to clinical areas within safe national time standards (30 minutes).

Method

Data was collected from the third party provider databases on multiple occasions between February 2015 and June 2016, correlating with changing the Agility Question set and introducing an education module based on BloodSafe eLearning, with assistance from the third party provider's education officers. As mandated by the third party provider, once the module was live on Learning Management System (LMS), it must be completed on day 3 of employment.

Results

900 jobs for blood product collection/delivery were initiated via HelpDesk between February 2015 and April 2016. 65 jobs were initiated in February 2015; baseline average time for job completion, 30 minutes. 96 jobs were initiated in March 2015, 1 month after changing the Agility question set; average time improved to 23 minutes.

Follow up post implementation of the education module in June 2015 to LMS, indicated between June and September 2015, 28 of 162 porters accessed the module (17%). Follow up in January 2016 revealed 130 of 162 porters accessed the module (80%). Some Porter positions were vacated between January and June 2016, total staff numbers fell to 140.

860 blood product collection/delivery jobs were initiated in April 2016; average time to completion, 20 minutes. This result fell within the key performance indicator (KPI) of 20 minutes, set by the third party provider and Transfusion Medicine Safety Nurses. As of June 2016, 140 porters completed the LMS module (100%).

Conclusion

Prior to Agility questions set changes and the introduction of LMS module, blood product initiation to delivery process was not streamlined for safe delivery of blood and blood products. As these improvements have demonstrated asking the precise questions at initiation of a job and educating the correct staff population can improve delivery.

P226. Standard Operating Procedures: How Small Alterations Can Improve Practice and Policy Adherence; RhD Immunoglobulins and an Obstetrics and Gynecology Service

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Fiona Stanley Hospital

Aim

To review prescribing, administration and documentation practices of medical and nursing staff within an Obstetrics and Gynecology (O&G) Service, to ensure adherence with National Standard 7 requirements and consistency with hospital policy when using RhD immunoglobulin (Anti-D).

Method

Retrospective data was collected via Transfusion Medicine Unit Database and Digital Medical Record, on all 275 patients who received Anti-D within the O&G Service, between February and November 2015.

Results

509 Anti-D doses were issued from Transfusion Medicine between February and November 2015. During this time, Anti-D was consented to on 6 different hospital consent forms, with 67% of patients having valid consent (N=340). Anti-D was prescribed on the correct blood product prescription form 6% of times (n=29), with 88% prescribed on a medication chart (inconsistent with the correct form in use) with 6% having no prescription. Compatibility labels were found on 7 different hospital forms, but only 7% (n=35) on the correct form.

Results were circulated to O&G Nurse Unit Managers. In collaboration with the Transfusion Medicine Safety Nurses, Standard Operating Procedure (SOP) for Anti-D was revised, staff were notified by email and further education.

Further review of prescription, administration and documentation practices post SOP revision indicated that Anti-D was consented to on 3 different forms, with 71% (n=42) of patients having valid informed consent. Post SOP change, Anti-D was prescribed on the correct blood product prescription form 73% (n=43) of times, with 3% having no prescription. Compatibility labels were found on 4 different forms, with 61% (n=36) on the correct form.

Conclusion

Prior to the SOP change, it was clear on analysis, prescription, administration and documentation practices were variable and not aligned with National Standard 7 requirements and hospital policy. With the SOP altered, adherence to using correct documents and post administration documentation remarkably improved.

P227. Lucky 13: identification of an antibody to a high-frequency Lutheran system antigen

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Australian Red Cross Blood Service

Background

In 2015 a 32-year-old female attended an Australian Red Cross Blood Service donor centre for a whole blood collection, following a five-year absence from blood donation. Routine mandatory testing revealed a positive antibody screen, in contrast to the donor's previous negative antibody history. Antibody identification testing was required to determine the antibody specificity and strength, and the resulting impact on blood component fate.

Method

Antibody identification was undertaken using red cell antibody identification panels, phenotyping and titration. DNA sequencing was performed using the TruSight™ One Sequencing Panel to confirm the presence of genetic variations associated with the donor's serological phenotype. Clinical records were reviewed to piece together a timeline of the donor's transfusion and pregnancy history, to hypothesise the likely stimulus for the antibody development.

Results

Anti-Lu13, an antibody directed against a high-frequency antigen in the Lutheran blood group system, was detected serologically by PEG-IAT method. An antibody titration result of 1 indicated a weak concentration of antibody in the donor's plasma, resulting in minimal restrictions to blood component usage. Phenotype testing demonstrated the donor's red blood cells to lack the Lu13 antigen, with these results corroborated by DNA sequencing of the *BCAM* (Lutheran) gene. Sequencing confirmed the donor to be homozygous for the c.1340C>T and c.1742A>T genetic variations that are associated with the absence of Lu13 antigens from the red blood cell surface, with a reported genotype of LU*02.-13/LU*02.-13. Clinical records indicated possible stimuli for the development of this anti-Lu13 antibody: transfusion of 6 units of homologous red blood cells in 1995, and two pregnancies between 2011 and 2014.

Conclusion

The clinical significance of anti-Lu13 red cell antibodies is not well understood, with very few examples having been reported due to the extreme rarity of the LU:-13 phenotype. Whilst Lutheran antibodies are generally considered to be clinically benign in transfusion and pregnancy, experts recommend transfusing LU:-13 blood to patients with anti-Lu13, or Lu(a-b-) blood if specific phenotype-matched blood is not available¹. Following the detection of this rare antibody, red cell components from two subsequent whole blood donations have now been cryopreserved for potential future transfusions.

P228. National Patient Blood Management Collaborative: Improving anaemia management for patients having elective surgery

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¹ Australian Commission of Safety and Quality in Health Care, ² KP Health

Aim

While blood and blood products can be lifesaving, their administration may also carry risks for patients. Blood transfusions can be avoided in many patients through effective patient blood management (PBM). PBM involves optimising blood volume and red cell mass, minimising blood loss and optimising the patient's tolerance of anaemia. The National Blood Authority's PBM Guidelines support clinicians to improve PBM. For elective surgical patients, pre-operative anaemia management reduces the likelihood a transfusion will be required. The Australian Commission on Safety and Quality in Health Care (the Commission) has developed the National Safety and Quality Health Service Standards including *Standard 7: Blood and Blood Products*. The intention of this Standard is that risks are identified and strategies are in place to ensure that a patient's own blood is optimised and conserved and that any blood and blood products they receive are appropriate and safe.

The Commission is leading the National Patient Blood Management Collaborative, to support improvements in the management of anaemia for patients undergoing specific elective gastrointestinal, gynaecological and orthopaedic surgery procedures. There are 12 Health Services from across Australia participating and the aim is to improve patient care by optimising haemoglobin and iron stores by the time of the elective surgery. The Collaborative applies the Model for Improvement to PBM across the patient journey, from the time the need for surgery is identified, through to when surgery is performed.

Method

Participating Health Service teams provide data on a monthly basis via a purpose designed qiConnect web portal. The measures used in the Collaborative include:

- The procedure performed (from an agreed range of diagnostic related groups).
- Whether the patient received a pre-operative assessment for anaemia, and/or iron deficiency
- The setting where assessment occurred (eg. hospital, general practice)
- Was the anaemia or iron deficiency confirmed?
- Where was it managed?
- Was there evidence of improvement?
- Units of red blood cells transfused (pre-, intra- and post- operatively).

Result

The Commission has led the learning workshops required for teams to share their experiences and learnings from their quality improvement processes, consult with experts in the field, gather new information and develop ideas for improvement. The Collaborative has data from over 5100 patient episodes for elective surgical procedures: gastrointestinal (20%), gynaecological (26%) and orthopaedic (54%). Of the total procedures, 90% received a haemoglobin test, 35% had iron studies and 34% had both tests prior to surgery.

Conclusion

By improving anaemia management for patients in the pre-operative phase of care, the Collaborative will contribute to: reduce the risk of post-operative infections and adverse reactions from blood products; reduce the risk of transfusion related inflammatory events; potentially reduce hospital length of stay; reduce the risk of readmission from infectious complications of transfusion, and reduce elective surgery cancellations. The Commission's Project Team will work with the National Blood Authority to share the resources developed by the Collaborative more broadly to support hospitals in adopting quality improvement approaches and improve PBM practices. The Collaborative will run to April 2017.

P229. The Role of Microparticles in Mediating Adhesion of Cryopreserved Platelets to Collagen

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Research and Development, Australian Red Cross Blood Service

The Role of Microparticles in Mediating Adhesion of Cryopreserved Platelets to Collagen

Aim: Cryopreservation of platelets is known to affect their *in vitro* quality and function. Despite having reduced recovery post-transfusion, there is evidence cryopreserved platelets may be more effective in reducing bleeding than conventional liquid-stored platelets. To better understand the mechanisms underlying this, the effect of cryopreservation and the role of microparticles (MPs) in platelet adherence to collagen under flow conditions were studied.

Method

Apheresis platelet concentrates were cryopreserved at -80°C by the addition of 5-6% DMSO. Thawed platelets were reconstituted in thawed fresh-frozen plasma. Pre-freeze and post-thaw samples ($n=8$) were flow perfused through collagen-coated micro-slide channels at a shear rate of 250 s^{-1} . The number of adhered MPs, platelets and thrombi was determined using Image Pro 7.1. Platelet adhesion markers and MPs ($<1\mu\text{m}$) were enumerated by flow cytometry. MP-depleted platelet samples were prepared by differential centrifugation. Non-depleted and MP-depleted post-thaw samples ($n=7$) were flow perfused as described above. For statistical analyses, paired t-tests were performed (significance $p<0.05$).

Result

Under shear stress, the number of platelets and thrombi adhered to collagen from pre-freeze and post-thaw samples were not significantly different ($p>0.05$), despite a significant decrease in the MFI of GPIIa, GPIIb, GPIIIa, GPVI, and GPIX post-thaw ($p<0.05$). However, there was a significant increase in the mean number of CD61+/annexin-V+ MPs ($p<0.0001$), and an increase in MPs adhered to collagen post-thaw. Depletion of MPs from the post-thaw samples resulted in a significant decrease in the number of platelets and MPs adhered to collagen under shear stress ($p<0.05$).

Conclusion

MPs from cryopreserved platelets facilitate adhesion of reconstituted cryopreserved platelets under shear stress conditions, despite decreased expression of platelet surface markers that mediate adhesion and aggregation. This study provides insight into the important functional role MPs may play in haemostasis when cryopreserved platelet products are transfused.

P230. Do donor- and/or processing- related differences impact on blood product quality?

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Background and Aim

During routine storage packed red blood cells (PRBC) undergo numerous biochemical and biophysical changes, including the accumulation of biological response modifiers (BRMs). We investigated changes in levels of BRMs relevant to storage, donor demographics and parameters associated with processing of PRBC units. We further assessed whether variation in BRM levels impacted on dendritic cell (DC) responses.

Methods

PRBC units (n=200) were stored at 2-6°C and sampled fortnightly. Donor- and processing- related parameters were recorded and potential BRMs were quantified in PRBC-supernatants (SN) using cytometric bead array (CBA) (Table 1). Multiple-regression analysis (95% CI) was used to determine whether differences in BRM levels were associated with donor- and/or processing- related parameters. Using an *in vitro* whole blood transfusion model, DC production of inflammatory mediators (Table 1) were assessed following exposure to PRBC-SN with either “high” or “low” BRM levels. Lipopolysaccharide (LPS) was included to simulate infection. Changes in immune profile were compared to matched “no transfusion” controls (P<0.05).

Results

Angiogenin, eotaxin and IL-9 levels declined during storage with no other storage related changes in BRMs evident. Of note, significant inter-unit variation in levels of BRMs was observed with a clear subpopulation of “high BRM” units identified. Longer processing times were associated with increased levels of E-Selectin, IL-1 α and IL-9. Both collection volume and PRBC volume were associated with increased levels of ICAM-1, VCAM-1 and P-selectin. PRBC volume was also associated with increased levels of RANTES and angiogenin. In the transfusion plus infection model, suppression of DC IL-1 α , TNF- α , MIP-1 α and MIP-1 β was observed following exposure to “high BRM” units compared to “low BRM” units.

Conclusions

Variations in BRM levels in PRBC units were associated with processing-related parameters rather than storage duration. High levels of BRMs may contribute to immune modulation in transfusion recipients. Further investigation into how processing-related parameters impact on BRM levels is warranted.

Table 1: Donor– and processing- related parameters for each PRBC unit, biological mediators assessed via CBA and inflammatory markers used to investigate immune modulation using *in vitro* transfusion assay.

Donor Parameters	Gender, Age, Blood group, BMI
Processing Parameters	Blood collection volume, PRBC volume, Time from collection to processing.
Biological Mediators	ICAM-1, VCAM-1, E-selectin, P-selectin, RANTES, sCD40L, Angiogenin, Eotaxin, IP-10, IL-1 α , IL-8, IL-9
DC Inflammatory Response	IL-6, IL-8, IL-10, IL-12, IL-1 α , TNF- α , MIP-1 α , MIP-1 β , MCP-1, IP-10

P231. Treatment of iron deficiency and iron deficiency anaemia with intravenous ferric carboxymaltose in pregnancy

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Treatment of iron deficiency and iron deficiency anaemia with intravenous ferric carboxymaltose in pregnancy

Iron deficiency, no anaemia (IDNA) and iron deficiency anemia (IDA) are common conditions in pregnancy.[1] Untreated they expose women to adverse outcomes, particularly should an obstetric haemorrhage occur.[2]

Aim

To evaluate the efficacy and safety of intravenous (IV) ferric carboxymaltose (FCM) administration to pregnant women with IDNA or varying severities of IDA.

Method

Prospective observational study. Data from 446 women with IDNA and IDA were analysed to assess the safety and efficacy of IDNA or IDA correction with IV FCM in the 2nd and 3rd trimester of pregnancy. Treatment effectiveness was assessed by repeat haemoglobin(Hb) measurements at 3 and 6 weeks post infusion, and ferritin levels where available. Safety was assessed by analysis of adverse events (AE), fetal heart rate monitoring before and after the infusion and newborn outcome data. Hb and ferritin data were analysed comparing across the four groups using Kruskal Wallis tests with post hoc analyses conducted using Mann Whitney U tests, adopting a Bonferroni correction. Frequency data were analysed using the Chi-Squared test. All analyses were conducted using GraphPad Prism 6 software (v0008); p values <0.05 were considered statistically significant.

Result

FCM significantly increased Hb values in women with IDNA and mild, moderate and severe IDA at 3 and 6 weeks post infusion (p<0.01 for all). In women with all severities of anemia, Hb values remained within the recommended range at 6 weeks post infusion. No serious AE were recorded, with minor effects occurring in 75 (17%) women. No adverse fetal or neonatal outcomes were observed. Ferritin significantly increased following the FCM infusion (p<0.01).

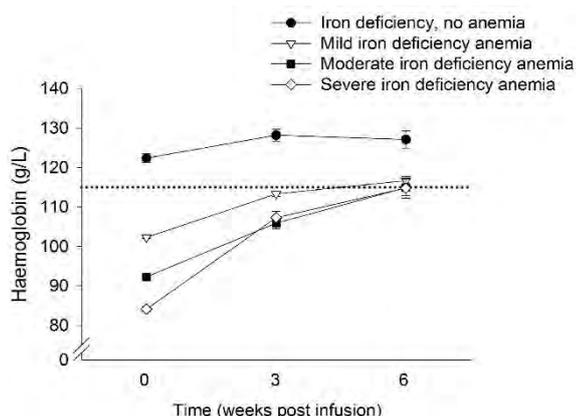


Figure1: Hb levels (mean ± SEM) Dotted line reflects Hb concentration (115 g/L). No anemia (n=50), mild anemia >95g/L (n=266), moderate anemia 90-64g/L (n=59), severe anemia <90 g/L (n=71).

Table1: Ferritin (ug/L) across testing period (median ± IQR).

	Antenatal screen	Pre-infusion	3 weeks post-infusion	6 weeks post-infusion	Post-partum
Ferritin (ug/L)	13 (7-28) n=297	6 (5-9) n=336	185 (95-312) n=75	136 (30-254) n=17	164 (88-120) n=20

Conclusion

This data supports the safe and effective use of FCM to treat and correct IDNA or IDA in pregnancy regardless of the severity of the anemia.

P232. Review of training to self-administer SCIg and the Health Related Quality of Life (HRQoL) impacts on patients changing from IVIg to SCIg treatment

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Background

Patients with immunodeficiency disease often require life-long immunoglobulin (Ig) support. This is provided by hospital-based intravenous Ig (IVIg) administered by nurse or patient-administered subcutaneous Ig (SCIg) following training.

Aim

To determine patient satisfaction with their training to self-administer SCIg and to measure the HRQoL impact for both modes of Ig treatment.

Method

Patients who consented to participate in the study, were mailed a project-specific questionnaire. Questions on patient satisfaction with training at the Sunshine Coast Hospital and Health Services (SCHHS) and on their HRQoL were based on a five point Likert Scales. The number of training sessions and infusion site was obtained from medical charts.

Results

Table 1: Satisfaction with SCIg training

Satisfaction (%)	n	very good	mostly good	ok	somewhat poor	very poor
written information	25	85	4	11	0	0
hospital training	26	92	4	4	0	0
support received from nurses	26	89	11	0	0	0

The mean number of training sessions required for patients to reach competency was 2.8 ± 1.5 sessions. The stomach was the preferred injection site for 21 patients and the upper leg in five patients. Data on HRQoL was only available for 20 patients. When comparing prior treatment (i.e. IVIg) to current SCIg treatment, patient reported an improved HRQoL (Table 2).

Table 2: Patient HRQoL response (n=20)

HRQoL (%)	best	good	neutral	bad	worst
Prior	5	0	30	20	45
on IVIg	9	48	38	5	0
on SCIg	71	9.5	9.5	5	5

Discussion and Conclusion

Patients achieved competency to self-administer SCIg earlier than the four scheduled training sessions and were very satisfied with the training. The SCIg program has improved the HRQoL for patients and none have asked to be switched back to IVIg. Further studies are underway to measure patient incurred cost for IVIg and SCIg treatment.

P235. Anti-U in a pregnant Sudan-born Australian

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PathWest

Aim

War and conflict displace millions worldwide from countries including Afghanistan, Syria, Somalia and Sudan. In response to global resettlement, ethnic diversity in Australia continues to change, impacting the blood group antigen and antibody diversity we now see in this country. We report a rare case of anti-U in a pregnant Sudan-born Australian.

Method

This case report concerns a 42 year old Sudanese pregnant woman (gravida 5, para 4) who presented at a Perth metropolitan hospital for blood group and antibody screen close to delivery. The blood group was O Positive and the antibody screen reactive (2+) with all three screening cells (Seqirus, Australia) by Biorad indirect antiglobulin test (IAT). The sample was referred to our tertiary laboratory for further testing. In preparation for caesarean section the clinicians were advised to avoid transfusion. At birth the neonate had normal Apgar scores. Mother and baby were discharged three days later with no clinical sequelae.

Result

The antibody was panreactive (2+) by IAT (Biovue, Ortho) with a titre of 1:8. The direct antiglobulin test (DAT) was negative. The antibody was resistant to papain, trypsin, AET and chymotrypsin. The patient's blood group typed as ccDee, K-, Jk(a+b-), Fy(a-b-), M+N-, S-s- U-. Additional IAT testing using ImmucorGamma Panocells revealed the presence of anti-U. The baby grouped as O Negative, U-positive with a negative DAT.

Conclusion

U-antigen is high frequency, found almost universally in Caucasians. However, in SubSaharan Africans it is absent (S-s-U-phenotype), due to deletion of the glycophorin B gene, in 1% to 35%. Whilst anti-U can cause mild to severe haemolytic disease (HDN) of the newborn, there was no evidence of HDN in this case. As provision of U-negative blood by the ARCBS requires advanced notification, it is important that the transfusion and obstetric community be aware of the immunohaematological problems of newer immigrant populations.

P236. Identification and Management of Anaemia in the Preoperative Patient - a Rollercoaster or Ferrous Wheel?

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Background

Undiagnosed anaemia is common in the surgical setting and is associated with increased perioperative morbidity and mortality, and longer hospital stays. The National Patient Blood Management guidelines Module 2 outlines the components of quality preoperative anaemia assessment and management, and provides a template for this purpose. Blood Matters audited the assessment and management of anaemia prior to elective surgery, and the impacts for patients going to surgery with untreated anaemia.

Aims

To determine what: processes are in place to assess and manage anaemia in elective preoperative patients is occurring in practice

Method

Part A audited processes for assessing and managing preoperative anaemia.

Part B assessed practice through retrospective audit of elective surgery patients.

Results

The existence of a preoperative haemoglobin optimisation pathway was reported by 18 health services (32%), 13 (72 %) of these included haemoglobin assessment/optimisation template.

*Health under-the of patients 11%)	In practice:	n (%)	services reported number anaemic (117, when
	• episodes audited	1142	
	• reportedly assessed for anaemia preoperatively	1057 (93)*	
	• assessed >28 days prior to surgery	335 (32)	
	• intra/post-operative transfusion rate (anaemic vs. non-anaemic)	30% vs 7%	
	• length of stay (anaemic vs. non-anaemic)	11 vs 7 days	

compared with Module 2 anaemia definition (212, 20%). This represents a missed opportunity to optimise red cell mass preoperatively.

Treatment for anaemia was instigated in 56 (48%) reported anaemic patients. Only 37 (66%) of the treated patients were re-assessed prior to surgery, with just 5 (9%) showing resolution of anaemia.

Conclusions

Although 93% (n=1,057) of preoperative patients were reported as screened for anaemia, few had quality assessment allowing timely identification and treatment. Untreated anaemic patients presenting to elective surgery were more likely to receive a perioperative transfusion and have a longer length of stay. Significant improvements are required for timely assessment and management of patients with preoperative anaemia.

P237. BloodSTAR – Australia’s immunoglobulin management system

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National Blood Authority

BloodSTAR is the new online system that is being implemented across Australia to manage patients who are authorised under the [Criteria for the Clinical Use of Intravenous Immunoglobulin \(IVIg\) in Australia](#) to receive government funded immunoglobulin (Ig).

BloodSTAR has been in development since early 2014, and had a comprehensive and wide-reaching stakeholder engagement process undertaken to ensure the system will work for different practices currently undertaken nationally. As well as process consultation throughout, the BloodSTAR team completed two rounds of user acceptance testing during the development phase to gather feedback on the system with users widely praising the ease of use.

BloodSTAR will be used by all prescribers, nurses, dispenser and authorisers across Australia who will submit and assess authorisation requests and manage the infusions and dispenses of Ig product to approved patients within their facility. BloodSTAR will streamline current paper based faxing processes increasing efficiency and transparency, while strengthening the approval process and improving data capture.

BloodSTAR is rolling out across Australia via a staged process, with each State/Territory going live between 14 July and 5 December 2016. The staged roll out is to ensure each jurisdiction has had the necessary training and support required leading up to their go live date, and to assist with the data migration from the existing database of patients that had a current authorisation beyond the go live date.

As demand for Ig products continues to increase by 10-12% every financial year it is imperative that Australia successfully implements changes and continues to work towards ensuring supply in the future by working collaboratively across the sector.

BloodSTAR will work towards product sustainability through standardising national practice and assist with the collection of data to ensure patients diagnosed with conditions that benefit from Ig will continue to always receive product into the future.

P238. Costs of Acute Care in Critically Bleeding Patients Requiring Massive Transfusion

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Aim

To describe the healthcare costs of critical bleeding events requiring massive transfusion (MT, defined as ≥ 5 units RBCs in 4h) and determine the factors associated with higher costs of care.

Method

Clinical costing units from sites participating in the Australian and New Zealand Massive Transfusion Registry (ANZ MTR) provided individual patient costs associated with acute episodes of inpatient care which included the MT. Costing data were linked with ANZ MTR data to obtain a dataset containing patient characteristics, transfusion and costs for patients requiring MT in 2011-2015. NZ costs were converted to \$AU using purchasing power parity and costs were converted to 2015 \$AU using ABS Health Consumer Price Indices. Where blood product costs were not included in the submitted costings, these were calculated using the number of units received and NBA published prices.

Results

15 hospitals provided costing data, with records from 14 sites linked to ANZ MTR data. The linked dataset contained records of 2782 MT episodes (from 2780 patients). The median [IQR] total cost for the acute episode of care was \$58,991 [\$31,502-\$108,745] with a mean (SD) cost of \$93,093 (\$119,897). The median total cost of blood products received was \$6,488 (mean \$9,050). There were significant differences in cost between the various bleeding contexts with liver transplant the most expensive and obstetric haemorrhage the least expensive. Regression analysis indicated that being male, younger, and having pre-existing comorbidities was associated with increased acute care costs.

Conclusion

Patients with critical bleeding requiring MT use significant financial resources, with the average cost of a MT admission 18 times more than the average cost of an acute hospital admission in Australia (\$4,966¹). Further research will model the economic impact of identified variation in practice and outcome, and estimate the potential cost savings with improved adherence to guidelines.

P239. A National Audit on the clinical use of Group O Rh(D) Negative Red Blood Cell Units in Australia

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Australian Red Cross Blood Service

Aim

The Australian Red Cross Blood Service has observed that group O Rh(D) negative red blood cell (RBC) units comprise an increasing proportion of the total RBC units ordered. Despite the overall decline in RBC usage, demand for this component is not abating. This audit was aimed at investigating how group O Rh(D) negative RBC units are used by Australian transfusion laboratories.

Method

Over a 5 week period, 9733 group O Rh(D) negative RBC units were issued to 305 laboratories. A one-page survey form was sent to the laboratories to capture data on each unit issued including the indication and urgency for transfusion or reason for discard, patient demographic information including their ABO and Rh(D) group and the component age at the time of transfusion.

Result

Data was returned on 7143 units (73% of surveys sent). There was balanced representation across laboratory size, geographic regions and public versus private sectors. The national discard rate was 8.2%. A total of 6387 units were transfused into an estimated total of 3008 patients (55% males, 44% females) with median patient age of 67 years. 47% of units were transfused to group O Rh(D) negative patients. 24.5% of the units were transfused into patients of other ABO groups. Of the units transfused into patients of other ABO groups, 58% were transfused to Rh(D) positive individuals. Median red cell age at transfusion was 21 days and 1565 units (24.5% of total number of units transfused) were chosen for transfusion as they were close to expiry, 81% of these were reported to be used in routine non-urgent transfusions.

Conclusion

The data appears broadly representative of the current Australian inventory management practices surrounding the use of group O Rh(D) negative RBC units. Strategies that might mitigate the demand for group O Rh(D) negative RBCs include increasing the panel of phenotyped blood units across all ABO groups and more regular rotation of units between sites.

Australian governments fund the Australian Red Cross Blood Service to provide blood, blood products and services to the Australian community.

P240. Application of a droplet digital PCR (ddPCR) assay for non-invasive detection of fetal *KEL*01.01* in isoimmunised pregnancies

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Australian Red Cross Blood Service

Aim

Prognosis for Haemolytic Disease of the Fetus and Newborn due to anti-K antibodies is complicated by poor correlation between antibody titre and disease severity. Prediction of fetal K antigen status in K negative mothers using cell-free plasma DNA can assist in patient management. Detection of the low abundance *KEL*01.01* single nucleotide variation (predictive of K antigen) against a background of maternal *KEL*02* (predictive of k) is challenging. This study aimed to assess accuracy of a fetal *KEL*01.01* ddPCR assay for two clinical cases.

Method

Maternal whole blood was collected from Case 1 and 2, 20⁺⁴ and 12⁺⁵ weeks gestation respectively. Cell-free DNA was isolated from 1mL plasma and 10 μ L added to a Taqman MGB assay optimised with the Bio-Rad ddPCR System. Amplitude threshold was set with reference to homozygous and heterozygous control DNA. Case 1 was tested in one replicate and Case 2 in 12 replicates.

Case 1 plasma was referred to the Molecular Diagnostics Laboratory of the International Blood Group Reference Laboratory, UK, for analysis.

Case 2 amniotic fluid was referred to Blood Service Red Cell Reference Laboratory for Kell genotyping using Immucor BioArray HEA Precise BeadChip TM.

Result

Case 1 produced 10 *KEL*01.01* droplets from a total of 16,953 (0.06% frequency). With a 9254 threshold, average amplitude was 12,791. Droplet amplitude and frequency predicted a K+k+ fetus, concordant with IBGRL results.

Case 2 produced 2 *KEL*01.01* droplets from 191,244 (0.001% frequency). Droplet amplitudes were 7409 and 7011, close to the 7000 threshold. This indicated a K-k+ fetus, concordant with amniotic fluid genotyping.

Conclusion

This report describes application of ddPCR for non-invasive detection of fetal *KEL*01.01*. The capacity of ddPCR to count single molecules overcomes some real-time PCR limitations, and fetal genotype was successfully predicted in two cases. Validation studies are continuing as clinical samples become available.

P241. Inappropriate Use of Fresh Frozen Plasma is All Too Common

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Aim

Fresh Frozen Plasma (FFP) is used by a range of clinical specialties in hospitals. Unfortunately, inappropriate FFP transfusions are common and may represent a large avoidable risk for patients. Given the availability of prothrombinex for warfarin reversal, we sought to assess the appropriateness of FFP transfusions given at a single tertiary centre.

Method

Retrospective audit of all FFP transfusions to inpatients at a single adult tertiary hospital (Sir Charles Gairdner Hospital) over a one month period (May 2015). Clinical indications were determined by review inpatient notes and laboratory data. Appropriateness of clinical indication was assessed by two doctors including a clinical haematologist, with reference to available guidelines. 'Transfusion episode' was counted as 24hr period from the first FFP transfusion.

Results

233 units of FFP were transfused to 43 inpatients in 59 'transfusion episodes'. Thirty-two of these were in the presence of bleeding (71.1%). The mean pre-transfusion INR was 2.01, while the mean post-transfusion INR was 1.83 (mean change in INR -0.21). Twenty-seven episodes (45.8%) were deemed to be consistent with guidelines, 12 (20.3%) were inconsistent with guidelines but nonetheless appropriate prescriptions, while 20 (33.9%) were inappropriate transfusions. The majority of inappropriate transfusions were for reversal of anticoagulants including warfarin (20%), or in patients with increased INR with no or only minor bleeding, or a minor procedure (55%).

Conclusions

One third of FFP transfusions at our centre were inappropriate. This exposes patients to avoidable transfusion risks and wastes a scarce resource. There is a need for better education of clinicians and improved governance of FFP use.

P242. Transfusion RCPAQAP survey scenario with passenger lymphocyte syndrome

Kahlyar H

RCPAQAP

Introduction

In 2016, Transfusion RCPAQAP sent out a survey based on a scenario in which a patient presented 12 days post liver transplant with a rapidly falling haemoglobin. The patient was B RhD positive and had received a donor liver from an O RhD positive female donor.

Method

The survey which included a patient sample, three donor units and the survey instruction sheet was sent to all laboratories enrolled in the general and basic compatibility modules. Participants were required to perform patient identification, ABO forward/reverse and RhD group reactions, ABO/RhD group interpretation, DAT, antibody screen and antibody investigation on the patient's sample. With the three donor units, participants were required to report ABO forward and RhD group reactions, ABO/RhD group interpretation, phenotyping for antigens corresponding to any clinically significant antibody identified and serological crossmatch against patient's plasma. Participants were also required to select units which were suitable for transfusion.

Results

Following the survey closing date, results were collated and reviewed by the Transfusion Advisory Committee. 420 participants returned the results for the survey. 17% of participants did not detect the presence of ABO antibody in the eluate screen, most likely indicating failure to include A and B cells along with the three group O screening cells.

Conclusion

The patient had Passenger Lymphocyte Syndrome (PLS) due to an ABO mismatched liver transplant (O RhD liver to a B RhD positive recipient). PLS occurs when viable immunocompetent donor lymphocytes are transferred in the transplanted organ during transplantation. Those immunocompetent donor lymphocytes may continue to produce alloantibodies (in this case anti-B) for a number of weeks post-transplant. Subsequently these antibodies react with the recipient's red blood cells resulting in severe haemolysis. A positive DAT may be observed in PLS requiring elution studies. A and B red cells should always be included when testing eluate samples.

P243. Red Cell Wastage Reduction in Western Victoria

Kerseboom M

Dorevitch Pathology

Background

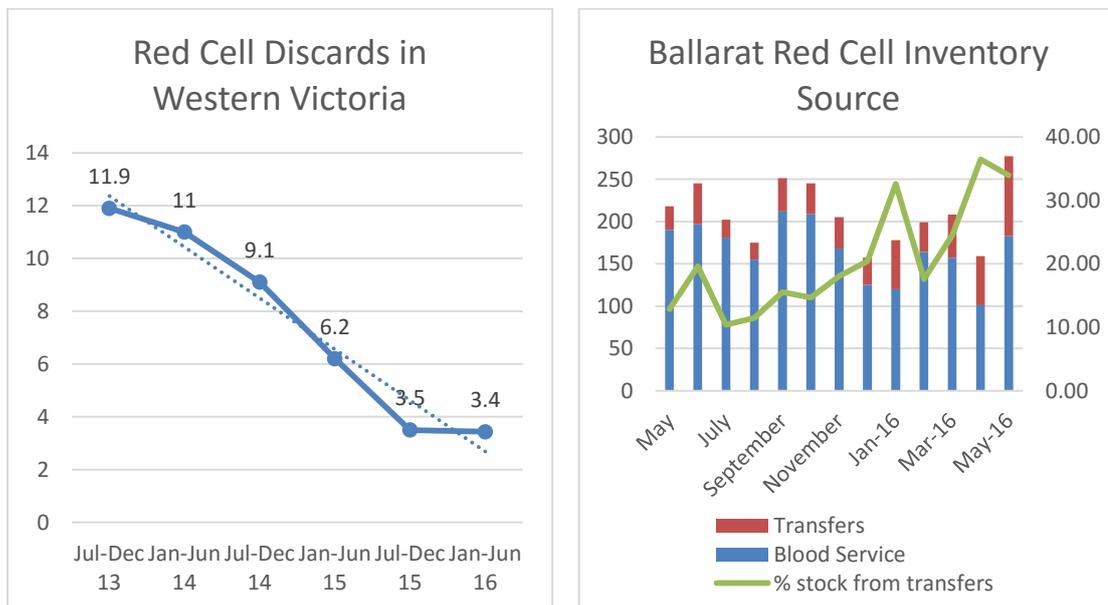
Red Cell wastage has become a major focus for all blood users in recent years, with greater accountability and the implementation of the National Blood and Blood Product Wastage reduction strategy 2013-17. While working in Adelaide I had seen firsthand how an effective Wastage Reduction plan could be implemented and decided to try and implement a similar plan when I moved to Western Victoria.

Implementation

Initially the plan was to focus on wastage reduction in one laboratory and then incorporate the smaller laboratories in the region. The first step was closer monitoring of what blood was crossmatched to patients and include this in our daily tallies. By including these units, we weren't over stocking our fridge if these units were not transfused. This change alone saw the wastage drop from 22.6% to 3.8% in one month. The next step was to reduce our overall stock holding. This was done in a gradual process over a period of 6 months. Overall we reduced our total holding from about 106 units to 82 units.

Stage two was to get the smaller labs in the region to return their short expiry blood to Ballarat, with the aim of reducing their wastage to zero, while not increasing Ballarat's wastage. In February 2015 we organised with the laboratories to use their routine couriers and the Blood Service shipper configurations to return red cells to Ballarat. For laboratories not on courier routes we planned to use V-line busses. After some testing with temperature loggers we were satisfied that the shipper and transportation worked, we began transporting units in May 2015.

Results



Future

Looking at moving platelets and FFP to reduce their wastage.

P244. Life in the fast lane- improving transfusion efficiency in transfusion dependent thalassaemia patients

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Aim

To improve current transfusion practice in Transfusion Dependent Thalassaemia patients at a tertiary Haemoglobinopathy centre, by determining whether increasing transfusion rates, reducing the use of frusemide and creating uniform practice across patients is possible.

Method

All patients receiving regular transfusions were assessed for cardiac function, current transfusion rates, use of frusemide, current weight and history of transfusion reactions. Patients with a history of cardiac impairment and transfusion reactions were excluded from the trial. Eligible patients' transfusion rates were increased to 5mL/kg/hr (in line with the American Cooley's Foundation guidelines) up to a maximum of 300mL/hr.. Concurrently, the use of post-transfusion frusemide was discontinued. Medical review was performed post-transfusion to ensure the increased rate was tolerated, to exclude fluid overload and document adverse reactions.

Results

54 patients (mean age of 37.4 years, 95% CI [35.3-39.6] with an average transfusion rate of 3.91mL/kg/hr, 95%CI [3.69-4.14]) were assessed. 4 patients were excluded due to documented cardiac impairment or previous transfusion reactions. 8 patients (16%) were on regular post-transfusion frusemide. This practice was discontinued in 7 with no documented transfusion-related fluid overload; one patient refused.

Outcome of Rate Change	n	%
No rate change, already at maximum	15	30
Rate increased to 5mL/kg/hr to a maximum of 300mL/hr	20	40%
Increased rate but refused to go to maximum calculated rate	6	12%
Refused change in practice	9	18%

During the trial, there was one documented transfusion reaction (palpitations). This patient was able to tolerate a higher transfusion rate (increased from 140 to 150mL/hr).

Conclusion

The transfusion rates were increased safely, with a calculated reduction in day-stay chair time of 17.45 hours per month and increased patient satisfaction. This confirms that the guideline of 5mL/kg/hr for Transfusion Dependent Thalassaemia patients with preserved cardiac function is safe and can be translated to other centres worldwide. We achieved uniform transfusion practice in 70 % of our patients and acknowledge that change in transfusion practice can be difficult.

P245. Routine prophylactic use of RhD immunoglobulin in Australia: comparison with international practice

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Background/Aim

Administering prophylactic RhD immunoglobulin (anti-D) to RhD negative mothers has decreased the incidence of haemolytic disease of the fetus and newborn (HDN) with its progressive introduction since the 1970s. Australian NHMRC *Guidelines on the Prophylactic Use of RhD Immunoglobulin (anti-D) in Obstetrics* was last published in 2003, whereas the National Institute for Clinical Excellence (NICE) reviewed its guidelines in 2008, influencing the 2014 update of British Committee for Standards in Haematology (BCSH) guidelines for the use of anti-D immunoglobulin for the prevention of HDN. The aim of the study is to compare current Australian RhD immunoglobulin prophylaxis guidelines with international guidelines such as BCSH.

Method

A literature and internet search of published articles and guidelines related to prophylactic anti-D in the prevention of HDN was undertaken, limited to articles 1970-2016, in English.

Results

Variations in guidelines and practice exist internationally. Similar recommended RhD immunoglobulin timeframes at 28 and 34 weeks gestation exist across Australian, UK, US and Canadian guidelines, although there are slight differences in dosage recommended. BCSH (UK) provides the possibility of one large dose at 30 weeks. Sweden has no routine antenatal anti-D prophylaxis program, but investigation into screening for fetal *RHD* genotype in RhD negative mothers is published. Offering RhD immunoglobulin to RhD negative mothers of *RHD* positive fetuses will match similar screening programs in Denmark and The Netherlands. The recent BCSH are considered the most comprehensive of the published RhD immunoglobulin guidelines.

Conclusion

Limited data are available on Australian obstetric patients for international comparison. The effectiveness and economics of a two-dose versus larger one-dose regimen is not yet addressed, neither is compliance with attendance and RhD immunoglobulin administration during pregnancy. Advent of fetal blood typing by free-fetal DNA testing warrants consideration in practice guidelines, catering for evolving technologies and possible reduction in RhD immunoglobulin requirements.

P246. Allogeneic and autologous serum eye drops - efficacy and tolerability compared

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Aim

Serum eye drops (SED) are used to treat severe dry eye disease and persistent corneal epithelial defect and are usually autologous. These may be inappropriate or impossible for some. Allogeneic SED are used for such patients. Potential problems with allogeneic SED include immunological reactions and blood-transmissible infections. We compared the efficacy and tolerability of allogeneic SED and autologous SED.

Method

The study population was New Zealand patients who switched from autologous to allogeneic SED, or vice versa, as of 30th April 2016. Patients were identified from the New Zealand Blood Service database. A questionnaire regarding indications, symptoms with each type of SED (modified from the standard OSDI questionnaire), reason for change, and side effects, was sent to participants. Non-responders were phoned after 3 weeks.

Results

Of 72 eligible participants, 8 had died and 3 had incorrect addresses. Questionnaires were posted to 61 patients. Thirty-three (54%), mean age 70 years, responded. Six (18%) were male, and 27 (82%), female. Age and M:F ratio was similar in non-responders. Indications included dry eye, exposure and herpes zoster keratitis, radiation keratopathy, limbal stem cell deficiency, neurotrophic cornea, other ocular surface disease, and promotion of healing post corneal grafting. Twenty-seven responders (82%) and 6 (18%), respectively, switched from autologous to allogeneic SED, or vice-versa. Broadly, reasons for switching SED included difficult veins, age/health issues, inefficacy and, logistic and technical issues. Four of 27 (15%) switching from autologous to allogeneic SED experienced some worsening of symptoms after changing. The remainder (23, 85%) experienced no change or an improvement. Two of 6 (33%) switching from allogeneic to autologous SED experienced some worsening after changing. The remainder (4, 67%) experienced no change or an improvement. Six percent of those on either autologous or allogeneic SED experienced minor side effects.

Conclusion

Efficacy and tolerability of autologous and allogeneic SED appear comparable.

P247. Severe acute haemolytic transfusion reaction to HLA/HPA compatible apheresis platelets due to high anti-A titre

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Background

The most severe acute haemolytic transfusion reactions occur when patients are transfused blood or blood products that result in isoantibodies binding to group A and B red cell antigens. These ABO-incompatible transfusion reactions usually occur with red blood cells, however can occur with platelet transfusions from single donors with high anti-A or anti-B titres. The ARCBS currently screens apheresis platelets for high anti-A and anti-B antibodies, but do not provide clinicians with titre quantification.

A Case Report

A 54 year old lady underwent a major-ABO mismatched, unrelated allogeneic stem cell transplant for erythroleukemia, transformed from RAEB-2 myelodysplasia, complicated by multiple HLA and HPA antibodies. She required CMV negative, HLA-matched, HPA-5b-matched platelets.

On Day +34, she received a unit of group O HLA/HPA-compatible platelets and developed an immediate, severe haemolytic reaction with severe back pain, fall in haemoglobin from 81g/L to 69g/L, raise in serum lactate dehydrogenase and bilirubin with undetectable haptoglobins. Pre-transfusion testing demonstrated mixed field of A and O cells due to engraftment and previous red cell transfusions, whilst post-transfusion testing demonstrated disappearance of all her native group A cells and the presence of a new anti-A antibody. The platelet unit was retrospectively crossmatched and was found incompatible. The anti-A titre of the platelet unit was found to be IgG 2048 and IgM 512.

Conclusion

This case illustrated a severe ABO incompatible haemolytic reaction to apheresis platelets. HLA/HPA matched platelets are scarce blood products, and transfusions across blood group are often required to meet demand.

We advocate national haemovigilance reporting for this under-recognised transfusion reaction, to justify whether there is a need for routine antibodies quantification. This would aid clinicians when deciding whether a unit of platelet is suitable for crossing blood groups, hence further improving patient safety.

	Pre-transfusion					Post transfusion				
Haemoglobin	81 g/L					69 g/L				
DAT	Negative					Negative				
LDH	-					831 U/L				
Bilirubin	32 umol/L					51 umol/L				
Haptoglobin	-					Undetectable				
Group	Anti A	Anti B	Anti D	A1 cells	B cell	Anti A	Anti B	Anti D	A1 cells	B cell
	M/F	0	4	0	3	0	0	4	2	3
Screen	Negative					Negative				

P248. Sustaining health's national treasures - blood, patients and staff

Lilley J

New Zealand Blood Service

Background

The Massive Transfusion Protocol (MTP) provides clinical teams with a system that delivers fresh blood components to the patient's side in an emergency. An incident occurred where emergency blood delivery was delayed via the pneumatic transportation system. This was the catalyst to review the MTP activation process in the high user areas: Emergency Department, Birthing Suite, Operating Theatres and Intensive Care Units.

Aim

This project aims to deliver the right treatment to the right patient in the right place at the right time by the right people who are supported by the right resources; to promote a responsive (rather than reactive) whole team approach to MTP activation; MTP cessation in a timely manner; and decrease wastage of blood components.

Method

Investigation included Root Cause, SWOT and Gap analyses. Results provided opportunities to improve the MTP activation process. Analysis methods, feedback, MTP simulation training, education sessions and post MTP audit were utilised to monitor and evaluate the pilot.

Results

The investigation identified confusion of existing process resulting in a gap in communication between the team on the scene and Blood Bank. It was also apparent that people were missing key steps in the process e.g. the role of the Guardian of the Box, the forms required to issue components, how to complete the forms, when to send a Group and Screen and how many units of emergency blood to order.

Conclusion

Findings resulted in the development of an emergency MTP paging system that would bring appropriate staff resources to the treating clinical team and patient. In addition an MTP Checklist guides the clinician through the correct process with Blood Bank so emergency blood and the MTP box 1 reach the patient in the shortest time possible. Early results indicate these systems add value to the MTP activation process.

P250. Multiplex real-time PCR genotyping of human platelet antigens HPA-1 to HPA-5 and HPA-15

Mahon D

Australian Red Cross Blood Service

Aim

Human platelet antigen (HPA) genotyping is performed as part of laboratory investigations into clinical conditions such as fetal-neonatal alloimmune thrombocytopenia, platelet refractoriness and post-transfusion purpura. We have developed, optimised and validated multiplex real-time PCR (RT-PCR) assays for simultaneous genotyping of HPA-1 to HPA-5 and HPA-15. This method provides cost and time improvements over existing RT-PCR and sequence-specific primer (SSP) PCR methods.

Method

Three multiplex RT-PCR assays utilising TaqMan dual-labelled MGB and QSY probes were developed. These assays are performed under identical PCR cycling conditions, which allows simultaneous genotyping of HPA-1 to HPA-5 and HPA-15 for 28 samples using a 96 well plate on the Applied Biosystems ViiA7 Real-Time PCR system. The performance of the new method was compared to our existing RT-PCR and PCR-SSP methods.

Result

This method demonstrated 100% correlation with routine, validated methods when testing 146 DNA samples. Examples of low frequency genotypes were included in this study and were all correctly genotyped. This method has also demonstrated success in genotyping DNA of concentrations as low as 1.8ng/ μ L. Our new method, with a total PCR reaction time of 45 minutes, has replaced 6 individual assays, each of which have a PCR reaction time of 2.5 hours.

Conclusion

Our validation testing of this multiplex RT-PCR HPA genotyping assay was successful and the results met all acceptance criteria. HPA genotyping by our new method is robust, rapid and cost-effective and has demonstrated its suitability for routine, high-throughput screening as well as urgent clinical testing.

P251. Simultaneous HNA-1 Genotyping and FCGR3B Gene Copy Number Determination Utilising Real Time PCR

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Background

Human neutrophil antigen genotyping is important when investigating cases of neonatal alloimmune neutropenia (NAIN), TRALI and autoimmune neutropenia. HNA-1 antigens are expressed on the Fcγ receptor IIIb glycoprotein (FcγRIIIb) encoded by the *FCGR3B* gene. Six single nucleotide polymorphisms within exon 3 of the gene differentiate the three common alleles *FCGR3B*01* (*HNA-1a*), *FCGR3B*02* (*HNA-1b & d*) and *FCGR3B*03* (*HNA-1b & c*) as well as less common genetic variants. Individuals can exhibit zero to four copies of the *FCGR3B* gene which leads to variable levels of FcγRIIIb and therefore HNA-1 expression.

Method

We have developed a real time PCR genotyping method using TaqMan probes targeting SNPs that differentiate the three common *FCGR3B* alleles. The probe sequences ensure that the highly homologous *FCGR3A* gene does not interfere with the results. The method incorporates primers to produce a single PCR amplicon to which allele specific probes can bind. A uniform amount of DNA starting template allows for comparison of test sample amplification curves with controls of known *FCGR3B* type and copy number.

Results

The distributions of *FCGR3B*01*, *FCGR3B*02*, *FCGR3B*03* alleles detected in 478 random blood donors were 60%, 83% and 5.2% respectively. 83% of blood donors exhibited two *FCGR3B* gene copies, 8.4% exhibited one copy and 8.6% three copies. Differing levels of FcγRIIIb expression predicted by the assay were confirmed using a CD16 specific monoclonal antibody against typed panel cells in the granulocyte immunofluorescence test.

Discussion

FCGR3B typing of neonates as part of familial studies in NAIN cases can be complicated when one or both parents do not exhibit two copies of the gene. Neonates can inherit a null type from a hemizygous parent or two different copies from the one parent. This can incorrectly suggest errors in typing when *FCGR3B* genotyping methods cannot detect gene copy number. The increase in resolution of the real time PCR method overcomes this issue and improves neutrophil cell panel selection when testing for HNA-1 alloantibodies.

P253. What's the "D"fference

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Aim

To determine the frequency, aetiology and clinical impact of 'weak D or mixed field' results on RhD grouping, due possibly to weak RhD expression or a RhD variant. Differentiation is important because those with weak D are reported not to form allo-anti-D¹, and so should be labelled as RhD positive whereas individuals who are D variants can form anti-D when transfused with RhD positive blood, should receive anti-D gammaglobulin antenatally and be labelled as RhD negative.

Method

Samples were initially screened using an automated platform (Ortho Innova). Between February and June 2016 all samples demonstrating a weaker than normal or a mixed field reaction on testing with anti-D, were reviewed and referred to the Reference Laboratory at Australian Red Cross Blood Service for investigation of weak RhD expression and clinical impact was assessed.

Results

Patient number	Gender	D Variant	Weak D	Clinical Implication
1	Pregnant female	DFR		Anti-D given at 28 & 36 weeks gestation.
2	Pregnant female		Type 2	Sent for investigation of discordant RhD typing with outside laboratory. Anti-D not administered.
3	Pregnant female		Type 2	Anti-D administered whilst awaiting ARCBS results
4	Pregnant female		Type 2	Anti-D not administered.
5	Pregnant female		Type 2	Previous anomalous results. Anti-D given in previous pregnancy. Anti-D not administered
6	Non-pregnant female		Type 1	
7	Male	DCS-2		
8	Male		Type 1	
9	Male		Type 2	

No patient required transfusion.

Conclusion

It is not possible to decide on initial testing if the patient expresses a weak RhD phenotype or is a RhD variant. Therefore a protocol should be established which allows the appropriate grouping for cross-match and the administration of anti-D gammaglobulin pending the resolution of the D status. Communication, whether directly or via the issued report, should be explicit, as clinicians may be unaware of the significance of such results.

P254. The great "D"ebate - to genotype or not

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Aim

The Bioarray BeadChip Genotyping system was implemented at the Australian Red Cross Blood Service on 1 June 2015. A total of 251 samples have been genotyped to date using the RhD BeadChip kit for patients who are pregnant or of child bearing age, and patients who have been transfused where the RhD blood group is unclear. This retrospective study aims to assess the requirement for molecular testing to resolve the RhD blood group status.

Method

Genotyping results were compared to the serological results from the ALBAclone Advanced Partial RhD typing kit (where available) to determine the level of correlation between the 2 methods. RHD BeadChip kit uses 35 Single Nucleotide Polymorphisms (SNPs) to determine 68 RHD genetic variants that are most commonly reported in literature. Approximately 2.9% of variants reported in the ISBT database are not identified by the current kit. The ALBAclone Advanced Partial RhD typing kit utilises 12 anti-D cell lines for the classification of 16 variants using Indirect Antiglobulin Technique (IAT) based on the reaction profile.

Result

ALBAclone Advanced Partial RhD typing kit results were available for 48% (121/254) of samples. Approximately 37% (45/121) in this group, gave inconclusive results requiring investigation with RHD BeadChip kit. The remaining 63% (76/121) were tested using the RHD BeadChip kit to confirm the results. The results for 9.2% (7/76) obtained were discordant results between ALBAclone Advanced Partial RhD typing kit and RHD BeadChip kit.

Conclusion

For the majority of samples tested results obtained using the ALBAclone Advanced Partial RhD typing kit was sufficient to resolve the patient RhD Status. Most remaining samples were resolved with RhD BeadChip analysis with a small number requiring further investigation to resolve the RhD status.

P255. KLF1 (InLu) mutations in Australian blood donors: changed red cell antigen profile and morphology?

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Aim

There are two genetic patterns associated with the red cell (RBC) Lu(a-b-) phenotype. The dominant pattern is due to mutations in *KLF1*, a key regulator of erythropoiesis. Mutations in *KLF1* result in the Lutheran Lu(a-b-) phenotype, and serotype In(a-b-). Other blood group antigens and RBC proteins are impacted by mutations in *KLF1*. The aim of this study was to measure the frequency of *KLF1*/In(Lu) mutations in the Queensland blood donor population and assess its impact on red cell morphology.

Method

Donors (n=1875) were screened for reduced or absent Lu^b antigen expression and those with weak or absent Lu^b were typed for Lu^a. Lu(a-b-) donors were analysed using flow cytometry to determine expression of selected RBC proteins. The samples were genotyped by MPS; CLC genomics workbench was used for variant detection. Whether mutations were novel was determined by comparison with online databases.

Result

Two donors serotyped as Lu(a-b-). Flow cytometry results showed reduced CD44 (Indian) and BCAM (Lutheran) in these donors. The first donor had a novel *KLF1* mutation causing a premature stop (c.421C>T/ p.Arg141*). The second donor was a compound heterozygote with mutations c.304T>C (p.Ser102Pro) and c.977T>G (p.Leu326Arg).

Conclusion

Two examples with an In(Lu) profile and *KLF1* mutations were found in this study of Queensland donors. There are a range of phenotypes associated with In(Lu), however, those with a premature stop mutation have been reported to have a poikilocytic morphology sometimes associated with poor red cell survival. In addition, mild haemolysis and very high levels of RBC protoporphyrin have been observed in In(Lu) individuals. The level of haemolysis for packed RBCs from donors with this genotype, after 42 days storage, is under investigation. Measuring the frequency of *KLF1* mutations in donors will assist in donor typing and management.

P262. An Audit of the Thromboelastometry Assay in a Large Tertiary Hospital in Australia

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Rotational thromboelastometry (ROTEM) measures the viscoelastic properties of haemostasis in whole blood by analysing the interaction of coagulation factors, inhibitors, anticoagulants and platelets during clotting and fibrinolysis. Key benefits of ROTEM use include improvement in patient bleeding and inventory management, and reduced risk of transfusion-related complications resulting from appropriate use of blood products.

Implementation at the RAH is laboratory-based with testing performed by the SA Pathology Transfusion Medicine Unit. Analysis is remotely performed in clinical areas using 'Secure Viewer', which transmits result images in real-time and enabling rapid targeted therapies and better outcomes.

The audit of all ROTEM assays was performed to determine clinical practice trends with information obtained being used for ongoing laboratory and clinical education.

Method

The audit was performed over a 24-month period commencing in July 2014. Data of all assays performed were retrieved and analysed using spreadsheet analytical methods. Parameters such as patient number, patient age, clinical area, and time of request were assessed.

Results and Discussion

A total of 486 patient assessments were analysed. Most requests were cardiothoracic aligned (65.2%), followed by Emergency (7.6%). Gradual increase in clinical application is observed.

Cardiothoracic patient age peaks between 60 – 80 years (52%) while emergency patients 20 – 30 years (25%). Greater gender distribution with males: 66.8% (cardiothoracic) and 61.3% (emergency). This reflecting the typical patient age and gender demographic in those areas.

Cardiothoracic requests peaked between 11:00 and 18:00 hours, coinciding with the likeliness of elective surgeries. Emergency requests typically were more sporadic throughout a 24-hour period.

Conclusion

This audit enabled data collation in a systematic manner, allowing information sharing with clinical areas to improve practices. This information will also allow efficient management of staffing resources in a 24-hour laboratory.

This audit will be the basis of a state-wide SA Pathology ROTEM Register capturing all test data, patient details, clinical information, and blood product usage.

P263. A retrospective audit of single unit red cell transfusion practice in South Australia

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Aims

As a result of ongoing programs to improve appropriate use and reduce wastage, red cell use in South Australia (SA) has reduced over time. However changes in the volume of red cell units used with each transfusion episode has not yet been studied at a state-wide level. The aim of this study was to assess rates of single unit red cell transfusion practice in a range of surgical procedures across metropolitan public hospitals in SA to determine targets for further implementation.

Method

A retrospective analysis of the number of units (no transfusion, single unit, two units and >2 units transfused per hospital stay in patients undergoing primary hip and knee arthroplasty, cardiac surgery (CABG, valve, and combined CABG & valve) and colorectal surgery at various public hospitals in SA, from 2006 - 07 to 2014 - 15 financial years were analyzed. Hip and knee arthroplasty and colorectal surgery data was from 4 main hospitals and cardiothoracic surgery data from 2 hospitals. Data was obtained from a database maintained at the SA Department of Health (DOH) with SA DOH ethics approval. Analysis of data was descriptive and presented as incidence (percentage).

Results

There was a consistent reduction in red cell transfusion rates across all surgical procedures over time when the results were combined for all SA hospitals studied. The overall rate of single unit transfusions per admission (a marker of definite single unit transfusion practice) was between 10 – 30% of transfusions. Single unit transfusions were present for each procedure for most years without a clear increase apart from in selected procedures at selected sites.

Conclusion

There are a significant number of single unit transfusions per admission but no clear increase across most hospitals. There is a potential for further improvement and implementation of this strategy in stable non-bleeding patients

P264. Chimerism in an IVF Multiple Birth - Six Years On

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Background

In 2009, blood grouping of a 25 year-old female who presented for routine antenatal screening at the Royal Hobart Hospital revealed a mixed field reaction with anti-A and anti-B. Further testing at the Red Cell Reference Laboratory confirmed the initial findings and the presence of chimerism. It was noted at this time that the patient was one of two surviving female IVF triplets. IVF has been reported as a risk factor for the occurrence of blood chimerism in utero¹

In 2016, a sample from the second surviving triplet, now 31 year-old female, was received for routine blood grouping. Marked mixed field with the anti-A, anti-B and complete agglutination with anti-AB reagents were observed and the sample was again forwarded to Red Cell Reference Laboratory for investigation

Method

Blood grouping and phenotyping were performed using standard serology techniques. HLA typing was performed using serology and/or molecular techniques. Red cell genotyping was performed using the BioArray BeadChip Precise Type™ HEA Kit on the BioArray AIS400C platform. Targeted MPS (Massive Parallel Sequencing) was performed using MiSeq Sequencing platform and the TruSight™ one sequencing panel (Illumina). Samples from both triplets were referred to the University and Regional Laboratories in Lund, Sweden for ABO genotyping and flow cytometry.

Results

The results for both triplets confirm the presence of A/B chimerism, with two distinct populations of A and B cells shown by flow cytometry. Phenotyping indicated 2 cell populations for ABO and Fy^a. Three alleles were noted in the HLA typings. ABO genotyping in both triplets revealed three ABO alleles.

Conclusion

This is the first confirmed example of chimerism in two triplets in our laboratories. Confirmation of a suspected chimerism can be complicated and may require extensive investigations using a wide array of techniques.

P266. Blood supply and traceability review of massive transfusions in the private sector in South Australia

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Aim

A private South Australian hospital in conjunction with the BloodSafe program and private transfusion service laboratory reviewed practices surrounding blood delivery/ receipt and communication between clinical and laboratory staff when a Massive Transfusion Protocol (MTP) was activated. The aim was to ensure the fate of all products was correctly recorded and to improve communication between hospital and laboratory staff.

Method

The review group was represented by critical clinical area staff, transfusion laboratory manager and the BloodSafe transfusion nurse. The review mapped out all the current steps occurring when MTP were activated. Each step was assessed for necessity and efficiency, the process was also analysed for any potential practise gaps.

Result

The review identified:

- A cumbersome retrospective documentation process requiring nursing staff to document all products delivered in two different registers.
- Use of Blood Service shippers for blood product delivery in a massive transfusion (MT) lead to multiple shippers being used to deliver components with different storage requirements, these shippers also don't maintain cold chain once accessed so staff may need to transfer blood if immediate use not certain, these shippers are also used for non-urgent deliveries and in MT their urgency is not easily distinguished.
- Staff activating MTP vary and MT occurrence is not regular which can lead to communication gaps between clinical site and laboratory

Conclusion

- New process have been developed to reduce retrospective documentation in registers
- New purpose built shippers have been designed (currently being validated) to maintain storage conditions for both red cells and platelets. These shippers, used only for MTP deliveries, are easily identified, have "just in time" reminders, negate the need to transfer products to the blood fridge, as such remain with the critical patient.
- A checklist-based notepad was developed for the laboratory staff to assist with telephone MTP activation by clinical staff.

P267. Patient Blood Management in Australia: An Update from the National Blood Authority PBM Guidelines Expert Working Group

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¹ National Blood Authority, ² on behalf of the PBM Guidelines Expert Working Group

Background

In 2008, the National Blood Authority (NBA) convened an Expert Working Group (EWG) to develop a suite of modules on Patient Blood Management (PBM). The initial aim was to develop a set of research questions on which to base a scientific review of the literature in six clinical settings (modules) of patient blood management. The suite of six module is now complete: Critical Bleeding/Massive Transfusion (2011), Perioperative (2012), Medical (2012), Critical Care (2013), Obstetrics and Maternity (2015) and Neonatal and Paediatric (2016).

Since the publication of the PBM Guidelines, the NBA has worked with Clinicians, Consumers and other Government agencies to develop a PBM Guidelines implementation strategy.

Methods

Clinical/Consumer Reference Groups (CRG) with experts from clinical colleges and societies were established to oversee the development of each of the PBM Guidelines module. For each module, there a number of recommendations supported by the level of evidence, practice points crafted by the CRG when insufficient evidence was available to support a recommendation and expert opinion points based on consensus decision making where relevant guidance that is outside the scope of the systematic review is required.

Results

The first PBM Guideline module was published in 2011. Since 2011-12 there has been a decline in red blood cell issues of 18% (in 2012-13 down by 5%, in 2013-14 - 8% and in 2014-15 - 5%). This represents a savings in excess of AUD 78 million. The publication and implementation of the PBM Guidelines underpins much of the success in improving appropriate use of fresh blood products.

Conclusions

Strategic collaborative partnerships between government, clinical and academic sectors has seen the development of high quality evidenced based guidelines and the rapid adoption of PBM practices in Australia.

P268. Validation and characterisation of sheep platelet units for transfusion research

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Aim

Platelet transfusions reduce the risk of bleeding and decrease complications associated with platelet-lowering treatments. We have previously developed sheep models (trauma and haemorrhage, cardiac surgery) that would benefit from the availability of sheep platelet units. Sheep models can also be used to investigate changes to platelet manufacturing and storage protocols (e.g. extended storage or cryopreservation). Therefore, this study aimed to develop protocols to manufacture sheep platelet units, and then to compare their characteristics and storage-related changes to human platelet units.

Method

Sheep platelet units (n=5) were prepared with minor modifications to standard procedures: for each unit 4 platelet-rich buffy coats were pooled, mixed with SSP+ additive solution, separated by centrifugation, leucodepleted by filtration and stored in the associated platelet storage bag. Both sheep and human platelet units (n=5; provided by the Blood Service) were stored at 22°C with agitation and underwent testing on days 2, 3, 5 and 7. Biochemical parameters were evaluated using blood-gas analyser, platelet activation markers were tested by flow cytometry, and platelet function was assessed by Multiplate using ADP and collagen activators.

Result

All sheep platelet units met human platelet unit quality control specifications. At day 2, sheep platelet units had higher phosphatidylserine and CD62P expression, higher ADP and decreased collagen aggregation responses when compared to human platelet units. During storage, similar changes in biochemistry (unchanged pH, decreased glucose and increased lactate), platelet activation marker expression (increased phosphatidylserine and CD62P) and platelet function (decreased ADP and collagen responses) were observed between sheep and human platelet units.

Conclusion

Production of sheep platelet units is feasible and storage-related changes are comparable to human platelet units. Based upon this evidence, sheep biomedical models should be able to incorporate sheep platelet transfusion, and provide a suitable pre-clinical model for the investigation of changes to platelet manufacturing and storage protocols.

P269. Zika Virus can be effectively inactivated in blood products by nucleic acid targeted adducts

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Background

The mosquito-transmitted Zika virus (ZIKV) causes usually asymptomatic infections, or mild symptoms, however the recent large outbreak in the Americas has highlighted ZIKV's infrequent neurologic conditions including microcephaly and Guillain-Barré syndrome. The additional potential for sexual transmission complicates control of transfusion-transmitted-infections (TTI) with standard measures. Four TTI cases have been reported. The risk of TTI may be reduced by use of the photochemical INTERCEPT™ Blood System pathogen reduction (PR) technology previously demonstrated to be effective for other arboviruses (CHIKV, WNV, DENV). The same PR system was effective for the inactivation of >6.57 log ZIKV in human plasma (Aubry M, *et al.*). The mode of action (MOA) of the technology is through formation of irreversible covalent adducts with nucleic acids, and a technology with the same MOA is under development for RBC, using amustaline and glutathione (GSH). Here we report that these PR technologies inactivate ZIKV in RBC, and platelet (PC) components irrespective of the suspension medium.

Methods

PC in either 65% platelet additive solution (PAS) or 100% plasma, or RBC prepared in AS-5 (N=3), were inoculated with ZIKV (CDC PRVABC59 strain; $\sim 10^7$ pfu/mL). PC were treated with amotosalen and low energy UVA (3 J/cm^2) light. RBCs were treated with amustaline/GSH (0.2 mM/20 mM) at RT for 18-24h. Inactivation was determined by comparing infectivity titers before and after treatment.

Results

Following inactivation in PC in PAS or Plasma, no infectivity was observed, consistent with inactivation of > 4.6 log. Following inactivation, a mean reduction of > 5.0 log pfu, or >4.3 log pfu/mL was measured in RBC. In all cases complete inactivation was observed.

Conclusions

Amotosalen/UVA and Amustaline/GSH PR technologies effectively inactivate ZIKV in Platelets, Plasma and RBC.

The INTERCEPT Blood System for RBC is not approved for use.

This research was supported by Cerus Corporation. The authors are employees of Cerus Corporation.

P270. Thrombocytopenia Secondary to Passive Transfer of Anti-HPA Antibodies from a Male Donor

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Background

Thrombocytopenia is a rare complication of blood transfusion. Pregnancy, transfusions and transplantation can lead to formation of platelet alloantibodies. Post-transfusion purpura occurs about a week following a transfusion in previously sensitized patients. Passive alloimmune thrombocytopenia secondary to the transfer of platelet antibodies occurs immediately after or during the transfusion of plasma containing products. We present a rare case of passive alloimmune thrombocytopenia associated with a sensitized male donor.

Case Study

A 58-year-old renal transplant recipient with acute antibody mediated rejection was commenced on plasma exchange with FFP replacement and low-dose IVIG (0.1g/Kg). 24 hours following plasma exchange she developed severe thrombocytopenia, fortunately without significant bleeding symptoms. Other potential causes of thrombocytopenia, such as heparin- or drug-induced thrombocytopenia, were excluded. Because of the close temporal relationship to plasma exchange, secondary thrombocytopenia due to passive transfer of platelet antibodies was considered. Additional testing identified that 1 of the 5 donors who provided FFP for the implicated plasma exchange had strong anti-HPA 1a antibodies. Genotyping of this donor revealed they were homozygous for HPA 1b. Platelet alloantibodies were not detected in the patient's serum, but her genotype was homozygous for HPA 1a. Review of the donor's details revealed a history of likely transfusion 18 years prior in the setting of trauma. The patient's platelet count improved over 10 days.

Discussion

Passive transfer of anti-platelet antibodies is a rare but serious complication of blood transfusion. This is the first reported case in Australia and only the second reported case worldwide from a male donor. The Australian Red Cross Blood Service has been supplying plasma for clinical use predominantly from male donors since 2007. The case highlights that this complication can still occur and should remain in the differential diagnosis for acute thrombocytopenia in the context of recent transfusion.

P271. Acute Haemolytic Reaction Secondary to an ABO Minor Mismatch Platelet Transfusion from a Group A Blood Donor

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Background

Platelet transfusion often requires non-ABO compatible platelets due to high demand and short shelf-life. ABO major mismatched platelet transfusion can be associated with platelet refractoriness. ABO minor mismatched platelet transfusion can rarely cause haemolytic transfusion reactions, most commonly associated with group O components. The Australian Red Cross Blood Service currently tests a proportion of group O apheresis platelets to identify components containing low titre anti-A and anti-B which may be selected preferentially for ABO incompatible transfusion. Apheresis platelets of other blood groups are not routinely screened for isoagglutinins.

Case Study

A 47 year old male post cardiac surgery received a component of platelets for mild post-operative bleeding. The patient was AB Rh(D) positive and received one unit of group A apheresis platelets. During transfusion, the patient had a cardiac arrest. Following successful resuscitation, markers for haemolysis were strongly positive (markedly elevated LDH, bilirubin and potassium; with reduced haptoglobin). The patient developed anuric renal failure and required dialysis. Bacterial infection and anaphylaxis were excluded. The patient was DAT positive (anti-IgG and anti-C3d) and anti-B was eluted from the patients red cells. Retesting confirmed the donor had an anti-B titre in excess of 32,000. The donor was deferred from donating fresh components for clinical use.

Discussion

This case demonstrates a severe acute haemolytic transfusion reaction from an ABO minor mismatch platelet transfusion. Notably not all ABO incompatible platelet donations (even with known high titre antibodies) are associated with haemolytic transfusion reactions, possibly due to dilution in patient plasma or neutralisation of antibodies from non-red cell ABO antigens (soluble or endothelial antigens). A risk-benefit analysis will be performed to determine if screening should be extended to include other ABO apheresis platelets and should components with high titres be reserved for ABO-compatible transfusions only.

P272. Homozygosity for the HLA-DRB1*15 allele is associated with a responder profile in RhD-immunised blood donors

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Cases of haemolytic disease of the newborn have dropped significantly since the successful administration of prophylactic anti-D immunoglobulin (Ig) to susceptible RhD-negative pregnant women. Our Blood Service conducts an Anti-D Program to actively immunise selected RhD-negative blood donors with small volumes of RhD-positive red blood cells to stimulate anti-D Ig production. Donors (n=431) were considered Responders if serum anti-D Ig concentrations >1 IU/mL were recorded. However, 39.2% were Non-Responders and did not produce any anti-D Ig despite multiple exposures. We sought to examine donor genetic factors associated with antigen presentation and to identify genetic characteristics that could be utilised to identify Responders prior to immunisation. We found that female donors were more likely to be Responders ($p < 0.0001$). DNA was extracted from a subset of donors (n=272) and genotyped by restriction fragment length polymorphism-polymerase chain reaction for polymorphisms in genes that play an important role in antigen presentation and pathogen recognition (TLR2, TLR4, CD14 and FcγRIIA), although no statistically significant association was identified. Further work examined the association between Responders and the HLA-DRB1 allele, and revealed that donors with DRB1*15 allele were significantly associated with a Responder profile ($p = 0.049$). High-resolution typing of donors with DRB1*15 alleles (n=78) demonstrated that 90% possessing two DRB1*15 alleles and 73.5% possessing one DRB1*15 allele had a Responder profile. This study has identified a potentially useful genetic characteristic (HLA-DRB1*15 allele) that could be used to screen new donors prior to entry into the Blood Service Anti-D Program.

P273. Serum growth factor and fibronectin concentrations in dry eye patients and healthy blood donors

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Australian Red Cross Blood Service

Background

Autologous serum eye drops (SEDs) are beneficial for patients suffering from dry eye syndrome due to their growth factor and fibronectin content. Patients are not always eligible to donate blood for autologous SEDs, and allogeneic SEDs from blood donors could provide an alternative. Limited studies have investigated the variability in serum growth factor concentrations from patients and healthy blood donors. Further, the minimum concentration of growth factors in SEDs to initiate corneal cell proliferation and wound healing is unknown. The aims of this study were to determine growth factor concentrations in serum from patients and healthy donors, and to investigate the ability of different serum samples to stimulate cell proliferation and wound healing.

Methods

Whole blood was collected from dry eye syndrome patients (n=9) and healthy blood donors (n=102) and left to clot at 2-6 °C for 24 hours. Serum was prepared by centrifugation, diluted to 20% with 0.9% sodium chloride and stored at -30 °C. Epidermal growth factor (EGF), platelet-derived growth factor (PDGF)-AA, PDGF-BB, transforming growth factor (TGF)- β 1, TGF- β 2 and fibronectin were measured using magnetic bead-based assays and ELISA. Growth factor concentrations were compared across donor age groups (one-way ANOVA) and gender (t-tests). Human corneal epithelial cells were incubated with serum from healthy blood donors and assessed using a CyQUANT cell proliferation assay and in a scratch wound assay.

Results

There was a wide range in growth factor and fibronectin concentrations from patient and blood donor serum samples (Table). Serum from younger blood donors (20-29 years) had significantly higher PDGF-BB concentrations ($p=0.0149$) than older healthy donors (60-69 and 70-79 years). There were no differences in growth factor concentrations between male and female donors. There was no correlation between individual growth factor concentrations and corneal cell proliferation. However, serum samples with higher fibronectin concentration were better able to stimulate cell migration in scratch wound assays than those with low fibronectin concentration.

Conclusion

Although serum contains many growth factors, correlation of a single factor with cell proliferation is difficult. However, fibronectin concentration does appear to influence cell migration, and may serve as a quality marker when producing eye drops from allogeneic serum.

Table: Serum growth factor and fibronectin concentrations (mean \pm SD).

Growth factor	Patients	Blood Donors
EGF (pg/mL)	75 \pm 30	79 \pm 36
PDGF-AA (pg/mL)	781 \pm 226	822 \pm 269
PDGF-BB (pg/mL)	4728 \pm 1396	4130 \pm 1607
TGF- β 1 (pg/mL)	7725 \pm 1533	8735 \pm 2249
TGF- β 2 (pg/mL)	1104 \pm 255	828 \pm 310
Fibronectin (μ g/mL)	192 \pm 46	188 \pm 46

P274. Blood and Blood Components ordering and utilization in Central Women Hospital, Yangon, Myanmar

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Aim

For many hospitals in Myanmar, over ordering of blood is a common practice. In this study, we assess on blood ordering for various types of conditions and diseases, and usage of blood and blood components in Central Women Hospital, Yangon, Myanmar.

Method

The study was approved by the Ethics Review committee at the Department of Medical Research, Ministry of Health, Myanmar. The research project was carried out at the clinical Pathology Department, Central Women's Hospital, Yangon, Myanmar.

Result

A total of 1542 units of blood and blood component requesting from of 721 patients from the 5 different wards of Central Women's Hospital, Yangon, Myanmar were included in this study. The actual usage of blood units per requested units in each wards were (24/ 335) 7.16 % in Obstetric ward, (190/ 431) 44.08% in Miscarriage patients ward, (43/ 210) 20.47% in Gynecological diseases ward, (70/ 142) 49.29% in Oncology ward and (35 / 424) 8.25 % in Emergency ward, respectively. We found that less than 10% of order blood units were actual used in Obstetric ward and Emergency ward.

Conclusion

In this study, we analyzed for the first time of blood ordering pattern according to patients' clinical diagnoses and different specialty wards in Central Women Hospital, Yangon, Myanmar. Monitoring of blood request forms can reduce rates of over ordering of blood and also inappropriate transfusions. This study will be great supported for transfusion policy especially for the changes of blood ordering pattern.

P275. Safety and efficacy of granulocyte transfusions in severe neutropenic sepsis

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Aim

The role of granulocyte transfusions (GTs) in patients with severe neutropenic sepsis remains contested. We assessed the efficacy and safety of granulocyte collection and transfusion in an adult haematology unit.

Method

A retrospective analysis of granulocyte donors and recipients from April 2002 to April 2016 at our institution was performed. Recipients included had severe neutropenic sepsis unresponsive to maximal anti-microbial therapy. Primary outcomes measures were; granulocyte dose; post-GT neutrophil count; infection control at 30-days and 30-day mortality.

Results

101 donors made 129 donations. 26 donors made more than one donation over an average of 5.4 days (2 – 46 days). GT collection was well tolerated.

42 patients with neutropenic sepsis (45.4% receiving ICU support) received 124 GTs for 45 septic events, with average of 2.8 transfusions per event (1-12). Patients were treated with anti-microbials for an average of 12.1 days (1-30 days) prior to GT initiation with median of 23 days (3-119 days) before neutrophil recovery. The average granulocyte dose was $1.4 \times 10^9/L/Kg$ ($0.1 - 14.4 \times 10^9/L/Kg$). Neutrophils were sustained greater than $0.5 \times 10^9/L$ for average of 1.6 days (0-13 days) after each GT.

61.4% achieved infection resolution at 30 days, 68.2% survived to hospital discharge and 30-day mortality rate was 31.8%. These results compare favorably with mortality rates of up to 48.9% reported in patients with neutropenic sepsis not administered GT^{1,2}. Only infection resolution ($p < 0.01$) and inotropic requirements ($p = 0.04$) correlated significantly with 30-day survival.

GT was well-tolerated although 5 patients developed adverse reactions (4.0%) with 2 developing severe transfusion-related acute lung injury leading to 1 death.

Conclusion

GT appears to be safe and beneficial in the management of neutropenic sepsis.

P276. Development of a platelet wastage audit tool identifies the need to improve patient HLA platelet inventory management

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Aim

Implementation of the BloodMove Platelet Program in July 2014 to all Adelaide metropolitan hospitals has significantly decreased platelet wastage. Using a new platelet wastage audit tool has found HLA matched platelets as a preventable cause contributing to platelet wastage. HLA matched platelets are usually required for patients who are refractory to random platelet transfusions due to the presence of HLA alloimmunisation. A further audit was undertaken to understand the allocation and use of HLA matched platelets for patients in one of the SA hospitals.

Methods

Patients requiring HLA matched platelets and the total platelets discarded between January and May 2016 were included. The total numbers of HLA matched platelet units allocated, units transfused and units discarded were analysed.

Result

A total of 193 HLA matched platelet units were allocated to 16 patients with haematological malignancy. Of the 193 units, 68 (35.2%) were transfused to the intended patient, 53 (27.5%) were discarded due to expiry and 72 (37.3%) were transfused to other patients to avoid expiry. The median HLA platelet units allocated to patients were 7 (IQR 3-17) and the median units discarded were 2 (0-5). Fifty three HLA platelet units discarded constituted 40.5% (53/131) of the total units discarded between January and May 2016.

Conclusion

Over a quarter of the HLA matched platelets allocated to patients were discarded due to expiry, as such wastage avoidance measures are being implemented ie. mandatory un-reserving HLA platelets on Day 5 at 12noon and expiry lists for the clinics.

The use of a novel platelet wastage audit tool has allowed to objectively identify causative factors leading to platelet wastage.

This spreadsheet based tool could be incorporated into the BloodNet Fate Module if extra fate fields are available, providing reports that identify wastage trends enabling active and targeted wastage minimisation intervention.

SA Pathology Platelet Wastage Audit Tool															MONTHLY TOTALS																
Month		APRIL													ALL	HLA	BANK	ARCBS	OTHER												
TOTALS															2	5	0	0	1	0	0	1	0	1	0	6	15	6	9	5	0
DONOR No	DISCARD DATE	RECEIVED DATE	RECEIVED TIME	ISSUE DATE	ISSUE TIME	RETURN TIME	TIME OUT OF TMU	US	ODC	HDC	GenH ThH	ED	RESUS PAIC	OTHER	NOT ISSUED	HLA	BANK	ARCBS DAY4 DELIVERY	INTER LAB DAY 4 DELIVERY AFTER 1700HRS	COMMENT											
2472740	07/04/2016	06/04/2016	01:14	06/04/2016	14:05	20:57	06:52	1										1	1	Rx sent on Day5											
2518104	08/04/2016	06/04/2016	01:14	07/04/2016	04:36	17:15	12:39	1										1	1	late trolley rtn											
2472756	08/04/2016	05/04/2016	10:50	07/04/2016	04:41	17:15	12:34	1										1		late trolley rtn											
2472758	08/04/2016	05/04/2016	11:04	07/04/2016	04:44	17:15	12:31	1										1		late trolley rtn											
2522527	11/04/2013	07/04/2016	18:05												1			1													
2474673	12/04/2016	10/04/2016	10:07	11/04/2016	00:02	16:42	16:40	1										1	1												
2474765	13/04/2016	09/04/2016	12:02	12/04/2016	19:41	20:09	00:28								1			1		1 of 2											
2474765	13/04/2016	09/04/2016	12:02				00:00								1			1		2 of 2											
2473331	14/04/2016	11/04/2016	15:39	13/04/2016	01:22	10:29	09:07				1							1													
2473279	14/04/2016	12/04/2016	10:35				00:00								1			1	1												
2929149	17/04/2016	14/04/2016	11:38				00:00								1			1		UR:xxxxxxxx											
2942924	19/04/2016	15/04/2016	11:28				00:00								1			1		UR:xxxxxxxx											
2939282	19/04/2016	15/04/2016	19:17				00:00								1			1		UR:xxxxxxxx											
2473692	19/04/2016	17/04/2016	18:33	18/04/2016	00:07	16:26	16:19	1										1		late trolley rtn											
2943371	23/04/2016	22/04/2016	19:43	23/04/2016	10:43	14:56	04:13	1										1	1	C6 late rtn											

P277. Audit of massive transfusion protocol activations in a tertiary level hospital in South Australia

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Aim

A Massive Transfusion Protocol (MTP) is an essential part of haemostatic resuscitation as laboratory parameters are not always immediately available to guide therapeutic decisions in exsanguinating patients. With haemostatic resuscitation being increasingly applied to patients with haemorrhage other than trauma, MTP use has proportionally increased. MTP was implemented in our institution in 2013. The aim of the audit was to examine MTP activations in a tertiary hospital in South Australia.

Methods: MTP activations between 2013 and 2015 were analysed. Data collected comprised activation reasons, number of MTP activations including unused, partially used or completely used massive transfusion packs, blood products used and patient outcome. Massive transfusion was defined as 10 or more red cell unit transfused in a 24hr period.

Results

A total of 334 activations were included: 138 (41.3%) in 2011, 125 (37.4%) in 2014 and 71 (21.3%) in 2015. Fifty six percent (186/334) of the activations were activated in Emergency Department, 24% (82/427) in the operating room and the remainder in Intensive Care Unit and other wards. MTP activations resulted in 19.5% (65) unused, 44.6% (149) partially used and 35.9% (120) complete use of massive transfusion packs. Forty eight unused (73.8%) and 52 (43.2%) of the completely used packs were activated in the Emergency Department. Within the 120 activations which were completely used, 85 activations had more than one massive transfusion pack ordered and 55.3% (47/85) of those activations resulted in a massive transfusion.

Conclusion

Over one third of the packs were used completely and nearly 25% were unused. Liberal MTP activations may reduce the risk of under treatment and help with rapid access of blood and products in bleeding patients but also has the risk of exposing patients with non-major haemorrhage to unnecessary blood. Regular review of MTP activations helping to define the activation criteria is warranted.

P278. Festive Excess: A Strategy to Reduce Red Cell Wastage in Victoria

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Background

Data from July 2012-February 2015 indicate that red blood cell (RBC) waste is decreasing; however, it highlights consistent 'festive spikes' occurring each year in January and February.

Aim

To assist health services/pathology providers to prepare for variations in blood product demand over the festive period to reduce waste in January- February.

Method

A 'stop the waste' checklist was circulated to key Victorian health services and laboratory stakeholders in October 2015 to raise awareness regarding:

- reduced activity (ward and theatre) that could influence demand
- absence of key personnel (e.g. waste champions, fridge monitoring/maintenance staff)
- inventory adjustment to meet expected demand.

A follow-up survey was distributed (n=72) in April 2016. It collected information regarding how the checklist was used, and whether it was helpful. It also sought suggestions for further improvement.

Results

Twenty surveys were returned (27%). Generally respondees indicated that the campaign was a good idea. Six (30%) discussed the checklist at blood management committees. Twelve (60%) adjusted RBC inventory over the festive period. Five (25%) found the checklist useful, and 11 (55%) unsure. The 2016 festive spike of 4.1% (January) and 3.6% (February), was lower than previous years, but still evident.

Suggested improvements included launching the campaign earlier to allow timelier implementation, and ensuring laboratories received the checklist. Some stakeholders commented that more reliable forecasting of activity is required from treating units.

Conclusion

The overall trend shows a pleasing decrease in RBC wastage in Victoria, including a lower festive spike than seen in previous years.

The 'stop the waste' festive campaign raised awareness of wastage related to changes in activity levels over the festive period, and prompted effective action. On-going collaboration with health/pathology services is crucial. The campaign will be repeated in 2016; however it will be commenced earlier, to allow for timely communication across departments.

P279. Clinical Scenario Learning. Is it the best way to learn Patient Blood Management?

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BloodSafe eLearning Australia

Introduction

The use of clinical cases to frame learning is widely used in adult education programs and can help link learning to clinical practice and develop problem-solving skills. However online learning has often taken a more didactic approach, with case-studies used only as examples.

In December 2015 BloodSafe eLearning Australia (BEA) released a patient blood management (PBM) course in the Critical Care setting. This was developed entirely as a clinical scenario with learners able to explore different options and treatments during the course. Uptake of the course was high with more than 2200 users completing the course in the first 6 months of its release.

In order to evaluate and compare the approach an online survey was sent to users who had completed this Critical Care course, and at least one other BEA course.

Results

1824 registered users were sent the survey and 110 (6.0%) provided a response.

Respondents main reason for completing the Critical Care course were:

- refresh/broaden/update their knowledge - 42%
- mandated/recommended by organisation - 30%
- relevant to their work - 21%
- obtain CPD/CME points - 5%
- other - 2%

54% of respondents stated they preferred the use of the case based approach, 10% preferred a more traditional theory-based approach, with 36% having no preference.

Respondents indicated that the course would assist them to:

- improve patient outcomes - 83%
- help prevent adverse events - 77%
- assist to change practice - 60%

Examples learners gave of personal practice change include:

- identifying patients at risk of anaemia
- consideration of herbal and over-the-counter therapies on bleeding risk
- more appropriate use of blood products
- Conclusion
- Learner's response to the Critical Care course was very positive with more than half the respondents preferring the case-based approach. This data will assist with future course development.

P280. Working smarter to minimise blood and blood product wastage – The collaborative success of a National Wastage Reduction Strategy

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Australian governments, through the National Blood Authority (NBA), spend approximately \$1 billion per annum funding the supply of blood and blood products. These products are provided to patients free of charge and based on clinical need and appropriate clinical practice. It is important that health providers work together and inventory is managed to ensure that product is available where it is clinically required and not unnecessarily wasted.

The *National Blood and Blood Product Wastage Reduction Strategy 2013-2017* (Wastage Strategy) was developed by the NBA to provide a basis for providing direction to Australian Health Providers in reducing their wastage. As part of the Wastage Strategy, the NBA has introduced number of initiatives and resources, to assist health providers with achieving these reductions. The initiatives achieve the broad categories of:

- collection, analysis and distribution of data,
- establish targets for discard rates,
- better practice inventory management,
- education and training,
- enhanced awareness of unit prices,
- enhanced collaboration,
- addressing systemic issues, and
- promotional campaigns.

It was important for the NBA to work closely with all levels of the health sector. Collaboration with jurisdictional governments to clinicians and scientists in the hospitals and laboratories ensured implementation success of the Wastage Strategy.

This collaborative approach has seen red cell wastage reduced from a Discards As a Percentage of Issues (DAPI) for red cells of 5.1% in the 2013/14 financial year to the current 2.8%. The downward wastage trend is being mirrored in other fresh blood products with platelet DAPI experiencing a similar pattern with rates reduced from 17.8% in the 2013/14 financial year to the current 13.0%.

Implementation of activities based on the Wastage Strategy has saved governments and the Australian taxpayer approximately \$15,795,781 in wastage of fresh blood products since its introduction. Additionally, our non-remunerated blood donor's altruistic gift is being well utilised.

P281. Comparison of additive solutions for the storage of thawed deglycerolised red cells

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Red cell concentrates are routinely glycerolised for cryopreservation of rare phenotypes and for anti-RhD immunisation. Following deglycerolisation, these components are resuspended in SAG-M with a limited shelf-life of 24 hours. The use of an alternative additive may enable this post-thaw shelf-life to be extended.

Aim

The *in vitro* quality of deglycerolised red cells stored in three additive solutions, SAG-M, AS-3 and ESol-5, was evaluated with the view to extend the shelf-life beyond 24 hours.

Method

Seven days post-collection, sets of three ABO-matched red cell concentrates were pooled and split to produce three equivalent components (n=9 replicates). These were glycerolised and frozen at -80°C. Upon thawing and deglycerolisation, one unit of each set of matched components was resuspended in a different additive, SAG-M, AS-3 and ESol-5. *In vitro* quality parameters were measured for 14 days or until haemolysis reached 0.8%. Data was analysed by two-way repeated-measures ANOVA; a p-value <0.05 was considered significant.

Result

Red cell quality parameters were not significantly different prior to glycerolisation. Post-thaw, haemolysis of red cells in SAG-M exceeded 0.8% on day 3, whereas haemolysis of red cells in AS-3 and ESol-5 increased more gradually, reaching the upper limit on days 10 and 14, respectively. The rate of LDH release correlated with haemolysis ($R^2=0.8252$, 0.5351 and 0.5530 for SAG-M, AS-3 and ESol-5, respectively). Red blood cell count, haemoglobin and 2,3-DPG concentrations were not significantly different between additives. Supernatant potassium ($p=0.0155$) and glucose concentrations ($p<0.0001$) varied significantly between the three additives. Haematocrit ($p=0.004$) and pH ($p=0.0012$) were also significantly different between AS-3 and ESol-5, and ESol-5 maintained a higher ATP concentration on days 10 and 14 post-thaw ($p<0.05$).

Conclusion

Alternative red cell additives such as AS-3 and ESol-5 may enable the post-thaw shelf-life of deglycerolised red cells to be extended to 10 or 14 days. These additives support lower haemolysis and LDH release compared to SAG-M.

P282. Learning from the Negative and Looking for the Positives: Review of RHD Immunoglobulin Events at Monash Health

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Background

RhD immunoglobulin (RhDIg)-related incidents are reportable to the Serious Transfusion Incident Reporting (STIR) program since January 2015.

Monash Health is a large multicampus university hospital in Melbourne's south-east. The weekly multidisciplinary Monash transfusion working group reviews all identified transfusion-related events to identify opportunities for feedback and improvement, and coordinates reporting to STIR.

Methods/results

Retrospective audit of RhDIg-related events identified at Monash Health, between May 2014-May 2015.

799 RhD negative women delivered during this period.

INCIDENT TYPE	NUMBER OF CASES AND CONSEQUENCES
RhDIg inappropriately issued	
RhD positive woman – transcription error	1 patient – RhDIg issued
RhD Ig administration omitted	
Specimen timing incorrect	3 patients – Kleihauer performed on pre-natal specimen
Specimen unsuitable	1 patient – clotted specimen, no repeat specimen, no Kleihauer performed (RhDIg issued)
Specimen not sent	3 RhD negative women with RhD positive or unknown babies
Weak D testing not performed	2 patients – RhDIg not issued
Transcription – RhD negative mother recorded as RhD positive	6 patients – RhDIg not issued
Transcription – RhD positive baby recorded as RhD negative	2 patients – 1 given RhDIg <72h, 1 not given RhDIg No Kleihauer performed
No prophylaxis, 28 weeks	2 patients – RhDIg administered at 31w in both
No prophylaxis, 34 weeks	2 patients – no follow-up dose given
No prophylaxis, 28 & 38 weeks	4 patients – 3 not given by GP, 1 not given by hospital
Prophylaxis at delivery	3 patients – 1 patient given RhDIg <72h, 1 given >72h and 1 not given
No prophylaxis, potentially sensitising event	1 patient – following miscarriage, given RhDIg >72h
Wrong dose	
Wrong dose for gestational age	1 patient, 250 IU instead of 625 IU at 13+3 gestation, no Kleihauer performed

Conclusion

RhD Ig-related events are not uncommon. Feedback to staff and reporting to STIR will help improve awareness and compliance with guidelines. Follow up audits are planned.

P283. The suitability of the seqirus ECHO analyser as a secondary analyser to the ortho autovue innova in a high throughput transfusion medicine laboratory

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Aim

To determine if a secondary, small, throughput analyser would enhance workflow for performing transfusion medicine testing at a large tertiary hospital.

Method

Comparison testing between the ECHO Capture technology and the Autovue Innova CAT were performed on >100 patient samples. Testing included, Group and Screens (GAS) 36; Blood Groups 20; Antibody Screens 18; Direct Antiglobulin Tests 18. Antibody identification panels (14 cells) were run on the ECHO for all ECHO positive 3 cell screens. Donor blood bags were tested on the Echo: Check groups 20, Rhesus/Kell phenotypes 20, and results compared to the ARCBS typing.

To assess the efficiency and run times of the ECHO, large batches of 20 GAS (Screen first, Group second) and 16 GAS (Group first, Screen second) were performed. For comparison 16 GAS were performed on the Autovue. Smaller batches of 3-4 samples were continuously fed to simulate Transfusion laboratory workflow.

Results

Test results between the Autovue and the ECHO were mostly concordant with some minor differences in reaction strengths (stronger in the ECHO). Test times were similar between the two analysers when running small batches however there were significant differences in run times when running large batches.

Conclusion

The ECHO is designed to be used in a low throughput laboratory and requires some manual interventions compared to larger analysers. Test results by Capture assay were comparative to CAT technology. In a high throughput laboratory, the ECHO could serve as an additional analyser particularly for performing non-routine testing such as donor bag checks or phenotyping. However, as antibody identification must be performed in the same medium as screen, the Echo could only be used as an additional testing tool.

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

P284. Reducing the rate of acute adverse reactions during rapid intravenous infusion of iron in pregnancy - a prospective comparative audit of Iron Carboxymaltose and Iron Polymaltose at Auckland City Hospital, New Zealand

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Auckland District Health Board

Purpose

Iron deficiency anaemia (IDA) in pregnancy is associated with adverse outcomes. We started IV iron infusion in May 2012 as part of an Iron In Pregnancy Guideline.

Treatment for IDA using intravenous (IV) Iron ferric carboxymaltose (IFC) via a rapid infusion delivers a higher concentration at a greater rate compared with a rapid infusion protocol of iron polymaltose (IP).

Methods

Following ethics approval, prospective data from 92 consecutive pregnant women having an IV iron infusion for IDA were audited for acute infusion related SE with rapid administration of IP (maximal dose 1.5g).

Once IFC became available in New Zealand in 2014, prospective data from 88 consecutive women having an IV iron infusion for IDA were audited for acute infusion related SE to rapid administration of IFC (maximal dose 1g).

We gathered data on demographics, details of adverse reactions, infusion completion, pre-, post infusion and postpartum haemoglobin, blood loss and blood products used in the peri-partum period.

Result

There was no statistical difference in the baseline characteristics between IFC and IP groups.

Only 2.3% of patients experienced an acute adverse reaction with IFC versus 28% with IP. The difference is statistically significant with p value 0.0001 (using Fisher's Exact test). Only two patients (2.3%) in our IFC group did not complete the infusion compared to 12 (13%) in the IP group because of side effects.

Implementation of IFC increased the number of iron infusions administered from 41.7 to 97.6 per annum, using the same staff resource.

Conclusions

IFC in pregnancy in our institution has a reduced side effect profile and provides a more time efficient way of administering iron in pregnancy and IV iron can be available to more women with no increase in staff resources. We have now adopted IFC as the standard iron formulation in pregnancy.

P285. Massive transfusion protocol: implementation and activation in a paediatric centre

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The Royal Children's Hospital (RCH) is the state trauma centre and treats neonates and children with complex surgical and medical conditions. In 2011 the National Blood Authority released guidelines on managing critical bleeding and massive transfusion and recommended hospitals develop a massive transfusion (MT) protocol (MTP). A neonatal and paediatric specific MTP was developed and implemented, with a definition of MT of > 40mls/kg RBC within 4 hours.

Key components of the protocol included safe, timely and appropriate provision of blood products, allocation of roles, effective communication, weight-based ratio therapy, pathology testing, and post procedure evaluation. Post MTP feedback is provided to all areas, highlighting processes that worked well and identifying issues.

Aim

To retrospectively review all episodes of MTP since implementation of the MTP with respect to key components in the protocol.

Methods

A retrospective review of all episodes of MTP at RCH from September 2014 to July 2015. Data collected included: demographics, indication for MTP activation, amount of blood components transfused, communication, documentation, and pathology results.

Results

28 MTP activations from September 2014 until July 2015 were reviewed. The median age was 4 years old (range 1 day to 16 years) and 12 (43%) were female. The most common indication for MTP was trauma (43%), followed by surgery (35%). A mean of 42 mls/kg RBC, 32 mls/kg FFP, 11 mls/kg platelets and 4 mls/kg cryoprecipitate was transfused during MTPs. 7 (25%) received tranexamic acid. 6 children (21%) died following resuscitation attempts.

Conclusion

MTP events occurred more frequently than anticipated. These children received large transfusion volumes of resuscitation. Areas identified for improvement included communication, documentation of events and completion of evaluation forms.

Future planning includes dedicated MTP simulation training.

P288. Phospholipid vesicle size and composition influence procoagulant activity

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Aim

Lipid membrane heterogeneity can differentially modulate the regulation of both procoagulant and anticoagulant reactions to varying degrees. Here we used a linear model based on factorial design, to prepare vesicles of varying lipid compositions, and investigated the associated differences in procoagulant activity. Further, by preparing the vesicles using a variety of techniques, we investigated if functional procoagulant activity was attributed not only to the lipid composition but also to the biophysical arrangement of these lipids in various sized vesicles.

Method

Phospholipid vesicles were prepared with 10 differing lipid constitutions containing phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), cholesterol (Chol) and sphingomyelin (SM) by using sonication, extrusion, dialysis, and hydration (3 runs for each technique). These different techniques produce phospholipid vesicles ranging in size from 100nm to 5 μ m, as confirmed using a Zetasizer (Malvern Instruments). Factor Xa-1-stage assays were used to examine the influence of vesicle size and composition on procoagulant activity in normal pooled plasma.

Result

Factor Xa-1-stage clotting times for each of the 10 different vesicle compositions decreased progressively from largest vesicles (those made by hydration, \sim 5 μ m), to the smallest (sonication \sim 100-200nm). There was at least a 10 second decrease for all lipid combinations that was dependant on vesicle size. For example, for PC:PS (90:10), clotting times decreased from (43 s) for hydration, down to (22 s) for sonication. When observing the influence of lipid composition, using sonicated vesicles, a significant decrease in clotting time was observed when cholesterol and/or phosphatidylethanolamine were added to PC:PS ($p < 0.05$).

Conclusion

The present study shows that the smaller rather than larger phospholipid vesicles preferentially stimulate procoagulant activity and that the addition of cholesterol and/or phosphatidylethanolamine potentiates this activity.

P289. Long-term safety and efficacy of recombinant Factor IX Fc fusion protein (rFIXFc) in adults/adolescents with haemophilia B: longitudinal analysis of B-LONG and B-YOND

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Aim

To report long-term efficacy/safety from the Phase 3 B-LONG study and B-YOND extension.

Method

This analysis includes cumulative data (interim data cut, 17-Oct-2014). In B-LONG, previously treated males aged ≥ 12 y with severe haemophilia B received weekly prophylaxis (WP: 50 IU/kg/7d, with dose adjustments), individualised prophylaxis (IP: 100 IU/kg/10d, with interval adjustments), episodic treatment (ET: 20-100 IU/kg), or perioperative management. Subjects in B-YOND received WP (20-100 IU/kg every 7d), IP (100 IU/kg every 8-16d or twice monthly), modified prophylaxis (MP, subjects not achieving optimal IP or WP dosing), or ET. Data were summarised according to treatment group in which each subject participated; thus, subjects may be included in >1 group.

Result

Of 123 subjects dosed in B-LONG, 115 completed and 93 of these enrolled in B-YOND (n=68 ongoing at cutoff). Among 123 subjects, median (range) cumulative rFIXFc exposure days was 123.0 (1-351) and the median cumulative duration on rFIXFc was 167.43 weeks. Median pooled annualised bleeding rates (ABRs) for were 2.41, 1.95, and 2.42 for WP [n=73], IP [n=33], and MP [n=13], respectively; for ET (16.28 [n=27]); for spontaneous bleeds/spontaneous joint bleeds, median ABRs were <1 in all prophylaxis groups. Overall median ABRs remained steady from Year 1 to Years 2 and 3 with WP (n=36; 2.05, 1.08, and 2.16, respectively) and IP (n=25; 1.00, 2.00, and 1.46, respectively). Overall, 97.3% of bleeding episodes were controlled with 1-2 infusions. With rFIXFc, 89% (32/36) of subjects increased and 11% (4/36) maintained dosing interval compared with prestudy FIX product, while median (IQR) total weekly prophylactic dose decreased (prestudy FIX: 79.4 [44.9, 107.5] IU/kg; on-study rFIXFc: 50.0 [40.0, 60.0] IU/kg). No inhibitors were observed; adverse events were typical of a hemophilia B population.

Conclusion

Data from B-LONG/B-YOND confirm long-term efficacy of rFIXFc in adults/adolescents with haemophilia B over a median of 3 years.

This research was supported by Biogen and Sobi. Biogen and Sobi reviewed and provided feedback on the abstract. The authors had full editorial control of the abstract and provided their final approval of all content

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P290. Long-Term Safety and Efficacy of Recombinant Factor IX Fc Fusion Protein (rFIXFc) Prophylaxis in Children With Haemophilia B: Longitudinal Analysis of Kids B-LONG and B-YOND

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Aim

To report long-term safety/efficacy data from the Phase 3 Kids B-LONG study and B-YOND extension study.

Method

This analysis includes cumulative Kids B-LONG and B-YOND data (interim data cut, 17-Oct-2014). In Kids B-LONG, previously-treated males aged <12y with severe haemophilia B received weekly rFIXFc prophylaxis (50-60 IU/kg) [dose and interval adjusted per pharmacokinetic/clinical assessment]. Subjects completing Kids B-LONG enrolled in 1 of 3 groups in B-YOND: weekly prophylaxis (WP: 20-100 IU/kg), individualised prophylaxis (IP: 100 IU/kg every 8-16d or twice monthly), or modified prophylaxis (MP, for subjects not achieving optimal prophylactic dosing with IP/WP). Data were summarised according to each treatment group in which subjects participated; thus, subjects may be included in analysis of >1 treatment group.

Results

At interim cutoff, 23/30 subjects had enrolled in B-YOND (n=21 ongoing in B-YOND). Among all 30 subjects, median cumulative rFIXFc exposure days was 90.0 (13, 164) and median cumulative duration on rFIXFc was 91.36 (11.9, 110.5) weeks. Median (IQR) pooled annualised bleeding rates (ABRs) were 1.60 (0.00, 2.90) for WP (n=30) and 2.37 (1.99, 6.28) for IP (n=5); for spontaneous bleeds/spontaneous joint bleeds, median ABRs were 0.00 in both groups. Among subjects on WP (n=19), overall median (IQR) ABRs were 2.00 (1.00, 3.12) and 1.15 (0.00, 3.44) in Year 1 and 2, respectively. Overall, 93.3% of bleeding episodes were controlled with 1-2 infusions. With rFIXFc, 78% (18/23) of subjects increased and 22% (5/23) maintained dosing interval compared with prestudy FIX product, while median (IQR) total weekly prophylactic dose decreased (prestudy FIX: 100 [62, 120] IU/kg; on-study rFIXFc: 60 [50, 70] IU/kg). No inhibitors were observed; adverse events were typical of a pediatric haemophilia B population.

Conclusion

Longitudinal data from Kids B-LONG/B-YOND confirm the long-term efficacy of rFIXFc in children with haemophilia B over a median of 1.8 years.

This research was supported by Biogen and Sobi. Biogen and Sobi reviewed and provided feedback on the abstract. The authors had full editorial control of the abstract and provided their final approval of all content.

P291. Audit of the use of Bethesda assays at a State Haemophilia Service

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Alfred Health

Aim

Bethesda assays are used to quantify coagulation factor inhibitors in congenital and acquired haemophilia. Our aim was to audit the use of Bethesda assays at a State haemophilia service and compare to published guidelines^{1,2}.

Method

Modified Nijmegen Bethesda assays were performed using buffered and ionised commercial normal control plasma. Bethesda assays ordered in an 18-month period from 12/04/2014 to 13/10/2015 were included. Clinical information for haemophilia patients (acquired and congenital) was collected from patient records regarding severity, indication, clinical setting, previous inhibitors, and subsequent changes in management.

Results

300 Bethesda assays from 95 different patients were included.

77 (81%) congenital haemophilia patients had 146 assays; 52% were performed as screening. Consistent with published guidelines, more severe haemophiliacs were screened (mild, moderate severe had 30, 20, and 50%, respectively). Also consistent was the median 12 months between assays. No management was changed by screening. 47% of assays monitored existing inhibitors in 18 congenital haemophilia patients. Median interval was 3 months. Only 2 assays (3%) changed management: one each of altered future haemostatic therapy and commencement of immune tolerance induction. 1 diagnostic assay was performed and was negative.

18 acquired haemophilia patients had 154 assays during the study period. 12 assays (8%) were diagnostic (9 new diagnoses and 3 relapses). 92% were monitoring known inhibitors. The test interval was between 7 days and one month in 82% (median 14 days). Testing was most frequent after diagnosis or a significant change in inhibitor level. Management was changed after 52% of assays, mainly titration of immunosuppression.

Conclusion

Use of Bethesda assays in screening and monitoring for inhibitors in congenital haemophilia is consistent with current published guidelines.

In acquired haemophilia guidelines do not recommend a frequency for monitoring inhibitors. Given the proportion of assays affecting management, this data suggests appropriate monitoring fortnightly after diagnosis or significant change in inhibitor titre until stability achieved, then less frequently.

P292. An investigation of the impact of discrete geometric variables on platelet aggregation at stenosis

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Aim

Atherothrombosis and thrombosis arising from stent implantation, LVAD, and extracorporeal perfusion devices (ECMO) represent significant clinical problems. Haemodynamic variables are thought to play a major role in thrombogenesis, however the effects of stenosis and device geometry on platelet function are poorly understood. This study investigated how stenosis entry angle impacts platelet-dependent adhesion and aggregation to von Willebrand factor (VWF) using a microfluidic platform with precisely controlled geometric variables.

Method

A high-resolution microfluidic platform with channel variations in entry angle, stenosis width and length, and exit angle was developed. Channels with stenoses with entry angles ranging from 85° - 30° were fabricated in polydimethylsiloxane using high-resolution (chrome mask) photolithography. Citrated human whole blood with DiOC₆ fluorescently-labelled platelets was perfused through VWF-coated microchannels at a range of constant flow rates (11.5 - 123µL/min). Platelet aggregation at the stenosis was monitored using real-time high-resolution epifluorescence microscopy. Aggregation rates and magnitudes were compared across geometries of varying entry angles at an arbitrary endpoint of 210sec.

Results

VWF-dependent platelet aggregation at stenosis was directly proportional to entry angle magnitude. Stenosis geometries with a constant peak shear rate of 45,000.s⁻¹ over 15msec, with entry angles ≥75°, induced platelet aggregation proportional to the angle. Stenosis geometries with an entry angle of 30° displayed minimal to no shear-dependent platelet aggregation despite a peak shear of 45,000.s⁻¹.

Conclusion

This study highlights the impact of discrete geometric parameters on haemodynamically-driven platelet aggregation and thrombosis, suggesting under conditions of short dwell time (≤15msec), rate of haemodynamic acceleration into stenosis is a critical factor in thrombogenesis. The critical role of the entry angle suggests that “platelet shear history” is a vital parameter in thrombosis development. Understanding the hydrodynamic and cellular scale parameters underpinning this will elucidate the role of haemodynamics in pathological thrombosis, and inform stent and ECMO device design(s) to prevent iatrogenic thrombogenesis.

P293. Management of Pregnancy Complications in a Patient with Combined Type 2N and Type 1 von Willebrand Disease

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Type 2N VWD is characterised by defective von Willebrand factor (VWF) with markedly decreased binding affinity for factor VIII (FVIII) resulting in disproportionately low FVIII level relative to VWF antigen (VWF:Ag) levels. Rarely, patients may exhibit both partial quantitative deficiency (type 1) and qualitative VWF abnormality (type 2). To our knowledge, this is the first report of a pregnant patient with combined type 2N/1 VWD, who has now had 2 successful vaginal deliveries at our institution.

Our patient had low pre-pregnancy levels of FVIII (6 U/dL), VWF:Ag (33 U/dL), VWF ristocetin cofactor (VWF:RCo; 34 U/dL) and VWF collagen binding (VWF:CB; 34 U/dL). The level of FVIII bound to VWF in a VWF-FVIII binding (VWF:FVIII B) assay was 1U/dL. Her levels did not rise appreciably during pregnancy. Sequencing of the *VWF* gene revealed 'homozygosity' for a novel missense mutation in exon 18, c. 2396G>T (p. Cys799Phe), which lies within the FVIII binding domain. We suspect our patient has a null mutation on the other chromosome, resulting in a combined 2N/1 phenotype. Her first pregnancy at age 21 was complicated by retroplacental haematoma at 28 weeks gestation. She received regular Biostate from 28 weeks onwards and Biostate infusion during labour, with an uncomplicated epidural for analgesia. Heavy vaginal bleeding postpartum was treated with additional Biostate.

During her second pregnancy at age 25, she was managed with prophylactic Biostate from 27 weeks gestation and early postpartum plus oral tranexamic acid. Both antepartum and postpartum bleeding were prevented.

Most published cases of 2N VWD did not require any haemostatic support [1-3], but none of these described cases were combined 2N/1 VWD. In patients with the most frequent type 2N mutation, Arg854Gln (R854Q), FVIII levels rise substantially during pregnancy. Our case highlights the need for careful monitoring and individualised treatment plans for patients with bleeding disorders.

P294. 2B or not 2B? A prothrombotic tendency masquerading as a bleeding disorder

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Type 2B von Willebrand Disease (2B-VWD) is a rare bleeding disorder in which increased affinity of von Willebrand factor (VWF) for platelets results in clearance of large VWF multimers and platelets. 2B-VWD is characterised by a positive low dose ristocetin-induced platelet agglutination assay (LD-RIPA), where patient platelet rich plasma aggregates at ≤ 0.6 mg/ml of ristocetin rather than the normal threshold of 0.8-1.2 mg/ml.

Although RIPA hyperresponsiveness is a defining feature of 2B-VWD (or its platelet counterpart – platelet type [PT]-VWD), the test can yield false positive and negative events.

We present a patient with metastatic clear cell renal cell carcinoma who had false positive LD-RIPA, and whom presented with a spinal metastatic mass requiring surgery. Routine pre-operative tests revealed prolonged APTT and prolonged closure times with the PFA-100. Comprehensive haemostatic testing then identified RIPA agglutination at 0.5 mg/ml of ristocetin; however, other platelet function tests, RIPA mixing studies, and VWF high levels with lack of functional VWF defect, suggested the positive LD-RIPA was due to non-specific hyperaggregable platelets rather than true 2B-VWD, PT-VWD or even acquired VWD. The patient had spinal surgery without any haemostatic support and this procedure was notably uneventful. He, however, developed a lower limb DVT on day 11 post-operatively, despite VTE prophylaxis.

Later genetic testing confirmed no VWD related defects in either *VWF* or *GP1BA* genes; however, a polymorphism associated with recurrent stroke was identified. This case aims to highlight potential issues with LD-RIPA testing and the importance of the patient's clinical history as well as additional laboratory evidence before making a diagnosis of VWD, particularly 2B or PT-VWD in this case. Notably, should the patient have been inappropriately identified as 2B-VWD based on his LD-RIPA response, any applied VWF concentrate therapy may have further promoted adverse thrombotic events.

P295. Role of Rotational Thrombelastometry (ROTEM®) in rapid estimation of Rivaroxaban drug level

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Background

Rivaroxaban, a direct factor Xa inhibitor, allows the use of fixed dosing schemes and eliminates the need for routine coagulation monitoring. However, rapid assessment of the degree of rivaroxaban anticoagulation is required in certain scenarios, e.g. major haemorrhage, before thrombolysis or urgent surgery. Clinically significant Rivaroxaban drug levels have been proposed between 30 – 50ng/mL using a calibrated anti-Xa assay, but the turnaround time for the test is slow. Therefore, rapidly available results using a point-of-care haemostasis test like ROTEM® might be beneficial in these scenarios.

Aim

The aim of this study was to evaluate the role of conventional rotational thrombelastometry (ROTEM®) and modified low tissue factor activated ROTEM® (LowTF-ROTEM®) in estimating rivaroxaban drug level in in-vivo setting.

Methods

In this multicenter study, we analysed 77 patients receiving Rivaroxaban.

Patients' citrated whole blood samples were tested on the ROTEM® delta analyser using commercially prepared EXTEM reagent according to manufacturer's instructions. Modified low tissue factor ROTEM® test was also performed, using a recombinant tissue factor reagent (Siemen's INNOVIN), used at a dilution of 1:1000 of the normal working concentration used for INR testing.

Patients' Rivaroxaban levels (ng/mL) were determined in citrated platelet poor plasma by analysis on the Stago STA-R Evolution analyser, using a chromogenic anti-Xa method (Diagnostica Stago Liquid anti-Xa).

Results

The relationship between ROTEM® clotting time (CT) and Rivaroxaban level is of moderate strength ($R^2 = 0.461$). For prediction of clinically significant Rivaroxaban levels of >50ng/mL, ROTEM® CT has a positive predictive value of 81% and negative predictive value of 100%.

The relationship between LowTF -ROTEM® clotting time (CT) and Rivaroxaban level is of a low strength ($R^2 = 0.142$).

Conclusion

ROTEM®, a point-of-care test, has utility in excluding clinically significant Rivaroxaban level, which may be critical for patients requiring urgent surgery or thrombolysis.

P296. Longitudinal analysis of annualised bleeding rates among adults/adolescents receiving weekly prophylaxis with rFVIII Fc in A-LONG and ASPIRE

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Aim

To report longitudinal annualised bleeding rates (ABRs) in subjects receiving once-weekly prophylaxis in A-LONG and/or the rFVIII Fc extension study ASPIRE.

Method

Previously treated males (≥ 12 y) with severe haemophilia A were eligible for A-LONG. Subjects enrolled into 1 of 3 arms: individualised prophylaxis (IP); weekly prophylaxis (WP; 65 IU/kg rFVIII Fc/7d) or episodic treatment (ET). Subjects who completed A-LONG could participate in 1 of 4 treatment groups in ASPIRE: IP, WP, modified prophylaxis (MP; subjects not achieving optimal prophylactic dosing with IP/WP), or ET. Subjects could change treatment groups at any point in ASPIRE; thus could be represented in ≥ 1 treatment group.

Result

From the beginning of A-LONG to first ASPIRE interim data cut (January 6, 2014), cumulative treatment time with rFVIII Fc among subjects ever on WP (n=37) was 1.51 (range, 0.02-2.08) years. Cumulative treatment time with rFVIII Fc among the subgroup of subjects remaining on WP from the beginning of A-LONG through ASPIRE interim data cut (n=17) was 1.99 (range, 0.02-2.08) years. Overall, spontaneous, and traumatic median (IQR) ABRs were 2.00 (0.72, 4.52), 1.00 (0.00, 2.66), and 0.60 (0.00, 1.93), respectively, among subjects ever in the WP group from the beginning of A-LONG to ASPIRE interim data cut. Among subjects treated episodically pre-A-LONG and remaining on WP from the beginning of A-LONG through ASPIRE interim data cut (n=17), overall median (IQR) ABR was 2.00 (0.98, 7.43). Among subjects in the IP group during A-LONG and switching to the WP group during ASPIRE (n=9), overall median (IQR) ABRs were 1.52 (0.00, 3.17) while on IP and 0.72 (0.00, 3.31) while on WP as of the interim data cut.

Conclusion

This analysis demonstrates that subjects dosing once-weekly with rFVIII Fc during A-LONG/ASPIRE maintained low ABRs over an extended time period, suggesting that once-weekly prophylaxis can control bleeding and may be appropriate for some patients.

This research was supported by Biogen and Sobi. Biogen and Sobi reviewed and provided feedback on the abstract. The authors had full editorial control of the abstract and provided their final approval of all content.

P297. Longitudinal modified Haemophilia Joint Health Scores (mHJHS) outcomes with recombinant Factor VIII Fc fusion protein (rFVIII Fc) prophylaxis in subjects with severe haemophilia A

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Aim

To report longitudinal joint health data from A-LONG and rFVIII Fc extension study, ASPIRE.

Method

Joint health was assessed using mHJHS at A-LONG screening and ASPIRE baseline (BL) and annually subsequently. The mHJHS differs from the standard HJHS Version 2.1; thus, the total score is lower (range=0-116; 0=normal function, 116=severe disease) compared with the standard HJHS (range=0-124). ASPIRE subjects who had mHJHS data at 4 time points (A-LONG BL, ASPIRE BL, ASPIRE Year 1 [Y1], and ASPIRE Y2) were included in this posthoc analysis.

Result

Among 150 adult/adolescent subjects (≥ 12 years) who completed A-LONG and enrolled in ASPIRE, 47 on rFVIII Fc prophylaxis in A-LONG/ASPIRE had mHJHS data at all 4 time points. Continuous improvement was observed over time with mean change of -1.6, -3.0, and -4.1 in mHJHS total score at ASPIRE BL, ASPIRE Y1, and ASPIRE Y2, respectively, compared with A-LONG BL (mean=23.4; SE=2.66; $P=0.001$ for total change from baseline). Among subjects with target joints at A-LONG baseline ($n=24$), mean changes in target joint mHJHS scores from A-LONG BL to ASPIRE BL, ASPIRE Y1, and ASPIRE Y2 were -0.9, -1.5, and -1.7, respectively. The mean changes in mHJHS scores of weight-bearing joints from A-LONG BL to ASPIRE BL, ASPIRE Y1, and ASPIRE Y2 were -0.4, -0.8, and -1.2, respectively; those in non-weight-bearing joints were -0.7, -1.5, and -1.9, respectively, over the same time points. All P values for ASPIRE Y2 were <0.05 . The mHJHS components that showed the greatest reductions in scores from A-LONG BL to ASPIRE Y2 were swelling (mean change [SE], -1.4 [0.52]) and range of motion (mean change [SE], -1.1 [0.48]).

Conclusion

Among subjects receiving long-term rFVIII Fc treatment, continued improvement in mHJHS scores for target joints and weight-bearing and non-weight-bearing joints was observed. Swelling and range of motion contributed most to the improvements in total score.

This research was supported by Biogen and Sobi. Biogen and Sobi reviewed and provided feedback on the abstract. The authors had full editorial control of the abstract and provided their final approval of all content.

P298. Efficacy and safety of rIX-FP in previously treated adult and adolescent patients with haemophilia B

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Background

A fusion protein genetically linking recombinant human coagulation FIX with recombinant human albumin (rIX-FP) has been developed with an improved pharmacokinetic profile which allows dosing every 7–14 days.

Aims

The safety and efficacy of rIX-FP was evaluated in previously treated male patients (12–61 years old) with severe haemophilia B (FIX activity $\leq 2\%$).

Methods

Subjects in the on-demand arm received on-demand treatment for 6 months before switching to 7-day prophylaxis. Subjects in the prophylaxis arm received 7-day prophylaxis for 6 months, and eligible subjects switched to either 10- or 14-day prophylaxis. Annualized spontaneous bleeding rates (AsBR) were compared between on-demand and prophylaxis treatment periods (on-demand arm), and between 7-day prophylaxis and 10- or 14-day prophylaxis (prophylaxis arm). Efficacy was evaluated by the number of injections to achieve haemostasis. Safety was evaluated by the number of inhibitors to FIX. The treatment period was 12 to 18 months.

Results

A total of 63 subjects were enrolled. In the on-demand arm, 19/23 subjects switched to 7-day prophylaxis treatment after completing 6 months on-demand treatment. The median (Q1, Q3) AsBR during on-demand treatment and prophylaxis treatment was 15.43 (7.98, 17.96) and 0.00 (0.00, 0.96), respectively, a reduction of 100% ($p < 0.0001$). Twenty-one subjects extended their prophylaxis treatment interval to 14 days. All prophylaxis subjects ($n=40$) on 7-, 10- and 14-day prophylaxis had a median AsBR of 0.00. Subjects on 14-day prophylaxis (50–75 IU/kg) reduced consumption by 50% over their prior FIX product. A total of 98.6% of bleeding episodes were successfully treated with ≤ 2 injections of rIX-FP, 93.6% with 1 injection. No subjects developed inhibitors to FIX and there were no related serious adverse events.

Conclusions

This phase 3 study demonstrated the clinical efficacy and favourable safety of rIX-FP for routine prophylaxis every 7-, 10- and 14-days and on-demand treatment of bleeding episodes.

This research was supported by CSL Behring. The company analysed the data and prepared the abstract.

P299. Diagnosis of factor IX propeptide mutation causing warfarin hypersensitivity

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Aim

To describe the investigation and diagnosis of a rare genetic defect causing severe bleeding in a therapeutically warfarinised patient following mitral valve replacement.

Case history

A 58 year old male presented 7 weeks post-op following mechanical mitral valve replacement with significant swelling and bruising to limbs caused by intramuscular haemorrhage. Hb on admission was 60g/L; the INR 3.0 and APTT 116s. Warfarin was reversed with Prothrombinex. Patient showed significant clinical improvement by day 5 of admission, commenced IV unfractionated heparin and was discharged day 15 on warfarin with a therapeutic INR.

36 days later the patient was re-admitted with severe bilateral lower limb and right upper limb bruising. Once again coagulation testing demonstrated a therapeutic INR but an excessively prolonged aPTT. The patient had no bleeding history to suggest a congenital disorder.

Methods

Coagulation investigations were performed on the patient at various intervals post warfarin cessation using a Stago Sta R Evolution analyser. Blood was subsequently sent for analysis of mutations within the propeptide of FIX.

Results

Testing revealed normal FVIII and vWF levels. However at presentation there was a marked deficiency of Factor IX (8%) with mildly reduced levels of other vitamin K dependant factors. Over 7 days the FIX returned to normal (71%) with the cessation of warfarin. Molecular testing confirmed that the patient was hemizygous for a missense variant in the coagulation Factor IX gene. This variant results in amino acid substitution in the pro-peptide domain of the Factor IX Protein. This mutation has been previously reported to be associated with bleeding complications during therapy with vitamin K antagonists.

Conclusion

Investigation of this patient's recurrent haemorrhage on warfarin revealed a rare FIX propeptide mutation. This has had important implications for the patient who has remained on twice daily injections of clexane to maintain anticoagulation.

P301. APS Auto-antibodies Creating Haemostatic Havoc

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Introduction

Antiphospholipid syndrome (APS) is an autoimmune disorder which is typically associated with thrombotic events. Uncommonly, bleeding diatheses may occur in APS, and are typically attributed to acquired coagulopathies such as severe thrombocytopenia or prothrombin deficiency. We report a case of long standing triple positive anti-phospholipid syndrome in a patient with recurrent pro-thrombotic events on combination anticoagulation and anti-platelet therapy, who presented with atraumatic intracranial haemorrhages and concomitant extensive cerebral venous sinus thromboses (CVT). We discuss the treatment dilemmas and strategies used to manage this case.

Case Discussion

A woman with primary APS with a longstanding history of recurrent thrombotic events despite combination anticoagulation and anti-platelet therapy presented in February 2015 with recurrent atraumatic intracranial haemorrhages (ICH). She subsequently developed concomitant extensive cerebral venous sinus thromboses (CVT). Extensive investigations did not identify any acquired coagulopathies, including those associated with APS. No structural abnormalities were detected on cerebral imaging and there was no evidence of cerebral vasculitis. Atypical pneumonia disrupting the pro-thrombotic equilibrium associated with her antiphospholipid auto-antibodies was considered a potential trigger.

This case highlights complex management dilemmas. Our treatment strategy was geared at rapid reduction in the APS auto-antibodies responsible for this life-threatening presentation, judicious anticoagulation, immune modulation and employment of hydroxychloroquine for its anti-thrombotic and anti-inflammatory properties. The patient is now well with no evidence of neurological sequelae clinically or on cerebral imaging.

Conclusion

We report a rare case of triple positive primary APS presenting with concurrent intracranial bleeding and thrombosis. She was successfully treated with an approach similar to that of catastrophic APS, employing a combination of judicious anticoagulation, plasma exchange, B cell depletion therapy and immune modulation.

P302. An attempt to "humanise" platelet PAR expression in mice by knocking PAR1 into the Par3 locus reveals expression of PAR1 is not tolerated in mouse platelets

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Aim

Anti-platelet drugs are the mainstay of pharmacotherapy for heart attack and stroke prevention, yet improvements are continually sought. Thrombin is the most potent activator of platelets and targeting platelet thrombin receptors (protease-activated receptors; PARs) is an emerging anti-thrombotic approach. Humans express two PARs on their platelets – PAR1 and PAR4. The first PAR1 antagonist was recently approved for clinical use and PAR4 antagonists are in early clinical development. However, pre-clinical studies examining platelet PAR function are challenging because the platelets of non-primates do not accurately reflect the PAR expression profile of human platelets. Mice, for example, express PAR3 and PAR4. To address this limitation, we aimed to develop a genetically modified mouse that would express the same repertoire of platelet PARs as humans.

Methods

To achieve this, human PAR1 preceded by a lox-stop-lox was knocked into the mouse Par3 locus, and then expressed in a platelet-specific manner (hPAR1-KI mice).

Results

Despite correct targeting and the predicted loss of PAR3 expression and function in platelets from hPAR1-KI mice, no PAR1 expression or function was detected. Specifically, PAR1 was not detected on the platelet surface nor internally by flow cytometry nor in whole cell lysates by Western blot, while a PAR1-activating peptide failed to induce platelet activation assessed by either aggregation or surface P-selectin expression. Platelets from hPAR1-KI mice did display significantly diminished responsiveness to thrombin stimulation in both assays, consistent with a Par3^{-/-} phenotype. In contrast to the observations in hPAR1-KI mouse platelets, the PAR1 construct used here was successfully expressed in HEK293T cells.

Conclusion

Together, these data suggest ectopic PAR1 expression is not tolerated in mouse platelets and indicate a different approach is required to develop a small animal model for the purpose of any future preclinical testing of PAR antagonists as anti-platelet drugs.

P303. Examination of Reduced Volume APCC (activated prothrombin complex concentrate- (FEIBA) for Accelerated Infusion in Adult Hemophilia A or B Patients with Inhibitors

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Introduction and Objectives

The use of bypassing agents such as activated prothrombin complex concentrate (aPCC: FEIBA) or rFVIIa has significantly improved treatment of hemophilia A and B patients with inhibitors. For the continued benefit of FEIBA prophylaxis in inhibitor patients, adherence to the prescribed regimen is critical. Product infusion time is an important consideration. A recent study of real-world FEIBA use (FEIBA PASS) showed faster infusion times (mean infusion rate = 3.8 U/kg/min) than currently indicated in the FEIBA SPC (2 U/kg/min). Presently, a study is underway to examine the efficacy of reducing the time of infusion through reduced volume and increased infusion rate of FEIBA.

Materials and Methods

A 2-part, phase 3b/4, open-label 2-way cross-over study, enrolling 32 adult hemophilia A or B patients with inhibitors (≥ 0.6 BU). The study aims at demonstrating safety and pharmacokinetic equivalence of FEIBA component Factor II in reduced volume versus FEIBA reconstituted in regular volume sterile water for injection (SWFI) at the standard infusion rate of 2 U/kg/min, followed by reduced volume FEIBA infused 2- and 5-times faster than the standard infusion rate. In part 1 of the study, subjects will receive 3 infusions of FEIBA reconstituted in regular volume, and 3 infusions of FEIBA reconstituted in 50% reduced volume, all administered at the current standard infusion rate (2 U/kg/min). In Part 2, subjects will receive 3 infusions of FEIBA reconstituted in 50% reduced volume at 4 U/kg/min followed by 3 infusions at 10 U/kg/min.

Results

The study is open for enrollment across Europe and the United States.

Conclusions

Prophylaxis with aPCC leads to improved outcomes in patients with inhibitors. The possibility of reducing infusion volumes and accelerating infusion rates could lead to increased adherence to FEIBA prophylaxis.

This study is sponsored by Baxalta US Inc./Baxalta Innovations GmbH, now part of Shire

P304. Results from a world-wide field study of FVIII activity assay variability of BAX 855, the PEGylated form of rFVIII ADVATE, in clinical hemostasis laboratories

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Background

Discrepancies were reported for BDD and some modified longer-acting FVIII products when one-stage clotting assays were used for FVIII activity analysis.

Aims

A multi-national collaborative field study among clinical and hemostasis laboratories to analyze plasma from patients with hemophilia A spiked in vitro with BAX 855 (ADYNOVATE/ADYNOVI) and ADVATE was performed.

Methods

FVIII was spiked at 0.80, 0.20 and 0.05 IU/mL based on labeled potencies. Samples were blinded and sent to participating sites. Laboratories analyzed samples with their routine FVIII assay and reported results.

Results

35 data sets were reported. All laboratories were using a one-stage clotting assays (OSCA) method, 11 also used chromogenic assays. No single laboratory performed a chromogenic assay only. At the highest FVIII concentration, the mean in vitro recovery relative to expected, using OSCA was 101% for ADYNOVATE and 114% for ADVATE. With decreasing FVIII concentrations, recoveries showed a trend to overestimation, however on average, recoveries were comparable for BAX 855 and ADVATE. Inter-laboratory variability increased with decreasing FVIII concentrations but was very similar for both products. For chromogenic FVIII activity assays, in vitro recoveries across all concentrations were comparable for BAX 855 and ADVATE. Variation increased with lower FVIII concentrations. At the lowest concentration it even exceeded variability of OSCA.

Conclusions

All types of OSCA and chromogenic assays can be used for FVIII activity measurement of BAX 855 and ADVATE in hemophilic plasma, resulting in similar accuracy and precision for both products. In general, at very low FVIII concentrations, higher variability should be expected with chromogenic assays. The results indicate no need for a product specific standard for BAX 855, similar to widely used and well established full-length rFVIII product ADVATE.

This study is sponsored by Baxalta US Inc./Baxalta Innovations GmbH, now part of Shire

P305. The AHEAD study: 2nd interim analysis results in 376 patients with Haemophilia A

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Introduction

We report 2 years of observation from the global long-term ADVATE Haemophilia A Outcome Database (AHEAD). The prospective cohort study collects data from patients on rAHF on-demand (OD), standard or pharmacokinetic -guided prophylaxis, or immune tolerance induction (ITI) therapy in routine clinical practice.

Methods

AHEAD will evaluate approximately 1000 severe to moderate haemophilia A (HA) patients, to be followed-for up to 8 years. Study endpoints include annualized (joint) bleeding rates (ABR/AJBR), factor consumption, and safety.

Results

The second interim analysis of September 2015 includes 376 patients from 18 countries; 243 completed year 1 and 154 year 2 visits. Median age at screening was 15 years (range 0–72) and 67% patients had severe HA (FVIII<1%); 78% were on prophylaxis, 20% were OD and 1% on ITI. Median ABRs/AJBRs were 1.4/1.0 and 11.1/6.5 in prophylaxis and OD groups, respectively for annual visit 1, 1.1/1.0 and 10.4/5.6, respectively, in the second year. 48% patients on prophylaxis and 22% patients OD had AJBR <1 in year 1 and 49% and 32% in year 2. In OD group 60% and 47% had AJBR \geq 6, after first and second year respectively, while only 9% and 7% in the prophylaxis group. Median annualized total dose in the prophylaxis group was 200,343 and 237,968 IU and 35,346 and 26,245 IU in OD group during year one and two,. Effectiveness of prophylaxis assessed by investigators was excellent/good in \geq 95% cases.. There were 8 treatment-related adverse events (AEs): one mild allergic cutaneous reaction with rhinitis and 7 low-titre inhibitors (4 positive at screening). All patients continued to receive rAHF.

Conclusion

Interim results of the AHEAD study show a - clear reduction in ABR/AJBR for patients on prophylaxis vs. OD. Treatment efficacy was excellent/good in the majority of cases. rAHF was well tolerated with very few treatment-related AEs

This study is sponsored by Baxalta US Inc./Baxalta Innovations GmbH, now part of Shire

P306. Evaluation of the Effectiveness of Eliquis (Apixaban) Risk Minimisation Tools in Australia

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Bristol-Myers Squibb

Aim

The Bristol-Myers Squibb Australia and Pfizer Australia Alliance have developed a healthcare professional (HCP) and patient educational program as a part of risk minimisation measures. The following risk minimisation (RM) tools were developed: **Prescriber Guide, Online Learning Module (OLM), and Patient Alert Card**. The primary objective of this study was to evaluate effectiveness of the RM tools assessing HCP's awareness, utilisation and knowledge.

Method

A non-interventional, cross-sectional study of Australian HCPs who received the RM tools and treated >1 patient with apixaban was conducted. A web-based online survey was designed with multiple-choice questions, descriptive comment fields and hypothetical risk-based scenarios. The primary goals were to calculate the proportion of HCPs who have used RM tools, assess HCP knowledge and comprehension, and behaviours related to bleeding risks associated with treatment. "Correct" knowledge was considered if ≥80% survey responses were "correct" and/or "partially correct".

Result

A total of 101 HCPs participated in the survey. The Prescriber Guide was used by 87 HCPs, the OLM was used by 43 HCPs, and the Patient Alert Cards were used by 68 HCPs. For the HCPs who did not use one of the RM tools, the primary reason was that were already aware of the risks of apixaban. Of the 13 questions related to HCP knowledge and comprehension on bleeding risks associated with treatment there were 3 questions with incorrect responses; 2%, 10% and 26%. The question with the most incorrect responses (26%) was related to management of apixaban overdose.

Conclusion

The RM tools distributed by the sponsors were effective in improving HCP knowledge of apixaban bleeding risks. The effectiveness of the RM tools will be assessed in a second study of Australian HCPs.

All authors are employees of Bristol-Myers Squibb Australia.

P307. Consequences of Tranexamic Acid on fibrinolysis in patients with hereditary bleeding disorders compared with healthy controls: a report on the control-arm

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Aim

To determine the peak and the duration of anti-fibrinolytic activity following oral Tranexamic Acid (TXA) in healthy volunteers using the clot lysis assay.

Method

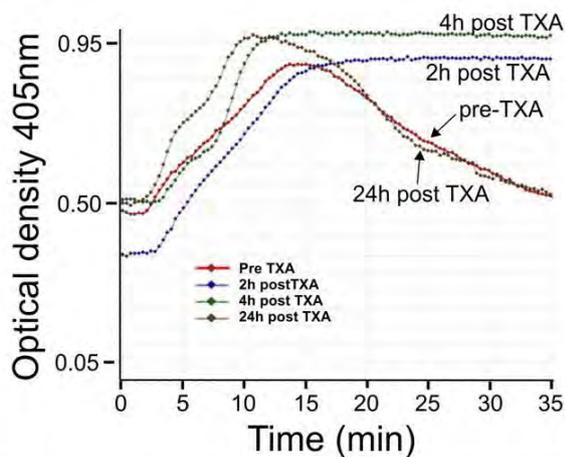
Ten eligible healthy volunteers were recruited after approval by the Hospital Research Ethics Board. Blood was taken at baseline, then at two, four, and twenty-four hours after ingestion of 1 gram of TXA. The Clot lysis assay was performed as described (Niego et al 2008), on plasma samples recalcified in the presence of tissue plasminogen activator (t-PA).

Results

8 Males (median age 35.5years, range 29-60years) and 2 Females (age 20 and 31 years) were recruited. One of the healthy volunteers had comorbidities and took regular medications which are not known to interfere with fibrinolysis. Preliminary analysis indicated typical normal clot formation and lysis profiles that were observed at baseline. TXA showed complete blockade of fibrinolysis at 2 hours and at 4 hours but returned back to normal at 24hours (see Figure).

Conclusion

In healthy subjects, oral TXA is effective at blocking t-PA mediated fibrinolysis up to at least 4 hours. Whether TXA has the same effect in patients with haemophilia who take prophylactic TXA for dental or endoscopic procedures in the absence of factor concentrate, will be investigated in the patient-arm of our trial.



Example of a plasma clot lysis assay showing the blocking effect of tranexamic acid (TXA) on t-PA-induced clot lysis 2 and 4h after administration, but returning to normal activity after 24h.

P308. Implementation of viscoelastic testing at a tertiary hospital in Western Australia

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The ROTEM® Delta analyser provides rapid measurements of the viscoelastic changes accompanying whole-blood coagulation and subsequent lysis and is proven useful in clinical situations where timely, targeted haemostatic therapy is required such as cardiac surgery and liver transplantation. Assessments were made of the correlation between ROTEM® and standard laboratory coagulation results. An audit of blood products given post ROTEM® analysis was completed.

Method

A retrospective audit of all ROTEM® results (n=603) was performed for the period 4 August 2014 to 22 September 2015 (13months). Correlations between ROTEM® and simultaneous plasma coagulation studies including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen levels and platelet counts were assessed using Spearman's rank correlation. Relevant transfusion information relating to subsequent administration and timing of haemostatic products was audited.

Results

A total of 603 ROTEM® analyses were performed on 364 patients. Correlation of ROTEM® results with simultaneous plasma coagulation tests and platelet counts are shown in Table 1. The proportion of results where fresh blood products were indicated and subsequently transfused guided by ROTEM® is shown in Figure 1.

ROTEM® Parameter	PT (s)	APTT (s)	FIB (g/L)	PLT COUNT (x10 ⁹ /L)
EXTEM CT	0.50			
CFT	0.37			
MCF				0.67
INTEM CT		0.33		
CFT		0.49		
FIBTEM A10			0.87	
MCF			0.80	

Table 1. Correlation studies (P<0.001)

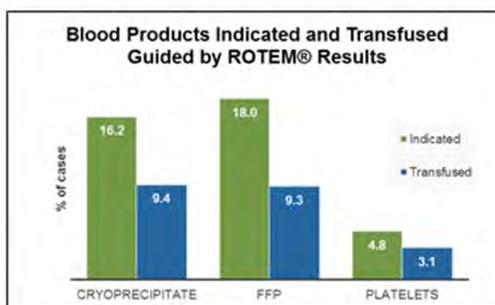


Figure 1. Transfusion audit.

Conclusion

ROTEM® parameters are derived from dynamic measurements accompanying whole blood coagulation and are therefore not directly comparable to standard plasma coagulation tests and whole blood platelet numbers. Despite this there was a very strong correlation between fibrinogen levels and FibTEM A10 results. There was also strong correlation between both the ExTEM CT and PT results and the ExTEM Maximum Clot Firmness (MCF) and platelet counts.

Not all fresh blood products indicated were subsequently transfused. This reiterates that transfusion therapy is a clinical decision, guided only by viscoelastic testing results.

P310. Prevalence of venous thromboembolism in lymphoma patients risk stratified according to Khorana score

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Aim

Several international guidelines have recommended that ambulatory cancer patients at high risk of venous thromboembolism (VTE), as stratified by the Khorana score, receive chemoprophylaxis while undergoing chemotherapy.^{1,2} Lymphoma has been identified as a high risk cancer¹. This audit aims to identify the prevalence of VTE in lymphoma patients at our institution, stratified according to risk.

Method

A retrospective chart review was performed on all patients treated acutely for a lymphoma in 2014 and 2015. Using the Khorana score, patients were identified as low (0 points), intermediate (1-2), or high risk (≥ 3).¹

Result

A total of 100 patients were included in the evaluation; four were excluded due to existing VTE at diagnosis. Of the remaining 96 patients, 81 were identified as intermediate risk and 15 were high risk. None received chemoprophylaxis. Six patients were diagnosed with VTE during chemotherapy for lymphoma (3 upper limb, and 3 PE); 2/6 were high risk (including a patient with concurrent renal cell carcinoma), 4/6 were intermediate risk. The prevalence of VTE in high risk patients was 13% versus 4.9% in intermediate risk patients ($p=0.2354$). Five patients (DVT or PE) were treated with enoxaparin, while one patient with superficial upper limb thrombosis was observed and received no pharmacological treatment. No bleeding was recorded despite two patients having platelet nadirs of $\leq 50 \times 10^9/L$.

Conclusion

The use of chemoprophylaxis for high-risk ambulatory patients remains low, likely due to concerns regarding increased bleeding risk. This audit demonstrates a higher rate of VTE in high risk lymphoma patients using the Khorana score (although not statistically significant due to low patient numbers) and confirms the potential role of chemoprophylaxis in the Australian setting. We also show the importance of considering other patient risk factors in addition to the Khorana score, such as permanent access lines and multiple malignancies.

P311. Patient Satisfaction After Conversion From Warfarin to a Factor Xa Direct Oral Anticoagulant (DOAC)

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Background

The direct oral anticoagulants (DOACs) are growing in popularity for the prevention and treatment of thromboembolism. There are a number of benefits of the DOACs over warfarin, including fixed dosing, linear pharmacokinetics, fewer food and drug interactions, a wide therapeutic window and a rapid onset of action. However, there is limited data assessing patient satisfaction after switching from warfarin to a DOAC.

Aims

To assess patient satisfaction after conversion from warfarin to a DOAC.

Methods

95 patients from a single centre were identified as being treated with a Factor Xa DOAC (rivaroxaban or apixaban) after switching from warfarin between August 2011 - December 2014. Satisfaction with their current anticoagulation therapy was prospectively assessed through the use of the Anti-Clot Treatment Scale (ACTS)¹. A supplementary questionnaire assessed the patient's relative satisfaction with their previous warfarin therapy compared to current DOAC therapy.

Results

At the time of writing, 72 patients had completed the survey and form the basis of this study (rivaroxaban=52, apixaban=18, unknown=2). The median ACTS score was 57 (range: 38-60) out of a possible 60, reflective of high satisfaction with DOAC treatment. 68 patients (94%) stated preference for the DOAC over warfarin. The leading perceptions driving this preference were reduction in frequency of medical contact and fewer side effects. Retrospective review of associated medical records identified seven surveyed patients (9.7%) that experienced an adverse event related to the DOAC. However, the majority of these individuals (n=6) maintained higher satisfaction scores with DOAC therapy compared to warfarin. The remaining patient stated neither treatment was better.

Conclusion

In this study, patient satisfaction with DOAC therapy after switching from warfarin was high. The incidence of adverse events after switching to a DOAC appears low. Improved patient convenience including reduced frequency of medical contact and fewer side effects are perceived as the most significant advantages of DOAC treatment compared to warfarin.

P312. Potential of Anthocyanins to Reduce Platelet-hyperactivation, Aggregation and Thrombogenesis in Obesity

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Aim

Obesity is becoming an increasingly widespread endemic, causing metabolic system dysfunction and resulting in endothelium damage, increased free radical production, lipid peroxidation, platelet hyperactivity and aggregation. Anthocyanins an antioxidant subclass of the polyphenol family, have been shown to exhibit anti-thrombotic properties in the obese or prothrombotic population.

Method

A randomised, double-blind, placebo controlled, crossover clinical trial was performed. Twenty-six overweight/obese subjects consumed 320mg/day anthocyanin capsule (bilberry and blackcurrant extract) or placebo for 28-days followed by a 2-week wash-out period. Fasting blood samples were collected at baseline and post supplementation and evaluated for haemostatic activity (coagulation pathway and full blood count analysis), biochemical profile, cardiac and oxidative stress markers (Lactate Dehydrogenase, Uric Acid, Creatinine Kinase and Troponin) as well as both platelet activation-dependent surface markers (CD14/CD42b, PECAM-1, PAC-1 and P-selectin) and platelet aggregation was used to evaluate possible blunting of specific pathways induced by (ADP, arachidonic acid and collagen). A one and two-way ANOVA was used to evaluate this data.

Result

Anthocyanin supplementation showed inhibition of platelet aggregation induced by ADP ($P<0.0001$), collagen ($P<0.0001$) and arachidonic acid ($P<0.0001$) as well as inhibition of platelet activation PECAM (CD31) ($P<0.001$) and PAC-1 ($P<0.05$).

Conclusion

Anthocyanin supplementation exhibited anti-thrombotic properties and a reduction in platelet activation and aggregation, suggesting that anthocyanin supplementation may benefit the pro-thrombotic population to reduce the risk of cardiovascular disease.

P313. The utility and safety of a nurse led pathway to transition patients with venous thromboembolism (VTE), treated with Rivaroxaban, into the community

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Background

Rivaroxaban, a factor X inhibitor, simplifies the treatment of patients with VTE. However the appropriate clinical monitoring of these patients to facilitate safe and early hospital discharge, whilst promoting treatment adherence, is yet to be defined.

Aim

To prospectively audit the safety and utility of a nurse led outpatient pathway to monitor patients treated with Rivaroxaban for venous thromboembolism (VTE).

Methods: Prospective audit of patients treated with oral Rivaroxaban for objectively confirmed VTE at Monash Health from August 2015-May2016. Patients were identified by emergency room or inpatient discharge referrals or by pharmacy discharge scripts. An experienced research nurse reviewed their inpatient and laboratory records' for suitability for receiving Rivaroxaban. Subsequently patients were followed with telephone calls and email for 90 days. All patients were referred for formal outpatients review

Outcome measures

The proportion of patients in whom an intervention was required to prevent potential major recurrence of VTE or major bleeding, and the proportion of patients adherent to medication at 90 days.

Results

One hundred and twenty three patients were identified with a mean age was 54 years, 55% were female and 59% of patients had PE, 48% had deep vein thrombosis. Three patients were treated for other VTE. Intervention to prevent major bleeding or recurrence was required in 11% of patients (6.5% and 4.5% respectively). One patient died prior to review on the pathway, a single possible recurrence occurred and no major bleeds. Of the 81 patients on the pathway at 90 days 83% were adherent with medication. Thirty two patients did not complete the 90 days follow-up for various reasons (changed anticoagulation (6.5%), declined follow up (15%), or planned cessation (12%)).

Conclusion

Our pathway potentially averted major bleeding or recurrence in 11% of patients and was associated with high rates of medication adherence.

P314. Poor adherence to intravenous heparin infusion protocols: do it well or not at all?

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Poor adherence to intravenous heparin infusion protocols: do it well or not at all?

Despite increasing anticoagulant choice, unfractionated heparin (UFH) retains an important role in the management of acute coronary syndromes, acute venous thromboembolism in patients with excessive bleeding risk or renal impairment, and peri-procedural anticoagulation. Due to the unpredictable anticoagulant response, heparin infusion protocols are used to guide dose adjustments based on the activated partial thromboplastin time (aPTT). We hypothesize that there is a lack of understanding of unfractionated heparin pharmacology and poor rates of compliance to the heparin infusion protocol. The consequence of this is an increased risk of thrombotic and bleeding events.

We conducted a retrospective audit of intravenous heparin use in 12 Blacktown District Hospital patients. Parameters investigated included timing of aPTT checks, percent of therapeutic aPTTs, appropriateness of dose adjustments and treatment location. Case notes were also reviewed to establish reasons for delays or protocol deviation, where possible.

37% of aPTT samples were not collected at the recommended time after dose change. 75% of all aPTT measurements were outside therapeutic range. Only 51% of dose adjustments were consistent with protocol recommendations. Our findings were consistent across treatment locations (cardiac step down, coronary care, high dependency and intensive care units).

Our results demonstrate that UFH protocol adherence is poor increasing risks of adverse events. Contributing factors identified include difficult intravenous access, delays in JMOs attending to aPTTs, sample processing or results being acted upon, poor understanding of heparin pharmacokinetics, lack of appreciation of implications of sub or supratherapeutic aPTTs, and lack of senior clinician input for complex scenarios not addressed by protocols.

These data from part of a quality improvement project and will be used as a comparator for a post-implementation audit. Quality measures to be implemented include electronic medication management with an IV UFH 'powerplan' and linked APTT orders, JMO education and senior clinician engagement.

P315. Acquired von Willebrand Disease Secondary to Haematological Malignancy: 2 Cases

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Aim

Acquired von Willebrand disease (aVWD) is a rare bleeding disorder with clinico-laboratory features of hereditary vWD that arises in previously normal patients. We describe two illustrative cases that demonstrate the variability in bleeding risk and management issues.

Method

We reviewed two recent cases of patients of aVWD that presented to our referral centre. Data was collected from case review and electronic results.

Results

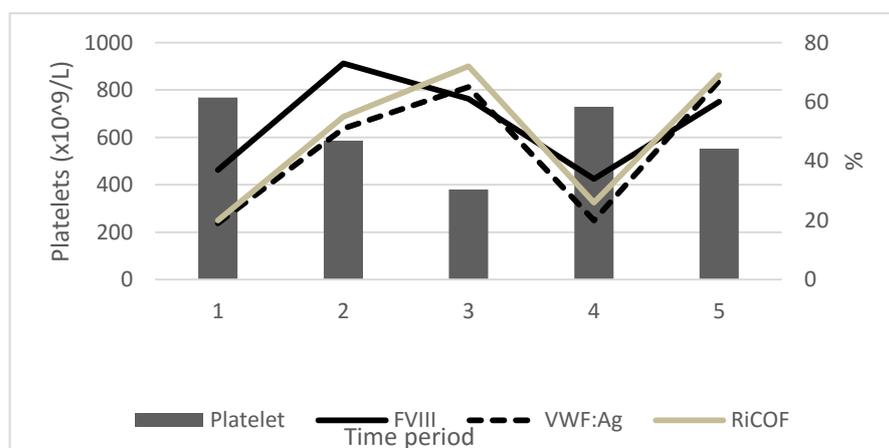
Patient #1: 42 year old lady has a 10 year history of essential thrombocytosis and diagnosed with aVWD as FVIII 37%, VWF:Ag 19%, RiCOF 20%, CBA 39%. There was an inverse relationship with platelet count and VWF levels (Figure), after therapy with either hydroxyurea, IFN or anagrelide. Factor replacement was required prior to surgical procedures.

Patient #2: 71 year old man with Waldenstrom's macroglobulinaemia (IgM lambda/kappa = 58.8g/L) has been under routine review with no indication for therapy. He was diagnosed with aVWD (Table). He underwent major facial surgery for excision of extensive squamous cell carcinoma and flap insertion. He was treated with Biostate pre-operatively and further doses in the week post-operatively. At 6 weeks post-operatively, there was significant secondary haemorrhage and bleeding was not controlled with further Biostate doses. There was successful haemostasis after therapy with plasma exchange (PEX) and prednisolone/cyclophosphamide.

	FVIII (%)	VWF:Ag (%)	VWF:RiCOF (%)	vWF:CBA (%)
Baseline	19	12	14	7%
Day 0 post-op.	75	104	81	
Delayed bleeding	24	14	11	13
Post-PEX	85	87	74	82

Conclusion

aVWD is a rare bleeding disorder and may be associated with an underlying haematological malignancy. The investigation of a bleeding disorder should consider aVWD in patients with a previous malignancy. The management of these patients may require a combination of tranexamic acid, replacement factor, chemotherapy or plasma exchange.



P316. Post hoc analysis to evaluate the effect of recombinant Factor IX Fc fusion protein (rFIXFc) prophylaxis in adults and adolescents with target joints and Haemophilia B

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Aim

To report longitudinal data from subjects with target joints at entry into B-LONG throughout the rFIXFc extension study, B-YOND.

Method

Subjects completing B-LONG could enroll in 1/4 treatment groups: weekly prophylaxis (WP; 20-100 IU/kg/7 days), individualised prophylaxis (IP; 100 IU/kg/8-16 days), modified prophylaxis (MP; subjects not achieving optimal prophylactic dosing with IP/WP), or episodic treatment. Subjects with ≥ 1 target joint at entry into parent study with on-study data were evaluated. Target joint was considered resolved if there were ≤ 2 spontaneous joint bleeds over 12 consecutive months. Outcomes were analysed over cumulative duration of B-LONG through B-YOND interim data cut (October 17, 2014).

Result

Among 117 B-LONG subjects with on-study data, 60 had a total of 166 target joints at baseline (knee [n=57; 34.3%], ankle [n=54; 32.5%], elbow [n=41; 24.7%], hip [n=7; 4.2%], shoulder [n=4; 2.4%], wrist [n=3; 1.8%]). In subjects with target joints at baseline, median (IQR) overall annualised bleeding rates (ABRs) with rFIXFc prophylaxis tended to be lower than those with prestudy (pre-B-LONG) prophylaxis (on-study: WP [n=13], 3.4 [2.1-5.9]; MP [n=6], 4.9 [1.3-6.3] vs, prestudy: WP [n=11], 6.0 [2.0-15.0]; MP [n=6], 8.0 [5.0-20.0]). On-study spontaneous target joint median (IQR) ABRs tended to be low among subjects receiving prestudy prophylaxis (WP, 0.4 [0.0-3.2]; MP, 0.2 [0.0-3.3]). 37.5% of WP, 8.3% of IP, and 45.5% of MP subjects had no target joint bleeds during B-LONG/B-YOND. 97.3% (36/37) of B-LONG prophylaxis subjects with baseline target joints and at 12 months follow-up had target joints resolved. In the WP/IP groups, median (IQR) average weekly prophylactic dose was 45.3 (37.3-54.7) IU/kg and 64.9 (47.1-82.3) IU/kg, respectively; median (IQR) dosing interval in the IP group was 10.4 (8.9-13.0) days.

Conclusion

Treatment with long-term rFIXFc prophylaxis resulted in low target joint ABRs with prolonged dosing intervals in adult/adolescent subjects with target joints and haemophilia B.

This research was supported by Biogen and Sobi. Biogen and Sobi reviewed and provided feedback on the abstract. The authors had full editorial control of the abstract and provided their final approval of all content.

P317. Quantification of neutrophil extracellular traps in sepsis and thrombosis

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Neutrophil extracellular traps (NETs) are networks of extracellular fibres produced primarily from neutrophil DNA, which has been shown to occur in conditions such as bacterial infection and thrombosis (1), as well as autoimmune conditions (2).

Easy and reliable assays for measurement of NETs are highly desirable. In particular, the potential for new treatments such as DNase in the treatment of clots is currently being explored, not only in DVT, but also in other conditions with aberrant or unhelpful NETs formation such in the context of myocardial ischaemia.

Gavillet et al has recently shown that flow cytometry can be used to detect NETs by showing increased leukocyte surface citrullinated histone H3 and myeloperoxidase in the context of sepsis (3).

We expanded on this method and confirmed its feasibility, demonstrating that the increased histone and myeloperoxidase appears to occur in the neutrophil population, and occurs not only in the context of sepsis, but also in patients with thrombosis. NETs as detected by flow cytometry is significantly increased in patients with sepsis and thrombosis compared with healthy controls.

In addition, we also explored the use of serum free double-stranded DNA as a surrogate marker for NET formation. This marker is also significantly increased in patients with sepsis and thrombosis. We were not able to demonstrate a strong correlation between these two methods of quantifying NETs.

The weak correlation between these methods could partly be the different compartments they measure – flow cytometry detects NETs still attached to neutrophils, while serum free dsDNA is a surrogate for NETs in the serum phase.

Further studies are required to determine the most biologically and clinically relevant marker of NET.

P318. A comparison of the Clauss fibrinogen and PT-derived fibrinogen in patients on dabigatran, rivaroxaban and apixaban

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Introduction and Aim

Previously we have demonstrated a close agreement between the Clauss fibrinogen (CF) and PT-derived fibrinogen (DF) within the normal fibrinogen range (1.5-4.0g/L). With increasing use of factor Xa and thrombin inhibitors and their variable effect on routine coagulation tests, we investigated if the CF-DF relationship was maintained with these anticoagulants. The DF, extrapolated from the PT, is more economical than CF to quantitate fibrinogen.

Methods

Plasma samples sent for dabigatran (n=23), rivaroxaban (n=28) or apixaban (n=26) level testing between 2012-2015 were retrospectively reviewed. Paired DF and CF testing was performed on each sample on the ACL TOP 700 (IL Werfen,) using RecombiPlasTin 2G (IL, Bedford USA) and Dade Thrombin Reagent (Siemens, Marburgh, Germany). Correlation with matched fibrinogen controls, median differences, and relationships to fibrinogen level and drug level were explored for each anticoagulant.

Results

The median differences in CF versus DF were: dabigatran 0.13g/L [-0.28 to 0.63], rivaroxaban 0.38g/L [0.02 to 0.66], apixaban 0.59g/L [0.2 to 0.8]. The DF overestimated the CF particularly in rivaroxaban and apixaban samples. Apixaban differences appeared to increase with higher drug levels, which was not seen in rivaroxaban. CF-DF correlation remained high within each group: apixaban $r = 0.97$, rivaroxaban $r = 0.91$, dabigatran $r = 0.87$, and when compared to matched controls - dabigatran (drug/control) $r = 0.97/0.95$, rivaroxaban $0.94/0.97$, apixaban $0.96/0.97$).

Discussion

In this laboratory with this instrument-reagent combination, in the normal fibrinogen range, DF appears to overestimate fibrinogen levels compared to CF for these DOACS. The difference is greatest with apixaban, followed by rivaroxaban and dabigatran. From these preliminary results we are routinely now performing CF for patients on apixaban, rivaroxaban and dabigatran, and suggest that other laboratories using DF assess the degree of discordance between the two assays.

P319. Very Low-dose prophylaxis in reducing bleeding episodes in adult patients with haemophilia A: a retrospective analysis

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Background

High-dose and intermediate-dose prophylaxis in haemophilia remain impracticable in resource-limited countries. Low-dose prophylaxis is feasible but limited data exists for tertiary prophylaxis in adult patients.

Aim

To assess the efficacy of very low-dose prophylaxis in reducing bleeding episodes in adults with moderate and severe haemophilia A.

Method

From July 2013, adults with moderate or severe haemophilia A without inhibitors with frequent bleeding episodes treated at our adult haemophilia clinic with “on demand” strategy were offered prophylaxis. We retrospectively analysed eight consecutive patients switched to prophylaxis from July 2013 to January 2016. Patients received FVIII concentrate at 5–10 units/kg body weight, rounded down to the nearest vial size, 1 to 3 days a week. Bleeding episodes were obtained from patients’ bleeding and infusion logs. Comparison was performed using Wilcoxon test.

Results

Seven patients, including one adolescent (3 severe, 4 moderate), were analysable. One patient withdrew. 3 moderate had 1% FVIII activity and one had >1–2% activity. Bleeding data 6 months before and 6 months (5 months in the most recent patient) following prophylaxis were analysed.

Age ranged from 17 to 51 years (median 30 years). Median annualised bleeding rate (ABR) reduced from 42.4 (range:23.8–66.6) before prophylaxis to 8.0 (range:2.4–26.1) after prophylaxis ($p<0.05$). Actual prophylactic doses of FVIII administered ranged from 2.8 to 9.4 (median:4.5) units/kg/infusion with a total of 5.6 to 18.1 (median:13.4) units/kg/week. 1 patient was on once a week, 4 on twice a week and 2 on three days a week infusions. All patients reported subjective improvement. Joint health and quality of life scores were not available prior to prophylaxis and could not be assessed.

Conclusion

Very low-dose prophylaxis is feasible and effective in reducing total bleeds in our adult patients and warrants further evaluation.

P320. Sensitivity of routine coagulation assays to Direct Oral Anticoagulants: Patient samples versus commercial drug-specific calibrators

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Aim/Background

Most studies on the sensitivities of coagulation assays to DOACS (Direct Oral Anticoagulants) are based on normal plasma spiked with anticoagulant in the laboratory. Recent studies have shown that reagent sensitivity varies significantly depending on whether spiked or patient samples are used. The aim of this study was to compare the sensitivities of routine coagulation assays in patient samples and commercial drug specific calibrators using commonly used Activated Partial Thromboplastin Time (APTT) and Prothrombin time (PT) reagents (i.e. Actin FS and Neoplastine CI Plus for APTT and PT respectively) in Australian laboratories.

Method

Samples collected at Pathology North Hunter (PN-H) for dabigatran (n=39), rivaroxaban, (n=56) or apixaban levels (n=22) between February 2013-November 2015 were analysed and compared to 2 different commercial drug specific calibrators from different manufacturers for each DOAC.

Results

Our results show that dabigatran (Hyphen and Technoclone) and rivaroxaban (Stago) calibrators tend to overestimate the APTT but are similar to patient samples for PT. A cut-off DOAC level of 50ng/ml based on results from patient samples within the laboratory can be used as the lower limit which will result in prolongation of APTT for dabigatran (sensitivity 96%, n=25) and PT for rivaroxaban (sensitivity 97%, n=29) respectively.

Conclusion

Individual laboratories should be familiar with the sensitivity of their coagulation reagents to different DOACs including differences between patient samples versus different commercial drug specific calibrators.

P321. Strategies for Enumeration of Circulating Microvesicles by Flow Cytometry: Forward vs. Side Scatter

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Aim

To determine if forward (FSC) or side scatter (SSC) is the better sizing parameter for enumeration of circulating microvesicles (MV) by flow cytometry.

Method

A FACS Canto flow cytometer (BD Biosciences, San Jose, CA, USA) was optimised for MV analysis using either FSC or SSC by calibration with Megamix or Megamix-Plus SSC beads (Biocytex, Marseille, France), respectively. A series of 74 platelet free plasma samples from patients with type II diabetes were analysed for MV expressing phosphatidylserine, CD41, CD235, CD31/CD42b, CD105 and CD62e. Events were acquired for 120s on low flow rate and a known amount of CountBright counting beads (Molecular Probes, Eugene, OR, USA) were included in each sample to enable calculation of absolute MV [MV/ μ l = (MV count/bead count) x (total # beads/test volume)]. Non parametric tests were used to determine differences and correlations between FSC and SSC results. A p-value <0.05 was considered significant.

Results

The Canto was able to adequately resolve 0.5 μ m beads by FSC and 0.16 μ m beads by SSC, but there were significantly more background events using SSC compared to FSC (3113, 2098-23860 vs 470, 404-3994; p=0.008). In general, sample analysis by SSC resulted in significantly higher numbers of MV (p<0.0001) but was nonetheless well correlated to enumeration by FSC for all MV subtypes (p=0.62-0.89, p<0.000001). However, there were significantly less counting beads enumerated when SSC was used (730, 595-826 vs 766,649-882; p=0.000001) and this was negatively correlated to the number of events in the MV gate (p=-0.366; p=0.001).

Conclusion

Although SSC may provide better resolution of small MV; high background negatively affects counting bead enumeration and overall MP calculations. Strategies to reduce SSC background would have to be employed in order to reliably use this technique.

P322. Comparison of Thrombin Generation and Markers of Clotting Activation After Conversion From Warfarin to a Direct Factor Xa Inhibitor

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Background

The direct factor Xa (FXa) inhibitor oral anticoagulants (e.g. rivaroxaban and apixaban) are widely used as alternatives to vitamin-K antagonists (e.g. warfarin) for the prevention and treatment of thromboembolism. Indirect comparison of *ex-vivo* thrombin generation (TG) and markers of *in-vivo* clotting activation (MoCA) between patients treated with agents from either therapeutic class suggest differences in their pharmacodynamic effects.

Aims

To directly compare intra-individual changes in TG and MoCA in patients after conversion from warfarin to rivaroxaban or apixaban.

Methods

Retrospective review of medical records from a single centre (August 2011 – December 2014) identified patients treated with a FXa inhibitor after switching from warfarin with paired plasma samples (taken within 3 months prior to and 6 months post switch) assessing TG and MoCA. TG was assessed by calibrated automated thrombography (CAT) and MoCA were assessed utilising D-dimer (DD), thrombin-antithrombin III complex (TAT) and prothrombin fragment 1+2 (PF1+2) plasma concentrations.

Results

36 patients (53% female, median age 57yo) were included (rivaroxaban n=33, apixaban n=3). Median duration of warfarin therapy prior to switching to a FXa inhibitor was 5.5 years. Median timing of paired samples pre- and post-switch was <1 and 3 months, respectively. Mean CAT parameters demonstrated evidence of TG suppression at both time points, however there was an increased endogenous thrombin potential with a prolonged lag time and time to peak with FXa inhibitors relative to warfarin. Mean values of all assessed MoCA were within the normal range. TAT and DD concentrations were similar for both anticoagulant classes, however there was an increase in PF1+2 levels associated with FXa inhibitors relative to warfarin.

Conclusion

Paired plasma samples from patients treated initially with warfarin and subsequently switched to FXa inhibitors both show pharmacodynamic evidence of effective anticoagulation. However, direct comparison of TG and MoCA in the same patients after a switch in therapy suggest differences in TG dynamics and background clotting activation between the different classes of anticoagulants.

P323. Current Practice regarding Prophylaxis in Patients with Moderate and Severe Haemophilia B in Australia

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Aim

To characterise current practice in patients with moderate or severe Haemophilia B (HB) in Australia regarding use of prophylactic clotting factor infusion.

Method

Data was derived from the Australian Bleeding Disorder Registry (ABDR) on patients from whom consent had been obtained. Data was obtained on patient diagnosis, disease severity, age, weight, treatment regime, expected and observed clotting factor usage during the Year 2015. The percentage of patients receiving prophylaxis according to severity of Haemophilia and age was calculated, along with average prophylactic dose (IU/Kg/Year). Compliance was assessed by calculating the percentage of patients in whom $\geq 75\%$, or ≥ 50 but $< 75\%$, of expected factor consumption was documented.

Result

Data was obtained on 128 HB patients, 70 with severe HB and 58 with moderate disease. 74% (52/70) patients with severe HB and 19% (11/58) of patients with moderate HB were on routine prophylactic therapy. The percentage of patients receiving routine prophylaxis decreased with age and in severe HB patients ranged from 100% (5-9years) to 33.33% (60 plus-years). The mean annual consumption of Factor IX in patients with severe HB was significantly higher in patients on routine prophylaxis (3781 IU/Kg/Year, SD 1708) in comparison to patients receiving on-demand therapy (869 IU/Kg/Year, SD 687), $p < 0.001$. 20/52 (38.46%) patients with severe HB receiving prophylaxis received $\geq 75\%$ of expected product, and 6/52 (11.53%) patients received ≥ 50 to $< 75\%$ of expected product.

Conclusion

Consistent with guidelines the majority of patients with severe HB are receiving routine prophylactic treatment, although the percentage decreases with age. The average prophylactic was similar to previous reports, and consistent with twice weekly dosing of 40 U/kg. A significant proportion of patients were not compliant with prophylactic treatment. Further information is required examining compliance and the continuation of prophylaxis into later adult life with joint health outcomes is required.

P324. Evaluation of platelet reactivity in patient on aspirin and clopidogrel undergoing Neurointervention procedures

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Background

Patients undergoing Neurointervention (NI) procedures for treatment of cerebrovascular disease are at high risk of complications of stent thrombosis, distal infarct and intracerebral bleeding. Dual antiplatelet therapy is routinely used with little evidence to support platelet reactivity testing or target thresholds.

Aims

1. To evaluate the utility of measuring Aspirin and Clopidogrel response in patients undergoing NI procedures.
2. To see whether abnormal results changed practice.
3. To correlate bleeding or thrombosis complications with platelet reactivity.

Method

Retrospective review of preprocedure testing in 62 patients who were taking Aspirin and Clopidogrel and who underwent NI procedures between 2012–2015 using the Multiplate Platelet Function Analyser (Roche).

Results

Sixty two patients (67 tests) were included in this study, 42 (66%) patients had procedures which required long term stents. Sixty five (98%) patients showed strong inhibition of COX-1 by aspirin on the ASPI test. Ten (15%) patients were non-responders to clopidogrel with AUC >45, a further 10 (15%) were considered partial-responders with AUCs of 30-45 on the ADP test. Six non-responders and 2 partial-responders to clopidogrel had management changes prior to procedure – these included substitution with prasugrel (n=5) and increasing clopidogrel dose (n=3) from 75mg to 150mg daily. Although 10 patients showed hyperresponse with AUC <10, only one had their clopidogrel dose decreased to 75mg alternate daily.

Clopidogrel ADP test		Aspirin ASPI (AA) test	
“High thrombotic risk in PCI” / Non responders AUC >45	10 (15%)	AUC >40	0 (0%)
AUC 19-45	22 (33%)	Inhibition COX 1 AUC 30-39	1 (2%)
“High bleeding risk in PCI” AUC <19	34 (52%)	Strong inhibition COX1 AUC <30	65 (98%)
Total	66 (100%)		66 (100%)

Three (5%) patients had bleeding complications which were presumed embolic converted to hemorrhagic strokes, all 3 patients had AUC <19 on ADP. Two (3%) patients had ischemic emboli, of which 1 was a partial-responder. One patient had an in-stent thrombosis, was a partial-responder to clopidogrel, and was restented after switching to Prasugrel. No in-stent thrombosis was identified in 34 patients for whom follow-up DSA were performed between 4 and 12 months at our institution.

Conclusion

30% of patients on dual antiplatelet agents undergoing NI procedures show sub-optimal platelet inhibition with clopidogrel. Aspirin resistance was not found. 10% patients experienced thromboembolic or hemorrhagic complications, emphasizing the importance of establishing thresholds for platelet inhibition. The Multiplate assay demonstrates feasibility for assessing platelet reactivity in NI patients.

P325. Modulation of endothelial adhesion by Omega-3 fatty acids in sickle cell disease patients

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Vasocclusive crises are the hallmark of Sickle Cell Disease. Endothelial activation and adhesiveness of blood cells are crucial for initiating this pro-inflammatory status. N-3(Omega-3) Polyunsaturated fatty acids are reduced in Sickle RBCs membranes proportionally to the degree of anemia. Omega-3(n-3) fatty acids have well established anti-inflammatory and anti-aggregatory roles. Soluble Vascular Adhesion Molecule (sVCAM -1) demonstrated to have the most significant increment in SCD during both steady state as well as crises and correlate with HB level inversely.

This study aimed to investigate the potential anti-adhesive properties of N-3 fatty acids in ameliorating SCD crises.

30 SCD patients (HB SS) were supplemented with 2-3 capsules of N-3 fatty acids (277.8 mg docosahexaenoic (DHA) and 39.0mg eicosapentaenoic (EPA)), according to age for one year. N-3 - treated patients were matched by age, gender and socioeconomic status to placebo- supplemented SCD patients and 24 healthy controls(HB AA) from their siblings. Levels of sVCAM-1 in serum were analyzed by Quantitative Indirect ELISA in duplicate.

The ameliorative effect of N-3 fatty acids on frequency of vasocclusive crises and need for blood transfusion was re-observed. HB level improved significantly by N-3 fatty acid. Mann - Whitney U test showed significant reduction in the level of sVCAM-1 in SCD patients treated with N-3 fatty acid compared to unsupplemented patients with P value of 0.001.

N-3 fatty acids are promising dietary modifiers of adhesiveness and inflammation in SCD. Further studies on gene expression and membrane integrin's to be carried out.

P326. A case of mistaken identity, a case of reclassification of a mild bleeding disorder 15 years on

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Aim

To highlight the utility of genetic testing to discriminate between Haemophilia A and type 2 von Willebrand disease (VWD).

Method

We report a case of a 63 year old man, presenting to The Royal Hobart Hospital for pre-operative assessment prior to a planned TURP. In 2000 a diagnosis of VWD had been made at another institution. Initial evaluation was prompted as part of investigation for a prolonged APTT. He had previously been managed with plasma derived von Willebrand factor (VWF) during a TKJR that proceeded without incident. Laboratory testing confirmed a reduced factor VIII level and abnormal qualitative VWF analysis. A previous desmopressin challenge confirmed normalisation of factor VIII levels and collagen binding activity. Multimeric analysis confirmed a normal multimeric pattern.

Results and Management

Initial pre-operative testing at our institution revealed a FVIII of 0.23 U/ml, a VWF:GPIbR 0.4 U/ml, VWF:Ag of 0.56 U/ml and VWF:CB of 48 U/ml. We treated him presumptively with plasma derived VWF given his past diagnosis. In view of the disproportionately low factor VIII, factor VIII and VWF gene sequencing was requested. Genetic testing confirmed the presence of a c.1573G>A mutation consistent with haemophilia A. Sequencing of exons 17-27 in VWF gene found no variant.

Conclusion

Genetic testing can discriminate between mild haemophilia A and type 2 VWD. This reclassification, in this case, has practical implications for future perioperative management, choice of factor replacement and genetic counselling. This case highlights the propensity of misdiagnosis in the pre genetic sequencing era and reinforces historical diagnoses should be revisited.

P327. PESI and simplified PESI in Monash Health

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Monash Health

Background

The pulmonary embolism severity index (PESI) score has been used to identify outpatients with acute pulmonary embolism (PE) at low risk of 30 day mortality. More recently a simplified version of the score (sPESI) has been developed. Our aim was to assess mortality in the low risk group (PESI class 1 and 2) compared to the high risk groups, and to compare this data with patients stratified by the sPESI score

Methods

Consecutive nonpregnant adult outpatients presenting to ED with symptomatic and subsequently objectively confirmed acute PE (segmental vessel or greater) between January 2013 and October 2013 were identified. A chart review was undertaken to extract data relevant to the respective PESI scores. Thirty day mortality was determined through hospital records or via the Victorian Births, Deaths, and Marriages registry.

Results

160 eligible patients were identified with complete data on 151 subjects. The mean age of the cohort was 61 and 45.6% were male. Sixty eight patients (45%) of the cohort were categorised as low risk (class 1 and 2) in whom the thirty day mortality was 0% (95% Confidence interval (CI) 0- 3.7%) Eighty three patients were categorised in the high risk groups (class 3,4,5) of whom 13 died within 30 days: 15.6% (95%CI 9.1- 24.7%), a mortality rate significantly higher than that of the low risk group (p=0.02). The corresponding 30 day mortality in the low risk sPESI groups was 0% (95%CI 0-5%) and these patients accounted for 32% of the cohort. For the higher sPESI groups the 30 day mortality was significantly higher at 12.7% (95%CI 7.3- 20.2%) (p=0.02).

Conclusion

These preliminary data suggest both the complete PESI score Classes 1 and 2 and the sPESI identify PE patients at low risk of 30 day mortality but the complete score may identify a greater proportion of patients than the sPESI.

PESIClass * Mortality Crosstabulation

PESI Class		Mortality		Total	95% C.I	Merging: Low / High
		Alive at 30 days	Dead at 30 days			
Very low risk	Count	37	0	37	0-6.5%	Low Risk (Class 1 +2): 0/68
	% within PESIClass	100.0%	0.0%	100.0%		
Low risk	Count	31	0	31	0-7.7%	95%CI: 0-3.7%
	% within PESIClass	100.0%	0.0%	100.0%		
Intermediate risk	Count	28	2	30	1.4-19.7%	High Risk (Class 3+4+5) 13/83 (15%) 95%CI:9.1-24.7%
	% within PESIClass	93.3%	6.7%	100.0%		
High risk	Count	11	3	14	6.4-46.9%	
	% within PESIClass	78.6%	21.4%	100.0%		
Very high risk	Count	31	8	39	10.2-35%	
	% within PESIClass	79.5%	20.5%	100.0%		
Total	Count	138	13	151	4.9-13.9%	P value: 0.002
	% within PESIClass	91.4%	8.6%	100.0%		

P328. Recovery of Clotting Factors Following Administration of Fresh Frozen Plasma in Russell's Viper (*Daboia russelii*) Envenoming

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Aim

Russell's viper bites cause severe envenoming with venom induced consumption coagulopathy (VICC), taking 24-48h to resolve post-antivenom. We investigated the recovery of clotting function and factor levels after administration of high-dose antivenom compared to low-dose antivenom and fresh frozen plasma (FFP).

Method

Serial citrate samples were collected for patients as part of a clinical trial, platelet poor plasma was prepared and frozen until analysis. Patients with confirmed Russell's viper bites on enzyme immunoassay, an INR >3 or fibrinogen <0.2g/L, with 4+ samples 12h post-antivenom available, were included. Prothrombin time [PT; international normalised ratio (INR)], fibrinogen, activated partial thromboplastin time (aPTT), D-Dimer, factor (F) V, FVIII and FX were measured using standard coagulometric or immunoturbidimetric methods. The two groups were compared using survival analysis, with time to fibrinogen >1g/L, INR <2, FV and FX >50% used as indicators for coagulation recovery. Area under the curve (AUC) of D-Dimer was compared to determine if FFP caused further consumption.

Results

Seventy-seven patients: 44 high-dose antivenom, and 33 low-dose antivenom and 4 units FFP within 6h, had a median highest INR and aPTT >12 (1.6 to >12) and 180s (28-180s), respectively. The median lowest fibrinogen, FV, FX, FVIII and median highest D-Dimer were <0.2g/L (<0.2-1.1g/L), 2.5% (2.5-45%), 25% (2.5-86%), 97% (2.5-292%) and 376mg/L (4.8-909mg/L), respectively. There was no significant difference in the time to recovery for INR or fibrinogen. There was a more rapid recovery of FV (Gehan-Breslow-Wilcoxon test; p=0.017) and FX (p=0.028) for low-dose antivenom and FFP group, which reached significance. There was no significant difference in the D-Dimer AUC between groups (median 4208 versus 3469g/L.h; p=0.47).

Conclusion

The use of FFP post-antivenom in Russell's viper induced VICC did not hasten the recovery of clotting times or factor levels except possibly for FV and FX. There was no evidence that FFP worsened coagulopathy.

P330. Importance of High Platelets Counts in Ovarian Cancer Patients at Central Women Hospital, Yangon, Myanmar

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Aim

In this study, we investigate the importance of high platelet count in women with ovarian carcinoma and evaluate its association with other known clinico-pathologic prognostic factors.

Method

The study was approved by the Ethics Review committee at the Department of Medical Research, Ministry of Health, Myanmar. The research project was carried out at the Oncology Ward, Central Women's Hospital, Yangon, Myanmar. A total of 57 newly diagnosed ovarian cancer patients of ages between 13 to 84 years were included in this study. Pre-operatively, platelet count was determined in these patients by using an automated hematological analyzer (Sysmex). Tumor marker protein CA 125 level in these patients was measured at the clinical pathology department, Central Women's Hospital. A blood film examination was also done by a pathologist. Post-operatively, patients were followed up to assess the histology report and other operative finding such as ascites and metastasis.

Results

49.1% (28/57) of patients showed normal platelet count (150,000 to 400,000 platelets per microliter (mcL) and 50.9% (29/57) showed high platelet count (>400,000 platelets per mcL). Among the patients with normal platelet count, 82.1% (23/28) were found with high CA 125 level (>21 U/mL) whereas all patients with thrombocytosis (100%, 29/29) were found with high CA 125 level ($p = 0.023$). Ascites was presented in 86.2% (25/29) of patients with thrombocytosis compared with 64.3% (18/28) patients with normal platelet count ($p = 0.06$). According to postoperative reports, metastasis were detected in 89.7% (26/29) in patients with thrombocytosis compared with 60.7% (17/28) patients with normal platelet count ($p = 0.01$).

Conclusion

In this study, we demonstrated the importance of elevated platelets counts at diagnosis in ovarian cancer patients, which is markedly associated with other known clinic-pathologic prognostic factors. Elevated platelet count may be used as a marker of aggressive disease in ovarian cancer patients. Platelet count is inexpensive, reliable, easy to interpret and can order routinely in patients and may be used as a biological marker related to cancer aggressiveness in hospitals with no facility to detect tumor markers.

P331. Intravenous immunoglobulin for recalcitrant Heparin Induced Thrombocytopenia/Thrombosis (HITTS)

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Aim

To describe the efficacy of IVIg in the treatment of a patient with refractory Heparin Induced Thrombocytopenia/Thrombosis (HIT/T)

Clinical History

We describe the case of a 50-year old female patient who presented with unprovoked left leg deep vein thrombosis (DVT) and bilateral pulmonary emboli (PE). She was initially treated with intravenous heparin due to rectal bleeding but was then transitioned to LMWH, and after negative malignancy screening to Apixaban 5mg twice daily. She represented 7 days later with extension of her DVT and thrombocytopenia ($63 \times 10^9/L$, baseline $237 \times 10^9/L$). HITS antibody testing (Acustar 17.8 IgG U/mL) and platelet aggregometry were both strongly positive. She was commenced on Argatroban infusion, with dose titration to keep her APTT $\sim 2x$ baseline. Despite therapeutic levels and no exposure to heparin, her platelet count remained low and after 9 days of Argatroban she developed an extensive right leg DVT. She was transitioned to Fondaparinux 12.5mg per day aiming for a trough level of 0.8-1.0 ug/mL, however despite therapeutic levels of Fondaparinux she remained thrombocytopenic. Fondaparinux antibody was negative. After international consultation IVIg at a dose of 0.5 g/kg for 2 doses were commenced. Within 48 hours the platelet count normalised. She was subsequently successfully transitioned to Warfarin therapy and her platelet count has remained normal.

Conclusion

There are few case reports showing efficacy of IVIg treatment in combination with anticoagulation as treatment for recalcitrant HIT. Rollin et al¹ have demonstrated in-vitro, the ability of normal plasma IgG to modulate HIT-antibody-induced platelet activation, and the immediate improvement in this case after administration suggests efficacy of iv IgG in controlling the underlying thrombotic process. Use of iv IgG should be considered in patients with refractory HIT with new thrombotic events despite standard therapy.

P332. Effect of anti-coagulants on procoagulant platelet formation

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Background and aim

It is increasingly clear that fibrin stabilisation of the athero-platelet aggregate is a key feature of occlusive coronary thrombosis. Analysis of thrombi retrieved from target vessels in acute myocardial infarction reveal fibrin stabilisation of platelet aggregate admixed with red cells. Procoagulant platelets provide the surface on the platelet aggregate for fibrin generation. We sought to understand the interaction between anti-coagulants and procoagulant platelet formation.

Method

We recently adapted a procoagulant platelet assay based on novel cell death agent, GSAO [1], to measure procoagulant platelet formation in *ex vivo* whole blood. Whole blood from healthy volunteers was pre-incubated with anti-coagulants at varying doses including: heparins, heparinoids, direct thrombin or FXa inhibitors or vehicle control prior to thrombin stimulation, noting that the level of anti-thrombin III in the assay is negligible. Procoagulant platelets were defined as being GSAO+ve/CD62P+ve by flow cytometry. Results were analysed with FlowJo and GraphPad Prism and analysed by paired or unpaired t-tests as appropriate.

Results

Unfractionated heparin and low molecular weight heparin both demonstrated a marked dose-dependent reduction in procoagulant platelets. The heparinoid danaparoid also showed a similar dose-dependent response. As expected, dabigatran, a direct thrombin inhibitor, inhibited thrombin-induced procoagulant platelet formation. In contrast, direct FXa inhibitor rivaroxaban was not associated with any change in procoagulant platelets.

Conclusion

Anti-coagulants were not *a priori* expected to have a significant effect on the procoagulant platelet formation and this was confirmed with the direct FXa inhibitor rivaroxaban. However, the heparins and heparinoids demonstrated a profound inhibitory effect on procoagulant platelet formation independent of either the anti-coagulation effect or the relative FIIa:FXa targeting. We speculate that the results indicate a direct interaction between thrombin and the platelet surface that is inhibited by heparins and heparinoids. This may have implications for the treatment of acute coronary events with anti-coagulants.

P333. Immune Thrombocytopenic Purpura associated with Fingolimod: 3 Case Reports

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Introduction

Fingolimod is an oral sphingosine-1-phosphate–receptor modulator which causes lymphocyte sequestration in lymph nodes. It was approved for relapsing Multiple Sclerosis (MS) following evidence it reduced relapse rates by 50%. The Therapeutic Goods Administration (TGA) of Australia as of September 2015 was aware of only one case where fingolimod preceded ITP by five weeks. Here we report three cases of ITP associated with fingolimod.

Cases

Cases are described in Table 1. None were on any medications known to cause ITP and routine investigations were non-contributory. All cases were treated with immunosuppression. Case 1 successfully weaned prednisolone after fingolimod cessation whilst case 2 had a slower wean whilst continuing fingolimod therapy. Case 3 had more refractory ITP and re-exposure to fingolimod worsened thrombocytopenia.

Possible mechanisms of the potential association between fingolimod and ITP remain unclear. One possible theory is immune dysregulation given fingolimod has been associated with autoimmune haemolytic anaemia and haemophagocytic syndrome. Another could be that it merely highlights autoimmune clustering.

Conclusion

In conclusion, our cases highlight that clinicians should be aware of the possible association between ITP and fingolimod although the mechanism for this remains unclear.

Table 1: Patient Characteristics

	Case 1	Case 2	Case 3
Year at ITP Diagnosis	2014	2013	2015
Age (years)	22	51	59
Sex	F	F	F
MS Duration (years)	3	2	10
Previous MS Therapy	Beta interferon	Beta interferon	Methylprednisolone Dimethyl fumarate
Other Autoimmune Conditions	-	Rheumatoid Arthritis Graves Disease	-
Duration of Fingolimod prior to ITP	12 months	2 months	19 months
Fingolimod post ITP	Continued	Continued	Discontinued
ITP Treatment	Prednisolone	Prednisolone IVIG Azathioprine Hydroxychloroquine	Prednisolone IVIG Azathioprine Hydroxychloroquine Elthrombopag Romiplostim

P334. Clinical champions of transfusion at the bedside

Catherwood A, Quested B, Goodwin J, Hunt R, Roberts T

BloodSafe and Central Adelaide Local Health Network

Background

Clinical champions are beneficial for improving patient safety, facilitating change and for influencing and improving local practice. **Central Adelaide Local Health Network (CALHN) implemented a Portfolio Nurse (PN) program for Standard 7 in 2013 and conducted an evaluation of the program during 2014-15.**

Aim

The aim of the study was to evaluate the effectiveness;

- of the program achieving
- Improvement in patient safety within the clinical domain
- enhanced support for accreditation activity requirements for NSQHCS, Standard 7 from the clinical domain
- of the education provided to prepare and support the PN role.

Method

Online questionnaires were completed by PNs and senior nursing colleagues to provide quantitative data with regard to PN activities and qualitative reflections with regard to

- the role and its impact on PBM practices
- assessment of the education and support provided to the PNs
- the most significant changes as a result of the program.

	Sample size	Responses	Response rate
Portfolio nurse	92	37	40%
Senior colleague	207	20	9%

Clinical Data Mining of the SA Health Datix Software for Patient Safety, Safety Learning System (SLS) was also used to provide quantitative data with regard to haemovigilance activity changes pre and post implementation of the PN program.

Results

SLS data demonstrated 46% increase in ward haemovigilance activity. Questionnaire responses demonstrated significant changes in practice with auditing, education provision and role development; 84% of PN's reported they had changed transfusion practice in their clinical area and 27% of senior colleagues stated the role had improved patient safety within their area.

The support and education provided to the PN's was positively received but indicated some refinement was required.

Conclusion

The results of the evaluation are informing further development of the program and it is currently being implemented across other SA Health sites.

P335. Application of an audit of all current Venesection procedures in the Haematology Day Ward, SCGH to determine outcomes and referral to the Red Cross to relieve pressure on the units resources

Cook D, Crawford J

Haematology Day Ward, SCGH

Aim

The Haematology Day Ward at SCGH is a busy 12 bed unit that treats a range of Haematological conditions predominantly with chemotherapy, transfusions and venesection procedures, specifically for Hemochromatosis and Polycythaemia Rubra Vera.

To alleviate the ever increasing demand placed on the unit's resources, we reviewed the current venesection procedures with a view to referring those eligible to the Red Cross venesection service.

To assess this proposition we performed an audit of all current venesection patients, looking at treatment outcomes and eligibility for referral to the Red Cross.

Method

To generate an audit sheet, we initially accessed the Red Cross online website to determine their specific referral criteria. Eligibility included age of the patient, Haemoglobin, pregnancy status, vascular disease, poorly controlled or complicated comorbidities, Hepatitis B, C and HIV status, cardiomyopathy, cirrhosis and previous venesection complications.

To assess treatment outcomes, we determined the ideal blood result parameters for Hemochromatosis and Polycythaemia Rubra Vera based on the Haematology Department SCGH guidelines, we looked at diagnosis, venesection frequency, clinic review frequency and current blood test results.

The audit sheet was completed for all active venesection patients attending the Haematology Day Unit and results correlated.

Results

Of the 58 current patients audited, 48 patients reached their treatment goals and 34 patients were eligible to transfer to the Red Cross service for their venesection procedures.

Conclusion

The results of the audit, along with the link to the Red Cross Referral webpage were emailed to the Haematologists, Senior Registrars and Registrars to promote awareness of the easy online referral system and the eligibility criteria but also ultimately to improve utilisation of the resources available in the Haematology Day Ward.

P336. “It is OK to Ask” Consumer Focused Patient ID Empowerment Project at a Perth Tertiary Teaching Hospital

Darby S

Sir Charles Gairdner Hospital

Aim

The patient identification check is a critical point in the transfusion process where errors are made. These mainly occur if staff do not follow policy, and result in no true check of the patient's identity at the bedside. The aim of the project was to empower patients to remind their caregiver to check their identity prior to any treatments. Involving patients in their care can help make it safer, and targeted use of these posters will remind both staff and patients of the critical importance of ensuring correct identity.

Method

Consumers were an integral part of the development of the posters and feedback resulted in changes to the content of the posters. The posters were placed hospital wide in patients' rooms, patients' bathrooms and in outpatient areas. A hospital advertising campaign was implemented to ensure that hospital staff and consumers were fully informed.

Result

The methods for evaluation were as follows:

- Comparing the number of patient ID related errors at one month, 3 months and six month
- Intervals against baseline data
- 30 Staff surveys at one and six month intervals
- 30 patient surveys at one month, 3 months and six month intervals against baseline data

Conclusion

There has been a 67% reduction in patient related ID errors after 3 month of implementation. 80% of patients felt that the poster campaign empowered them to ask hospital staff to check their identity. 11% of staff surveyed, stated that patients reminded their caregiver to check their identity.

P337. A Quality Improvement Activity to Inform Service Delivery Development for Haematology Patients in their Post Treatment Phase

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Cancer Care Services Royal Brisbane & Women's Hospital

Background

Survivorship or post treatment care is recognised as a priority in the continuum of care to patients receiving treatment for Haematology malignancies. The literature clearly articulates and calls for further strategic direction in the coordination and advice of Haematology patients in their post treatment phase. Our experience at the RBWH is that there is a need to educate and prepare patients for their post treatment phase and integrate this aspect of care into standard care delivery models.

Aim

Our aim was to identify unmet needs of patients at the completion of treatment at a local level. The data gathered will directly inform future development and design our service delivery in the phase of completion of treatment. To provide further accuracy to local resource development a literature review was undertaken to explore requirements for patients at the completion of treatment in the haematology setting.

Structure

A survey tool aimed at patients who have completed their treatment was developed in collaboration with the local Cancer Care Enhancement Team. The tool was developed using available research to ensure it was comprehensive in assessing all areas relevant to this cohort of patients. Consumer engagement was sought on the content and layout. Fifty patients post their acute treatment were approached within the clinic to complete the survey. The survey assessed consumer needs for physical, emotional, psychosocial, spiritual, living well post treatment and organisational requirements upon treatment completion.

Findings

Results identified if the patient received information and found it relevant, if the patient did not receive the information and whether this information would have been useful or not. Patients were asked how they would like to receive this information and at what stage of their treatment journey this would have been beneficial. Results have informed design and development of resources in this essential component of care delivery.

P338. What is the role of the haematology outpatient nurse in educating and supporting patients living with chronic lymphocytic leukaemia about the risks of secondary skin cancers

Foster A

Waikato Hospital

Aim

To establish if there is a specific role for the outpatient nurse in assessing the risk of secondary skin malignancies in people living with chronic lymphocytic leukaemia.

Methods

A literature review was conducted utilising several electronic databases (CINAHL, Medline, PubMed, Nursing Reference Centre, Cochrane Library, and EBSCO). Keywords searched were chronic lymphocytic leukaemia, secondary malignancy, skin cancer, risk factors, survivorship, treatment, chemotherapy, nurse's role, patient education, learning styles, patient teaching, Ottawa charter. Time period of articles was ten years. The final literature review was conducted with a total of 22 articles.

Results

The reviewed literature unanimously supported the finding that people living with chronic lymphocytic leukaemia (regardless of how effectively the disease is controlled) are at a higher risk of developing skin cancer. The leading causes for this is thought to be due to both the immunocompromised status associated with chronic lymphocytic leukaemia, its treatment, together with exposure to UV light. Mortality rates are higher in patients with a chronic lymphocytic leukaemia diagnosis who then develop skin cancer. The role of the nurse is acknowledged in recent literature; however there is limited research in this area as to exactly what that role consists of. In other nursing specialities it has been proven that nurses are in the prime position to provide ongoing preventative health education.

Conclusion

A framework of care based on the Ottawa Charter has been developed in the long term/follow on maintenance approach in chronic lymphocytic leukaemia. Nurse led health management clinics will be held in the outpatient setting. This will enable the outpatient nurse to offer patient support and education in prevention, self-screening and management of secondary skin cancers.

P339. Spitting chips at splitting PICCs: a sudden increase in catheter fracture rates in one institution treating acute myeloid leukaemia

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¹ *Royal Melbourne Hospital*, ² *Victorian Comprehensive Cancer Centre*

Aim

Central venous access is a requirement for curative treatment in acute myeloid leukaemia. Increasingly, peripherally inserted central catheters (PICCs) are being used. Complications from the use of PICCs such as infection, malposition and venous thrombosis are not uncommon. PICC fracture was rare in our institution, which we considered to be an advantage of the 'power injectable' nature of the type PICC used (Cook Turbo-Ject). Anecdotally, we noticed a sudden increase in the incidence of PICC fracture on the distal tip in PICCs inserted in 2015 despite no obvious change in PICC management policy or types of line attachments.

Method

We retrospectively analysed the medical records for all patients diagnosed with acute myeloid leukaemia that had a PICC line inserted between 1/1/2013 to the 31/1/2016. Insertion date, removal date and reason for removal were recorded. Statistical significance was calculated using Fisher's exact test.

Results

69 patients, 103 PICCs, 3908 PICC days were included in the analysis. For the two years from 1/1/2013 to 31/12/2014 there were 51 patients, 86 PICCs, 2899 PICC days, 0 lines were removed due to fracture. From 1/1/2015 to 31/1/2016 there were 17 PICCs, 11 patients 6 fractures (35%). There was a statistically significant increase in fracture rates ($p = 0.0001$). The median days to fracture was 47, range (22-149).

Conclusion

In this uncontrolled retrospective analysis we were not able to determine the cause of the increase in fracture rates. Despite this, the rate of fracture was high and subsequently there has been a change in the type of PICC line used in our hospital. Monitoring of central line infection is a common practice, but the monitoring of other complications is also important. PICC fracture generally occurred late after insertion and so may only be evident in populations that require long term access.

P341. Implementation of BloodTrack - an electronic inventory management system for satellite blood fridges at the Randwick Campus

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Background

The Randwick Campus had 6 satellite blood fridges and relied on a hand written documentation process. Audits over a 12-month period showed less than 100% compliance in record keeping of products in and out of the fridges despite engaging key stakeholders, education and re-formatting documentation. Randwick Campus satellite fridges failed to comply with NATA Standards and consequently 2 fridges were closed March 2016. It was agreed at the Randwick Transfusion Committee Meeting to implement a technology solution.

Aim

- Review usage of each satellite fridge
- Deploy an electronic system to improve traceability of red cells in satellite fridges and reduce wastage

Method

- Inventory registers were examined to review storage rates
- Project managed by Transfusion CNC, liaising with Blood Bank, Randwick Transfusion Committee and Haemonetics® (supplier of BloodTrack®)
- Site preparation, installation and education were the key stages to ensure a safe system delivery
- BloodTrack Manager™ was installed in Blood Bank and BloodTrack Courier® Kiosks in four satellite blood fridges
- Training needs and assessment were conducted Campus wide ensuing development of a training framework for over 700 staff

Outcomes

- 1 fridge closed due to low usage
- 2 fridges amalgamated due to their close proximity
- BloodTrack Courier® went live in three satellite fridges in June 2016, awaiting server connection in fourth fridge
- Early review shows improved tracking and therefore safety and quality of products in and out of satellite blood fridges
- Increased confidence of Blood Bank staff to return products to stock
- At present no wastage
- Positive feedback from staff due to improved workflow, reduction in workload and easy access to blood when it is needed
- Fridge locks are now being activated, ensuring a fully secure system. Blood can only be put in or taken out of the fridges if the correct process has been followed.

P342. Evaluation of low dose cyclophosphamide (LDC) for haematopoietic progenitor cell collection (HPCC)

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Aim

Compare the efficacy, toxicity and impact on resource usage of outpatient LDC (1.5g/m²) mobilisation and inpatient High Dose Cyclophosphamide (HDC) (3.5g/m²) mobilisation protocols.

Background

Haematopoietic Progenitor Cell (HPC) mobilisation and autologous HPC transplantation remains an important treatment option for patients with Multiple Myeloma (MM) and relapsed chemosensitive Lymphoma. The optimal method for HPC mobilisation remains debated in the literature. After an in-house presentation of literature our unit changed our mobilisation protocol from HDC to LDC.

Method

All patients (pts) receiving Cyclophosphamide mobilisation for HPCC over a 17 month period (Feb 2015-June 2016) received the LDC protocol. Data was compared to a retrospective cohort of patients receiving HDC (Oct 2013 - Jan 2015). Data collected included; hospital bed days, collection efficiency, blood/blood product usage, febrile neutropenia admission and intravenous antibiotics (IV abs) post chemotherapy.

Results

35 pts received LDC (n=35) and 43 pts received HDC (n=43). Fewer total bed days were used for LDC (3 v 129). One LDC pt (3%) required hospital bed for 3 bed days post chemotherapy for febrile neutropenia and required IV abs. Eight pts required admission post HDC for febrile neutropenia and IV abs (19%). No pts in the LDC group required blood/blood product support compared to 12 pts (28%) who received HDC.

Mobilisation	LDC (n=35)	HDC (n=43)
*Failure (%) (per pt)	2 (6%)	6 (14%)
number of collections to meet target mean (range)	57 (1.6) (1-3)	61 (1.4) (1-3)
Peak pre-CD34 mean (range)	173.66(7.3-173.66)	733.15 (1.63-733.15)
Total pt collections \geq CD34 $< 2 \times 10^6$ /kg (%) (per patient)	6(17%)	7 (16%)
Total pt collections \geq CD34 $< 5 \times 10^6$ /kg (%) (per patient)	8 (23%)	5 (12%)
Total pt Collections \geq CD34 $< 7 \times 10^6$ /kg (%) (per patient)	19 (54%)	28 (58%)
**Good mobilisers (%) (per pt)	22 (63%)	30 (70%)
Plerixafor usage (per pt)	8 (23%)	8(19%)

*Failure = CD34 $< 2 \times 10^6$ /kg

**Good Mobilisers $\geq 5 \times 10^6$ /kg CD34 in ≤ 2 collections

Conclusion

LDC is an effective mobilisation regimen with reduced toxicity and resource usage, without an increase in collection failures. We Acknowledge that this a single centre review with small numbers.

P343. Transfusion Education on Wheels Initiative

Monk A

Joondalup Health Campus, Glengarry And Attadale Private Hospitals

Background

In accordance with National Safety and Quality Health Service Standards 7 blood and blood products, there is a requirement to educate the workforce in safe transfusion practices. However, challenges for staff to attend face to face sessions due to rostering and workload issues in clinical areas are common place. This is compounded by accessibility to hospital computers restricting the ability of staff to complete online e-learning modules. Therefore it was necessary to 'think outside the square' and develop other strategies to ensure that the hospital staff were able to receive relevant education.

Aim

The aim is to provide an innovative and effective way of educating hospital staff in transfusion safety, by providing mobile education.

Method

- The trolley is adorned with transfusion education and is rotated between clinical areas throughout the entire hospital.
- The information provided on the trolley can be generic or area specific as determined from a training needs analysis or recent audit results.
- Staff sign that they have read the information and are encouraged to provide feedback
- Modifications to education are made following feedback from the clinical area.

Results

Feedback has been extremely positive and the initiative has increased the number of hospital staff who have received transfusion education. The transfusion education trolley has been particularly beneficial for night staff who tend to find it difficult accessing education.

Conclusion

The transfusion education trolley will continue to be used as an innovative education method at both hospital sites. Ongoing staff feedback will ensure that the content and overall look of the trolley will remain relevant and interesting.

P344. Low Density Lipoprotein Apheresis (LDL-A) for the Treatment of Patients with Refractory Homozygous and Heterozygous Familial Hypercholesterolaemia: The Austin Apheresis Unit Experience

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Austin Health

Aim/ Introduction

To describe the Austin Health Apheresis Unit experience with Low Density Lipoprotein Apheresis (LDL-A) and assessment of efficacy of the program. LDL-A is the selective removal of low density apolipoprotein B (apoB) containing lipoproteins from the blood. Treatment criteria are:

1. Homozygous Familial Hypercholesteremia (FH)
2. Heterozygous FH with progressive coronary disease despite maximal drug therapy where the LDL-C is >3.5 mmol/L
3. Lp(a) > 0.6 g/L (3x ULN) with progressive coronary disease despite maximal drug therapy.

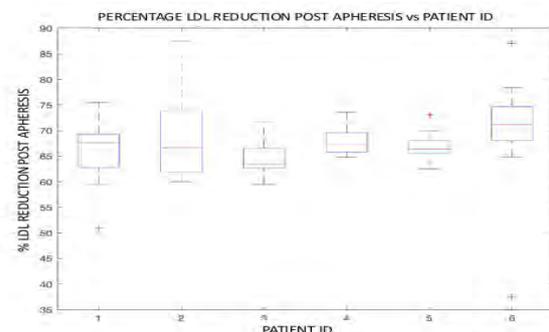
Method

Each LDL-A procedure is performed using the Com.Tec (Fresenius Kabi) continuous flow cell separator and the addition of a Evaflux 5A Plasma Fractionator (Kawasumi Laboratories, Inc). The Evaflux filter selectively removes LDL molecules based on size. One plasma volume is processed through the filter each treatment to a maximum volume of 3.2 Litres. Treatments are performed at two weekly intervals and usually take 2-3 hours. Retrospective analysis of pre and post apheresis low density lipoprotein levels over the first six months of treatment were obtained in 7 patients meeting the above treatment criteria. Adverse events were recorded.

Results

Of the 7 patients treatment is ongoing in 4. Of the patients that have ceased treatment, 1 patient went on to liver transplant, 2 patients were stopped due to vascular access issues.

Each LDL-A achieved a mean reduction in LDL of 67.31% \pm 7.03%. See graph below:



Vascular access was obtained peripherally in 4 patients, via permacath in 2 patients and via a double lumen apheresis infusaport in 1 patient. Side effects experienced are minor and include vascular access issues, citrate toxicity and ACE inhibitor induced hypotension and flushing. No new coronary events have occurred in the patient cohort once on the LDL-A program.

Discussion and Conclusion

Our LDL-A program allows reduction of LDL by 67%, with these results being comparable to other centres¹. LDL-A is an effective treatment in patient groups mentioned above and has acceptable tolerability. In the future evolocumab (PCSK9 inhibitor) may help many of our FH patients be controlled without LDL-A, which will be the subject of a randomised trial at our institution.

P345. Blood culture contamination as a measure of haematology nursing quality

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¹ Royal Melbourne Hospital, ² Victoria Comprehensive Cancer Centre

Aim

Contamination of blood for culture with commensal bacteria is a common problem and potentially results in unnecessary antibiotic administration, prolonged hospital admission and increased healthcare costs. Research studies have shown improvements in the rate of blood culture [BC] contamination via multiple interventions such as individual practitioner education, unit policy changes and type of disinfectant used. Taking blood for culture in our unit is a frequently undertaken and an exclusively nursing practice. Unit policy is often changed without monitoring of its effects. We aimed to track the rate of BC contamination over time in the context of policy changes.

Method

We prospectively and retrospectively monitored BC contamination from January 2013 until December 2015 in our 20 bed haematology/oncology inpatient ward. BC contamination was determined using the definition of the College of American Pathologists. Statistical significance was determined using Pearson's chi-squared test.

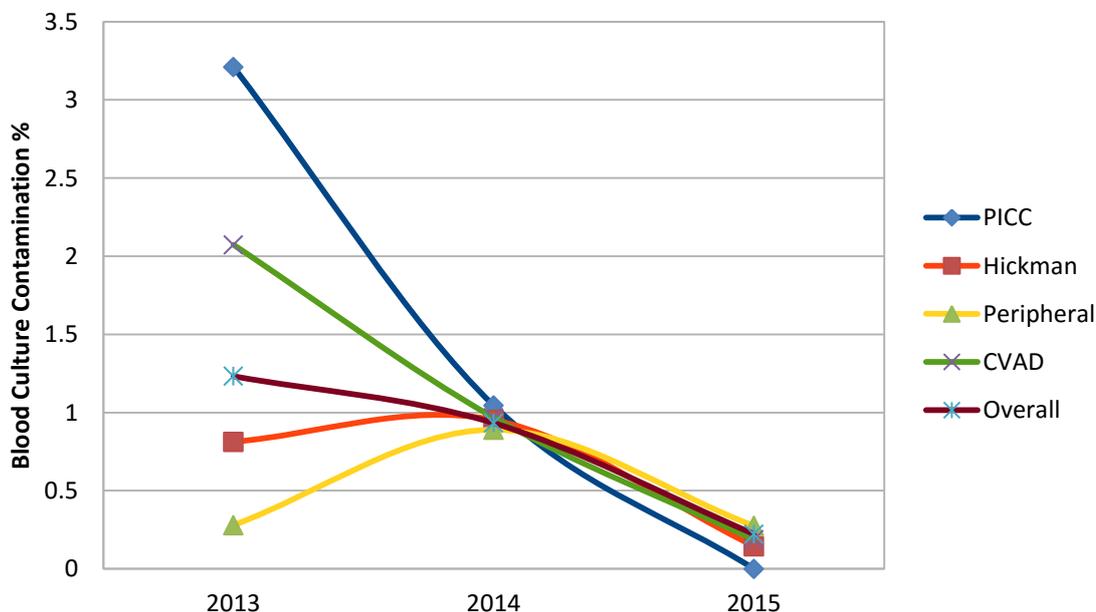
Over this period ward policy changed including type of needleless connector, equipment used to inoculate blood culture bottles and percentage of chlorhexidine in disinfection fluid.

Results

In total, 4838 sets of blood cultures were taken, 37 of which met the definition for contamination. There was a statistically significant reduction in the rate of BC contamination; 2013 1.2%, 2014 0.9%, 2015 0.2%, $p = 0.002$. Reductions most notably occurred in the blood taken from central venous catheters.

Conclusion

Blood culture contamination is an easily collected quality measure of both individual nursing and collective nursing practice and can be modified through policy changes.



P346. Long Weekend at the Coast? Don't forget to Pack your Buffy Coats and bring your Grannie as well

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University Hospital, Geelong

Aim

In a regional setting managing complex haematology patient's requires a coordinated approach from many specialities. This is our story of how we identified and implemented a process improvement. In 2016; two young adult patients with acute myeloid leukaemia, critically unwell both requiring initial provision of buffy coat preparations (BCP) while granulocytes were organised. We explore the 'lessons we learnt' from the events.

Method

Our first experience involves an 18 year old girl. Its Friday afternoon, long weekend, critically unwell with overwhelming sepsis. The decision is made to provide granulocytes and BCP to be sourced while awaiting granulocyte collections. This requires expert coordination from external and internal clinicians. The transport time required from Melbourne means the BCP are minutes away from expiry on arrival, blood bank first needs to process and test the BCP, ICU staff are unfamiliar with administering them. The Apheresis coordinator needs to organise donors and send the granulocyte collections to Melbourne for irradiation, the clock is ticking while these precious cells are in transit. Can we do better next time?

Results

Despite the challenges, effective teamwork and coordination enabled this treatment to be provided. Following this case, we identified an opportunity to: 1) review and update the screening and collection guidelines, 2) explore local irradiation to save time, 3) provide standardised donor information, and 4) update product release labels. An agreement to undertake the irradiation of the granulocytes locally was paramount to reduce time from granulocyte collections to patient use.

Conclusion

The long weekend in June a 24 year old male critically unwell septic, this time we were able to implement our changes and provide more timely specialised products. Providing complex haematology patients with the best possible care in a regional setting can be a challenge.... especially on a long weekend!

P347. Quality performance indicators for the clinical management of acute leukaemia; a single centre experience

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¹ *The Royal Melbourne Hospital*, ² *Victorian Comprehensive Cancer Centre*

Aim

Quality performance indicators (QPIs) are increasingly used as a method of measuring and maintaining improvements in medical and nursing care. In our own institution we feel that the hospital mandated indicators (such as discharge summary completion time and inpatient falls) are not sufficiently relevant to the complexity of acute leukaemia management.

The Scottish Cancer Taskforce (SCT) developed 12 evidence-based QPIs, all clearly measurable as a percentage of patients diagnosed and with a nominated target level. Examples include overall mortality rate (QPI 5) and the proportion of patients enrolled in a clinical trial (QPIs 8 and 11).

Method

We conducted a retrospective analysis of 110 patients diagnosed with acute leukaemia in our haematology department from Jan 2014 to Dec 2015. Each patient's care was measured by applying all 12 indicators, and we calculated the percentage of each indicator against the target rate of the QPIs from the SCT.

Results

Overall we reached the target rates in 10 of the 12 QPIs. We failed to reach the target in QPI 6 (timing of initiation of tretinoin in acute promyelocytic leukaemia) and QPI 11 (trial enrolment in age > 65).

Conclusion

Although we question the relevance of some of the QPIs used, we value the process of evaluating performance systematically with indicators that are relevant to acute leukaemia. Areas of low performance, such as rates of enrolment in clinical trials, are of particular concern. Though fed back to the multidisciplinary team, we feel that nursing has a natural role in QPI selection and measurement.

P348. Haematopoietic Progenitor Cell Proficiency Testing

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Royal Prince Alfred Hospital

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.

The benefits of proficiency testing are widely recognised. These include:

- Monitoring of a long-term laboratory performance.
- Improvement in the performance of tests/calibrations following investigation and identification of the cause(s) of unsatisfactory PT performance, and the introduction of corrective action to prevent re-occurrence.
- Staff education, training and competence monitoring.
- Evaluation of methods, including the establishment of method precision and accuracy.
- Estimation of measurement uncertainty.
- Contribution to the laboratory's overall risk management system.
- Confidence building with interested parties, e.g. customers, accreditation bodies, regulators.

The Transplant Laboratory in Cell and Molecular Therapies (CMT) employs both clonogenic and CD34 enumeration tests to evaluate the viability of HPC that is collected and cryopreserved for future transplantation and form the basis of the target specifications for the harvested product.

The CMT laboratory is enrolled in the Proficiency Testing Program – Human Bone Marrow (two samples per year) and the StemCell QC – Human Bone Marrow (12, monthly tests) assays supplied by StemCell™ Technologies. Staff in the CMT laboratory perform the two PT assays on a rotational basis for their continued competency assessment.

P349. Validation of HPC Marrow Harvest Procedure

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¹ SEALS BMT Laboratory, ² Sydney Children's Hospital

Background & Aim

HPC transplant using bone marrow (HPC-M) as the cell source has been performed at this facility for more than 40 years. More recently, HPC have also been sourced from mobilised peripheral blood by apheresis (HPC-A) or from cord blood (HPC-CB). Current NATA requirements mandate validation of harvesting procedures for HPC-A, but not for HPC-M or HPC-CB. This study was performed to review HPC-M harvest procedures performed and determine adequacy of current procedures.

Methods

A review of all 54 HPC-M harvests (14 autologous and 40 related allogeneic) performed at this facility between 2010 and 2015 was performed. Data obtained from 14 unrelated donor HPC-M harvests performed at external sites was included for comparative purposes where relevant.

Results

HPC-M harvests performed at this facility had a similar nucleated cell concentration to harvests performed at external facilities. Nucleated cell concentration (NCC) correlated directly with the CD34 count, and inversely with donor weight. Multi hole harvesting needles were found to yield similar NCC as standard needles, but significantly lower CD34 concentration. Failure to meet the target cell dose was associated with a peripheral WCC of $<5 \times 10^9/L$ in autologous harvests, or recipient weight $>$ donor weight in allogeneic harvests.

	Autologous	Related allogeneic	Unrelated allogeneic
HPC-M volume/donor kg	16 (12 – 22)	19 (13 – 22)	NA
NCC ($\times 10^9/L$)	20 (3 – 34)	16 (8 – 53)	15 (9 – 21)
Infused NC ($\times 10^8/kg$)	7 (6 – 7) n=2	5 (2 – 15)	8 (3 – 14)
Days to ANC $>0.5 \times 10^9/L$	14 (12 – 15)	20 (12 – 28)	15 (8 – 20)
Microbial contamination	7.8%		28%

Conclusion

HPC-M harvesting procedures at this hospital generally obtain the target cell dose, have a low contamination rate & result in timely engraftment.

P350. The addition of cyclophosphamide to G-CSF increases progenitor cell yield following mobilisation – Royal Adelaide Hospital experience

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SA Pathology

Our laboratory undertook a retrospective review of data collected from patients with multiple myeloma who underwent mobilisation with cyclophosphamide/ G-CSF or G-CSF with the aim of collecting at least 4×10^6 CD34/kg.

The aim of this review was to compare the efficacy of both mobilisation regimes.

The dataset comprised 83 patients who were mobilised with cyclophosphamide/ G-CSF and 64 patients mobilised with G-CSF. The collections took place between 2012 and 2016. The findings are summarised as follows:

- Starting CD34/ μ l
- We analysed the number of patients in each cohort who were collected when their CD34/ μ l count was less than 20, 15 and 10.
- The results show that there were more collections commenced when the CD34 count was below the target starting point of 20/ μ l in patients mobilised with G-CSF alone than in patients mobilised with cyclophosphamide/ G-CSF.
- The number of runs required to reach 2×10^6 CD34/kg per transplant
- Significantly more apheresis collection procedures were required to reach the target dose in patients mobilised with G-CSF than in patients mobilised with cyclophosphamide/ G-CSF (mean of 1.8 vs 1.2, $p < 0.0001$).
- Total number of runs performed and total number of CD34 cells collected
- Significantly more apheresis collection procedures were performed to collect the target CD34 dose in patients mobilised with G-CSF than in patients mobilised with cyclophosphamide/ G-CSF (mean of 2.5 vs 1.4, $p < 0.0001$).
- There was also a significant difference in the number of progenitor cells collected per apheresis procedures in patients mobilised with cyclophosphamide / G-CSF group (median CD34 cells 8.36×10^6 /kg) than in those mobilised with G-CSF (median CD34 cells 5.21×10^6 /kg).

Conclusion

The addition of cyclophosphamide to the G-CSF mobilisation regime increases the yield of progenitor cells collected in a single mobilisation.